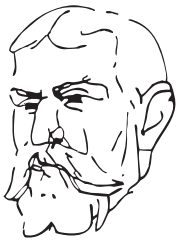
Wiadomości Lekarskie

Czasopismo Polskiego Towarzystwa Lekarskiego



Pamięci
dra Władysława
Biegańskiego

TOM LXXII, 2019, Nr 8, sierpień

Rok założenia 1928

Aluna Publishing

W ogłoszonym 31 lipca br. przez Ministra Nauki i Szkolnictwa Wyższego wykazie czasopism naukowych Wiadomości Lekarskie otrzymały 20 punktów (pozycja 27088).

Wiadomości Lekarskie is abstracted and indexed in: PubMed/Medline, EBSCO, SCOPUS, Index Copernicus, Polish Medical Library (GBL), Polish Ministry of Science and Higher Education.

Copyright: © ALUNA Publishing.

Articles published on-line and available in open access are published under Creative Common Attribution-Non Commercial-No Derivatives 4.0 International (CC BY-NC-ND 4.0) allowing to download articles and share them with others as long as they credit the authors and the publisher, but without permission to change them in any way or use them commercially.

Zasady prenumeraty miesięcznika Wiadomości Lekarskie na rok 2019

Zamówienia na prenumeratę przyjmuje Wydawnictwo Aluna:

– e-mailem: prenumerata@wydawnictwo-aluna.pl
– listownie na adres:

Wydawnictwo Aluna
ul. Z.M. Przesmyckiego 29, 05-510 Konstancin-Jeziorna

Prosimy o dokonywanie wpłat na numer rachunku Wydawnictwa:
Credit Agricole Bank Polska S. A.: 82 1940 1076 3010 7407 0000 0000

Cena prenumeraty dwunastu kolejnych numerów: 240 zł/rok (w tym 5% VAT)

Cena prenumeraty zagranicznej: 120 euro/rok.
Cena pojedynczego numeru – 30 zł (w tym 5% VAT) + koszt przesyłki.
Przed dokonaniem wpłaty prosimy o złożenie zamówienia.

Wiadomości Lekarskie

Editor in-Chief:
Prof. Władysław Pierzchała

Deputy Editor in-Chief:
Prof. Aleksander Sieroń

Statistical Editor:
Dr Lesia Rudenko

Polskie Towarzystwo Lekarskie:
Prof. Waldemar Kostewicz – President PTL
Prof. Jerzy Woy-Wojciechowski – Honorary President PTL
Prof. Tadeusz Petelenz

International Editorial Board – in-Chief:

Marek Rudnicki — Chicago, USA

International Editorial Board – Members:

Kris Bankiewicz	San Francisco, USA
Christopher Bara	Hannover, Germany
Krzysztof Bielecki	Warsaw, Poland
Zana Bumbuliene	Vilnius, Lithuania
Ryszarda Chazan	Warsaw, Poland
Stanislav Czudek	Ostrava, Czech Republic
Jacek Dubiel	Cracow, Poland
Zbigniew Gasior	Katowice, Poland
Andrzej Gładysz	Wroclaw, Poland
Nataliya Gutorova	Kharkiv, Ukraine
Marek Hartleb	Katowice, Poland
Roman Jaeschke	Hamilton, Canada
Andrzej Jakubowiak	Chicago, USA
Oleksandr Katrushov	Poltava, Ukraine
Peter Konturek	Saalfeld, Germany
Jerzy Korewicki	Warsaw, Poland
Jan Kotarski	Lublin, Poland
George Krol	New York, USA
Krzysztof Łabuzek	Katowice, Poland
Henryk Majchrzak	Katowice, Poland
Ewa Małecka-Tendera	Katowice, Poland
Stella Nowicki	Memphis, USA
Alfred Patyk	Gottingen, Germany
Palmira Petrova	Yakutsk, Russia
Krystyna Pierzchała	Katowice, Poland
Tadeusz Płusa	Warsaw, Poland
Waldemar Priebe	Houston, USA
Maria Siemionow	Chicago, USA
Vladyslav Smiianov	Sumy, Ukraine
Tomasz Szczepański	Katowice, Poland
Andrzej Witek	Katowice, Poland
Zbigniew Wszolek	Jacksonville, USA
Vyacheslav Zhdan	Poltava, Ukraine
Jan Zejda	Katowice, Poland

Managing Editor:
Agnieszka Rosa — amarosa@wp.pl

International Editor:
Lesia Rudenko — l.rudenko@wydawnictwo-aluna.pl

Distribution and Subscriptions:
Bartosz Guterman — prenumerata@wydawnictwo-aluna.pl

Graphic design / production:
Grzegorz Sztank — www.red-studio.eu

Publisher:
ALUNA Publishing
ul. Przesmyckiego 29, 05-510 Konstancin – Jeziorna
www.aluna.waw.pl www.wiadomoscilekarskie.pl
www.medlist.org

REGULAMIN PRZYJMOWANIA I OGŁASZANIA PRAC W WIADOMOŚCIACH LEKARSKICH

1. Miesięcznik Wiadomości Lekarskie jest czasopismem Polskiego Towarzystwa Lekarskiego, ma charakter naukowo-edukacyjny. Zamieszczane są w nim prace oryginalne, kliniczne i doświadczalne oraz poglądowe w języku polskim lub angielskim oraz innych językach (za zgodą redakcji).
2. Publikacja pracy w Wiadomościach Lekarskich jest płatna. Od stycznia 2017 roku koszt opublikowania artykułu wynosi 1000 zł plus 23%VAT. Jeżeli pierwszym autorem pracy jest członek Rady Naukowej czasopisma lub zespołu recenzentów – za druk nie pracy nie pobieramy opłaty, jeśli zaś jest kolejnym współautorem – opłata wynosi 500 zł plus 23%VAT. Wydawca wystawia faktury. Opłatę należy uiścić po otrzymaniu pozytywnej recenzji, przed opublikowaniem pracy. Z opłaty za publikację zwolnieni są członkowie Polskiego Towarzystwa Lekarskiego z udokumentowaną opłatą za składki członkowskie za ostatnie 3 lata.
3. Prace zapisane w formacie DOC (z wyłączeniem rycin, które powinny stanowić osobne pliki) należy przesłać pocztą elektroniczną na adres redakcji: Agnieszka Rosa - amarosa@wp.pl.
4. Objętość prac oryginalnych – łącznie z rycinami i piśmiennictwem – nie może przekraczać 21 600 znaków (12 stron maszynopisu), prac poglądowych – do 36 000 znaków (20 stron).
5. Strona tytułowa powinna zawierać:
   – tytuł w języku angielskim i polskim,
   – pełne imiona i nazwiska autorów,
   – afiliację autorów,
6. Praca oryginalna powinna mieć następującą strukturę: wstęp, cel pracy, materiał i metody, wyniki, dyskusja i wnioski, które nie mogą być streszczeniem pracy. Przy zastosowaniu skrótów konieczne jest podanie pełnego brzmienia terminu przy pierwszym użyciu. W pracach doświadczalnych, w których wykonano badania na ludziach lub zwierzętach, a także w badaniach klinicznych, należy umieścić informację o uzyskaniu zgody komisji etyki badań naukowych.
7. Streszczenia zarówno w języku polskim, jak i angielskim powinny zawierać 200-250 słów. Streszczenia prac oryginalnych, klinicznych i doświadczalnych powinny posiadać następującą strukturę: cel, materiał i metody, wyniki wnioski. Nie należy używać skrótów w tytule ani w streszczeniu.
8. Słowa kluczowe (3-6) należy podawać w języku angielskim i polskim, zgodnie z katalogami MeSH (Medical Subject Headings Index Medicus http://www.nim.nih.gov.mesh/MBrower.html). Słowa kluczowe nie mogą być powtórzeniem tytułu pracy.
9. Materiał ilustracyjny - ryciny, wykresy, rysunki, fotografie, slajdy - powinien być opisany cyframi arabskimi i zapisany jako pliki JPG, TIFF lub EPS o rozdzielczości 300 DPI (nie w plikach tekstowych). Ich opisy należy przesłać w osobnym pliku. W tekście muszą znajdować się odniesienia do wszystkich rycin (w nawisach okrągłych).
10. Tabele – ich tytuły (nad tabelą) i treść - powinny być zapisane w programie Microsoft Word, ponumerowane cyframi rzymskimi. Wszystkie stopki dotyczące tabeli powinny znajdować się poniżej tekstu tabeli. W tekście pracy należy umieścić odniesienia do wszystkich tabel (w nawiasach okrągłych).
11. W wykazie piśmiennictwa ułożonym według kolejności cytowania należy uwzględnić wyłącznie te prace, na które autor powołuje się w tekście. W pracach oryginalnych nie powinno być więcej niż 30 pozycji, a w poglądowych nie więcej niż 40 pozycji. Każda pozycja powinna zawierać: nazwiska wszystkich autorów, pierwsze litery imion, tytuł pracy, skrót tytułu czasopisma (wg Index Medicus), rok, numer, stronę początkową i końcową. Przy pozycjach książkowych należy podać: nazwisko autora (autorów), pierwszą literę imienia, tytuł rozdziału, tytuł książki, wydawnictwo, miejsce i rok wydania. Dopuszcza się cytowanie stron internetowych z podaniem adresu URL i daty użycia artykułu oraz o ile to możliwe nazwisk autorów. Każda pozycja piśmiennictwa powinna mieć odwołanie w tekście pracy umieszczone w nawiasie kwadratowym, np. [1], [3–6]. Pozycje zapisuje się w sposób zaprezentowany w Załączniku nr 1 do niniejszego regulaminu.
12. Po piśmiennictwie należy podać adres do korespondencji, nazwisko i imię pierwszego autora, adres, numer telefonu oraz adres e-mail.
13. Do pracy należy dołączyć oświadczenie podpisane przez wszystkich autorów określające udział poszczególnych autorów w przygotowaniu pracy (np. koncepcja i projekt pracy, zbieranie danych i ich analiza, odpowiedzialność za analizę statystyczną, napisanie artykułu, krytyczna recenzja itd.), a także oświadczenie, że biorą oni odpowiedzialność za treść. Ponadto należy zaznaczyć, że praca nie była publikowana ani zgłaszana do druku w innym czasopiśmie.
14. Jednocześnie autorzy powinni podać do wiadomości wszelkie inne informacje mogące wskazywać na istnienie konfliktu interesów, takie jak:
   – zależności finansowe (zatrudnienie, płatna ekspertyza, doradztwo, posiadanie akcji, honoraria),
   – zależności osobiste,
   – współzawodnictwo akademickie i inne mogące mieć wpływ na stronę merytoryczną pracy,
   – sponsorowanie całości lub części badań na etapie projektowania, zbierania, analizy i interpretacji danych lub pisanie raportu.

   Konflikt interesów ma miejsce wtedy, gdy przynajmniej jeden z autorów ma powiązania lub zależności finansowe z przemysłem bezpośrednie lub za pośrednictwem najbliższej rodziny. Jeśli praca dotyczy badań nad produktami częściowo lub całkowicie sponsorowanymi przez firmy, autorzy mają obowiązek ujawnić ten fakt w załączonym oświadczeniu.
15. Każda praca podlega weryfikacji w systemie antyplagiatowym (zapora ghostwriting).
16. Redakcja przestrzega zasad zawartych w Deklaracji Helsińskiej, a także w Interdisciplinary and Guidlines for the Use of Animals In Research, Testing and Education, wydanych przez New York Academy nof Sciencees' Adhoc Resarch. Wszystkie prace odnoszące się do zwierząt lub ludzi muszą być zgodne z zasadami etyki określanymi przez Komisję Etyczną.
17. Czasopismo recenzowane jest w trybie podwójnej, ślepej recenzji. Nadesłane prace są oceniane przez dwóch niezależnych recenzentów, a następnie kwalifikowane do druku przez Redaktora Naczelnego. Recenzje mają charakter anonimowy. Krytyczne recenzje autorzy otrzymują wraz z prośbą o poprawienie pracy lub z decyzją o niezakwalifikowaniu jej do druku. Procedura recenzowania artykułów jest zgodna z zaleceniami Ministerstwa Nauki i Szkolnictwa Wyższego zawartymi w opracowaniu „Dobre praktyki w procedurach recenzyjnych w nauce" (Warszawa 2011) i szczegółowo została opisana na stronie http://www.nauka.gov.pl/g2/oryginal/2014_02/307f933b1a75d6705a4406d5452d6dbf.pdf
18. Redakcja zastrzega sobie prawo redagowania nadesłanych tekstów (dokonywania skrótów i poprawek). Prace są wysyłane do akceptacji autorów. Poprawki autorskie należy przesłać w terminie 3 dni od daty wysłania wiadomości e-mail (pocztą elektroniczną). Brak odpowiedzi w podanym terminie jest równoznaczny z akceptacją przez autora nadesłanego materiału.
19. Przyjęcie pracy do druku oznacza przejęcie praw autorskich przez Redakcję Wiadomości Lekarskich.
20. Autorzy otrzymują nieodpłatnie plik PDF wydania, w którym znajduje się ich praca, a na życzenie - egzemplarz drukowany. Plik elektroniczny przeznaczony jest do indywidualnego użytku autora, bez prawa do rozpowszechniania bez zgody redakcji.
21. Prace przygotowane niezgodnie z regulaminem zostaną zwrócone autorom do poprawienia.
22. Redakcja nie odpowiada za treść zamieszczanych reklam.

CONTENS / SPIS TREŚCI

ORIGINAL ARTICLES / PRACE ORYGINALNE

Lyudmila A. Vygivska
MATRIX METALOPROTEINASE-2 AND PLACENTARY DYSFUNCTION DEVELOPMENT IN PREGNANCY WITH PERINATAL INFECTIONS 1421

Justyna Rosińska, Joanna Maciejewska, Robert Narożny, Wojciech Kozubski, Maria Łukasik
ASSOCIATION OF PLATELET-DERIVED MICROVESICLES WITH HIGH ON-TREATMENT PLATELET REACTIVITY IN CONVALESCENT ISCHEMIC STROKE PATIENTS TREATED WITH ACETYLSALICYLIC ACID 1426

Pavel Dyachenko, Igor Filchakov, Anatoly Dyachenko, Valeria Shahinian, Victoria Kurhanskaya
DIAGNOSTIC VALUE OF SPECIFIC ANTIBODY SYNTHESIS IN BRAIN OF PATIENTS WITH NEUROINFECTIONS 1437

Oleg Mokryk, Svitlana Ushtan, Yuliya Izhytska
CLINICAL EVALUATION OF THE EFFECTIVENESS OF THE DEVELOPED METHOD OF CONDUCTIVE ANESTHESIA OF THE FACIAL BRANCHES OF GREAT AURICULAR NERVE 1442

Olena O. Dyadyk, Serhiy I. Savolyuk, Roman A. Herashchenko, Valentyn A. Khodos, Vladyslav S. Horbovets
DEVELOPMENT OF OBLITERATION IN THE GREAT SAPHENOUS VEIN AFTER INFLUENCE OF HIGH-FREQUENCY ENDOVENOUS WELDING BY DATA OF MORPHOLOGICAL AND ULTRASONIC STUDIES 1447

Mariusz Jaworski, Mariusz Panczyk, Aneta Binkowska, Piotr Leszczyński, Robert Gałązkowski, Joanna Gotlib
MALADAPTIVE PERFECTIONISM AND AUTHENTIC LEADERSHIP SKILLS IN EMERGENCY MEDICINE STUDENTS 1453

Nataliia M. Adamchuk, Olena Y. Sorokina
ANALYSIS OF PSYCHO-EMOTIONAL STATE AND THE SEVERITY OF ASTHENIC SYNDROME IN CHILDREN WITH ACUTE LEUKEMIA AFTER THE FIRST STAGE OF CHEMOTHERAPY 1460

Mariia A. Krykhtina, Kseniia O. Bielosludtseva, Larysa A. Botvinikova, Nataliia M. Matikina
LUNG VESSELS THROMBOSIS IN HOSPITALIZED PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA: ROLE OF ENDOTHELIAL FUNCTION, HEMOSTASIS, FIBRINOLYSIS AND INFLAMMATION ON DIFFERENT PHASES OF TREATMENT 1463

Grażyna Kobus, Jolanta Małyszko, Hanna Bachórzewska-Gajewska
ACUTE KIDNEY INJURY IN ELDERLY PATIENTS 1466

Nataliya Matolinets, Helen Sklyarova, Eugene Sklyarov, Andrii Netliukh
CHANGES OF NITRIC OXIDE SYNTHASE AND HYDROGEN SULFIDE IN BLOOD LYMPHOCYTES IN THE ACUTE PERIOD OF POLYTRAUMA 1473

Kateryna V. Pivtorak, Natalia A. Shevchuk, Natalia A. Pivtorak, Iryna V. Fedzhaga
CORRECTION OF ADIPOCYTE SECRETION DISORDERS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE WITH OVERWEIGHT AND OBESITY 1477

Nataliia Kuzniak, Tetiana Protsak, Oleg Marchuk, Larysa Fedoniuk, Alex Kamyshnyi, Nataliia Penteleichuk, Denys Stoliar, Roman Dmytrenko
HISTOTOPOGRAPHY OF THE OVIDUCTS IN FETUS 1481

Olga I. Zalyubovska, Tetiana I. Tiupka, Victor V. Zlenko, Yulia N. Avidzba, Mycola I. Lytvynenko, Alina O. Minaieva
STRUCTURAL AND FUNCTIONAL STATE OF THE SEMINAL GLANDS IN THE DYNAMICS OF ACUTE INFECTIOUS INFLAMMATION 1486

Viktor P. Boriak, Svitlana V. Shut', Tetiana A. Trybrat, Olena V. Filatova
CONDITION OF HEMODYNAMICS IN THE PULMONARY CIRCULATION OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) CONCURRENT WITH METABOLIC SYNDROME WITH HYPERTROPHY AND ATROPHY OF THE MYOCARDIUM 1491

Maryna Kochuieva, Valentyna Psarova, Larysa Ruban, Nataliia Kyrychenko, Olena Alypova, Olha Matlai, Anna Shalimova
ASSOCIATIONS OF IRS-1 POLYMORPHISM WITH VARIOUS COMPONENTS OF THE METABOLIC SYNDROME IN HYPERTENSIVE PATIENTS 1494

Alina M. Yelins'ka, Lilia I. Liashenko, Vitalii O. Kostenko
QUERCETIN POTENTIATES ANTIRADICAL PROPERTIES OF EPIGALLOCATECHIN-3-GALLATE IN PERIODONTIUM OF RATS UNDER SYSTEMIC AND LOCAL ADMINISTRATION OF LIPOPOLISACCHARIDE OF SALMONELLA TYPHI 1499

Oleksandr Yavorovskiy, Sergii Omelchuk, Lyudmyla Sokurenko, Tetyana Zinchenko, Nina Solokha, Vasyl Aleksiichuk, Roman Brukhno
ENVIRONMENTAL AND OCCUPATIONAL HAZARDS OF METAL NANOCOMPOUNDS PRODUCTION AND APPLICATION: HYGIENIC, CLINICAL AND TOXICOLOGICAL ASPECTS 1504

Viktoria A. Petrashenko, Andrii M. Loboda, Olexandr I. Smiyan, Sergii V. Popov, Svetlana N. Kasyan, Igor E. Zaitsev, Elena K. Redko
LABORATORY CRITERIA OF PERINATAL DAMAGE OF CENTRAL NERVOUS SYSTEM AT PREMATURE NEWBORNS 1512

Inga R. Tymofiychuk, Ihor K. Churpiy, Tetiana P. Savchuk, Lilia D. Boreyko, Xenia V. Slobodian
COGNITIVE DISABILITY IN ESTROGENECTOMITED AND OLD RATS WITH DEVELOPMENT OF DIABETES MELLITUS 1517

Oleksandr O. Lytvynenko, Volodymyr F. Konovalenko, Anton Yu. Ryzhov
LOCAL RECURRENCES AFTER THE TREATMENT OF SOFT TISSUE MALIGNANT FIBROUS HISTIOCYTOMA (UNCLASSIFIED PLEOMORPHIC SARCOMA) OF THE LIMBS 1523

REVIEW ARTICLES / PRACE POGLĄDOWE
Anna Lis-Święty, Dorota Milewska-Wróbel
FACTS AND SPECULATIONS ON VITAMIN D IN PREVENTION AND TREATMENT OF ATOPIC DERMATITIS 1527

Borys Palamar, Tetiana Gruzeva
THE ESTIMATION OF ECONOMIC EFFECTIVENESS OF PREVENTIVE MEASURES OF NON-INFECTIOUS DISEASES 1532

Beata Janoszka, Agnieszka Nowak, Magdalena Szumska, Ewa Śnieżek, Krystyna Tyrpień-Golder
HUMAN EXPOSURE TO BIOLOGICALLY ACTIVE HETEROCYCLIC AROMATIC AMINES ARISING FROM THERMAL PROCESSING OF PROTEIN RICH FOOD 1542

Jakub Warakomski, Lucyna Siemińska
THE ROLE OF ADIPOSE TISSUE WITH PARTICULAR EMPHASIS ON CYTOKINES IN THE PATHOGENESIS OF NEOPLASTIC DISEASES 1551

Viktor Konoplitskyi, Ruslan Shavliuk, Dmytro Dmytriiev, Kostiantyn Dmytriiev, Oleksii Kyrychenko, Bohdan Zaletskyi, Oleksandr Olkhomiak
PILONIDAL DISEASE: CHANGES IN UNDERSTANDING OF ETIOLOGY, PATHOGENESIS AND APPROACH TO TREATMENT 1559

Mateusz Mizgalski, Krzysztof Zub, Karolina Dorobisz, Tomasz Zatoński
THE ROLE OF 1,25(OH)$_2$D$_3$ AND ITS ANALOGS IN PROLIFERATION AND DIFFERENTIATION OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK – LITERATURE REVIEW 1566

Antonina H. Bobkova, Maryna V. Trotska
SAFE NATURAL ENVIRONMENT AS GUARANTEE OF EXERCISING THE RIGHT TO HEALTH 1571

Oksana V. Kaplina, Olha H. Shylo, Ivan A. Titko
USING THE SAMPLES OF HUMAN BIOLOGICAL MATERIALS IN THE CRIMINAL PROCEDURE: THE PRACTICE OF THE EUROPEAN COURT OF HUMAN RIGHTS 1576

Mykhailo A. Anishchenko, Olexandr G. Aleksieiev, Inna A. Hamburh
INTERNATIONAL LEGAL STANDARDS FOR CONDUCTING BIOMEDICAL EXPERIMENTS ON ANIMALS: STATUS AND PROSPECTS OF DEVELOPMENT 1582

CASE REPORT / OPIS PRZYPADKU
Joanna Płonka, Jarosław Bugajski, Piotr Feusette, Agata Duszańska, Marek Gierlotka
LEVOSIMENDAN – A VALUABLE PLAYER IN THE TREATMENT OF A RIGHT-SIDED HEART FAILURE 1586

LETTER TO THE EDITOR OF "WIADOMOSCI LEKARSKIE"
Łukasz Wojdyga 1592

RESPONSE TO THE LETTER TO THE EDITOR OF "WIADOMOSCI LEKARSKIE"
Unislawa Williams, Janusz Kasina, Zbigniew K. Wszolek
PROGRAMY EDUKACYJNE DLA POLAKÓW CHCĄCYCH STUDIOWAĆ/PODNOSIĆ KWALIFIKACJE ZA GRANICĄ 1594

ORIGINAL ARTICLE
PRACA ORYGINALNA

MATRIX METALOPROTEINASE-2 AND PLACENTARY DYSFUNCTION DEVELOPMENT IN PREGNANCY WITH PERINATAL INFECTIONS

Lyudmila A. Vygivska
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Introduction: The search for a statistically significant link between sFasL and their potential regulators MMP-2 is necessary to optimize the tactics of managing and treating pregnant women with perinatal infections.

The aim: To determine the level of MMP-2 in pregnant with perinatal infections and its role in the development of placental dysfunction.

Materials and methods: The study involved examination of 230 pregnant, who were divided into 3 groups (60 people each): Group I with viral (CMV, HSV-1,2,6 types), Group II with bacterial (chlamydia, ureaplasma, mycoplasma) and Group III with mixed infection. Group IV was the control one, and included 50 pregnant with a physiological course of pregnancy. The study implied determination of serum levels of MMP-2 and sFasL, the level of caspase-3 in the placenta, ultrasound was performed by the MINDRAY M7 (China) scanner. The data obtained were statistically processed using the STATISTICA software.

Results: Pregnant women with perinatal infection were found to have a significant increase in sFasL and caspase-3 levels in all the studied groups as compared to corresponding values in the control group (Kruskal-Wallis test, $p<0.001$). In all groups of pregnant women with perinatal infection the level of MMP-2 in serum was elevated with respect to control values approximately 3-4 times (Kruskal-Wallis test, $p <0.05$). According to ultrasound data, hypoplasia of placenta was observed in (46.6 ± 6.4)% of cases in Group I and in (30 ± 6.4)% in Group III. In Groups II and III, placental hyperplasia was detected in (20 ± 5.2)% and (26 ± 5.7)% of cases, respectively.

Conclusions: The findings showed that secondary to the impact of perinatal infection among a number of indices in the peripheral blood of pregnant women, a significant role is played by an increase in levels of MMP-2 and sFasL. These indices reflect and determine the induction of apoptotic response from the placenta in this category of pregnant women. The revealed increase in caspase-3 level means that pregnant women with perinatal infections undergo caspase cascade of apoptosis in placental cells. Thus, the obtained results indicate that pregnant women with perinatal infections develop an increase in the level of MMP-2 and activation of the Fas-dependent signaling pathway of programmed cell death, which leads to the launch of a caspase cascade of apoptosis in placental cells. An increase in the loss of placental cells due to apoptosis may be one of the key factors triggering the development of placental dysfunction and, as a consequence, unfavorable conditions for fetal development.

KEY WORDS: placental dysfunction, matrix metaloproteinase-2, perinatal infections

Wiad Lek 2019, 72, 8, 1421-1425

INTRODUCTION

The process of formation and functioning of the "mother - placenta – fetus" system is controlled by common in their biological nature and opposite in their biological effects processes of cellular proliferation, caused by the action of growth factors and apoptosis [1]. Cell proliferation and programmed cell death are multidirectional processes that determine the fate of a particular cell in a cell population. It was stated [2] that the timing of implementation and severity of placental dysfunction (PD) are due to the degree of maladaptation of the endothelial system, activation of immunopathological reactions in the placenta, manifested in the trophoblast induced programmed cell death of lymphocytes and changes in the regulation of cell proliferation. The presence of a correlation between markers of endothelial dysfunction, apoptosis and cell proliferation indicates a close pathogenic relationship between angiogenesis disorders, vascular endothelial functions, immunopathological and proliferative processes in pregnant women with PD, especially of infectious origin [3].

The formation of the placenta, which is closely related to the destruction of basement membrane and extracellular matrix, is an invasive process. It involves a number of enzymes, in particular matrix metalloproteinases (MMP), whose main purpose is to regulate activation and intensive tissue rebuilding. Presented in an inactive form, after transport to the intercellular space, MMP is activated by a number of other proteinases [4, 5].

Formation of the placenta is associated with cell proliferation and reorganization in the local vascular system, accompanied by the increased activity of enzymes. This is important for the successful development and progress of pregnancy [6]. MMP-2, along with other MMPs, is involved in the transformation of the apoptotic ligand FASL into its soluble form of sFasL and thus may affect apoptosis [7,8]. The system of the Fas receptor membrane and its FASL ligand are one of the main ways of launching apoptosis in the placenta, which leads to the development of the fetoplacental complex dysfunction and, as a consequence, the pathological course of pregnancy. Assessment of the

level of MMP-2 is not only important for prognosis, but also represents the monitoring of the subcellular index reflecting the conditions for the formation of the placenta [8]. In view of this, the study of MMP-2 activity in pregnant women with perinatal infections (PIns) and the search for a statistically significant relationship between the level of sFasL and MMP-2 as its potential regulator is relevant and necessary to optimize the tactics of management and treatment of this category of women

THE AIM

To determine the level of MMP-2 in pregnant women with perinatal infections and its role in the development of placental dysfunction.

MATERIALS AND METHODS

For this purpose the study involved examination of 230 pregnant women after 22 weeks of gestation, who were divided into 3 groups (60 women each): Group I with viral (CMV, HSV-1,2,6 types), Group II with bacterial (chlamydia, ureaplasma, mycoplasma) and Group III with mixed infection. Group IV was the control one and included 50 pregnant women with a physiological course of pregnancy.

In the blood serum of pregnant women, the level of matrix metalloproteinase-2 (MMP-2) was measured by the immune enzyme method using the kits of human MMP-2 reagents produced by RD Systems (USA). A quantitative determination of the level of sFasL in blood serum and caspase-3 in the placenta was performed by ELISA according to the manufacturer's instructions using the diagnostic kits for sFasl and caspase-3 produced by eBioscience (USA). Ultrasound study was conducted by the MINDRAY M7 scanner (China)

The resulting data was statistically processed using the STATISTICA software. Quantitative indices were given as median (Me), interquartile scale (LQ - lower quartile, UQ - upper quartile) and sample size (min - minimum value, max - maximum value) or in the form X ± m, where X is the average value, and m is the standard error of the mean. For indices that characterize qualitative characteristics, the absolute number and relative value in percentage (%) was indicated. The percentage error Δ was calculated by the formula: $\Delta=\sqrt{n1/n(1-n1/n)/n}\cdot 100\%$, where n is the sample size, n1 is the number of patients with the presence of the sign being investigated.

To check the coincidence of the distribution of quantitative indices with norm in groups, we used the Kolmogorov-Smirnov test. Since the law of distribution of the numerical indices under study was different from the norm, the statistical significance was checked with the Mann-Whitney U-test (in case of paired independent entities), the Kruskal-Wallis test (in the case of multiple independent entities). In case of dependent entities, the Wilcoxon W-criterion was used. Qualitative characteristics were compared with the use of Pearson criterion and Fischer's exact test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Pregnant women were found to have a significant increase in apoptotic indices in all studied groups compared with the corresponding values in the control group (Kruskal-Wallis test, $p < 0.001$). The apoptotic ligand sFasL in Group IV patients was practically not detected: median – 0.00; the interquartile range was 0.00-0.40 ng / ml, while the median value of this indicator in Groups II and III was 1.00 ng / ml and 1.05 ng / ml, respectively (with an interquartile range of 0.83 ÷ 1.14 ng / ml in Group II and 0.81 ÷ 1.17 ng / ml in Group III) (Fig. 1). In Group I, the median sFasL value was 0.64 ng / ml (with an interquartile range of 0.44 ÷ 1.04 ng / ml). The maximum value of sFasL was recorded in Group III, where it was 3.00 ng / ml, while the maximum value of this indicator in Group IV was 1.00 ng / ml [9].

Concentration of MMP-2 in serum of women in the control group is presented in Table I.

Subsequently, the dependence of the content of the MMP-2 enzyme in groups was determined using a discriminant analysis of the grouping of variables, which demonstrated that this parameter depended on the group at a statistically significant level (Kruskal-Wallis test, $p < 0.01$).

Indices of MMP-2 in blood serum of patients with PIns depending on the group are presented in Fig. 2.

The given data show that in all groups of pregnant women with PIns, the level of MMP-2 in serum was elevated with respect to the control values approximately 3-4 times (Kruskal-Wallis test, $p < 0.05$).

Thus, in Group I the median MMP-2 was 31.18 ng / ml, and the interquartile range was 27.87 to 35.50 ng / ml. In Group II the median was 30.12 ng / ml, and the interquartile range was 29.87÷33.18 ng / ml. Group 3 was found to have the highest rates of MMP-2: median – 36.05 ng / ml, interquartile range – 29.84 ÷ 43.29 ng / ml.

The level of the effector unit of apoptosis – caspase-3 in the placenta was studied to understand whether there was a high activation of the cell death program in placental cells in pregnant women with a PIn. Results of biopsies of the placenta were obtained after labor, and were examined for the contents of this apoptotic marker. In all studied groups, there was a significant increase in the number of caspase-3 in the placenta tissue compared with control (Kruskal-Wallis test, $p < 0.01$) (Fig. 3).

Assessment of the findings showed the following tendency: the most marked increase in caspase-3 levels was found in Groups II and III, the median values of which were 5.42 ng / ml and 4.60 ng / ml, respectively, and the interquartile range was 5.20 ÷ 5.55 ng / ml in Group II and 3.51 ÷ 6.40 ng / ml in Group III. Moreover, it should be noted that the interquartile rangee in Group III covered the range of corresponding values of this indicator in Groups II and I. Such a feature may be explained by the combined effect of bacterial and viral infectious agents present at the same time in pregnant of Group III, which affects the activation of programmed death of placental cells. The level of caspase-3 in Group I was relatively high in relation to control, but to a lesser extent as compared with the Groups

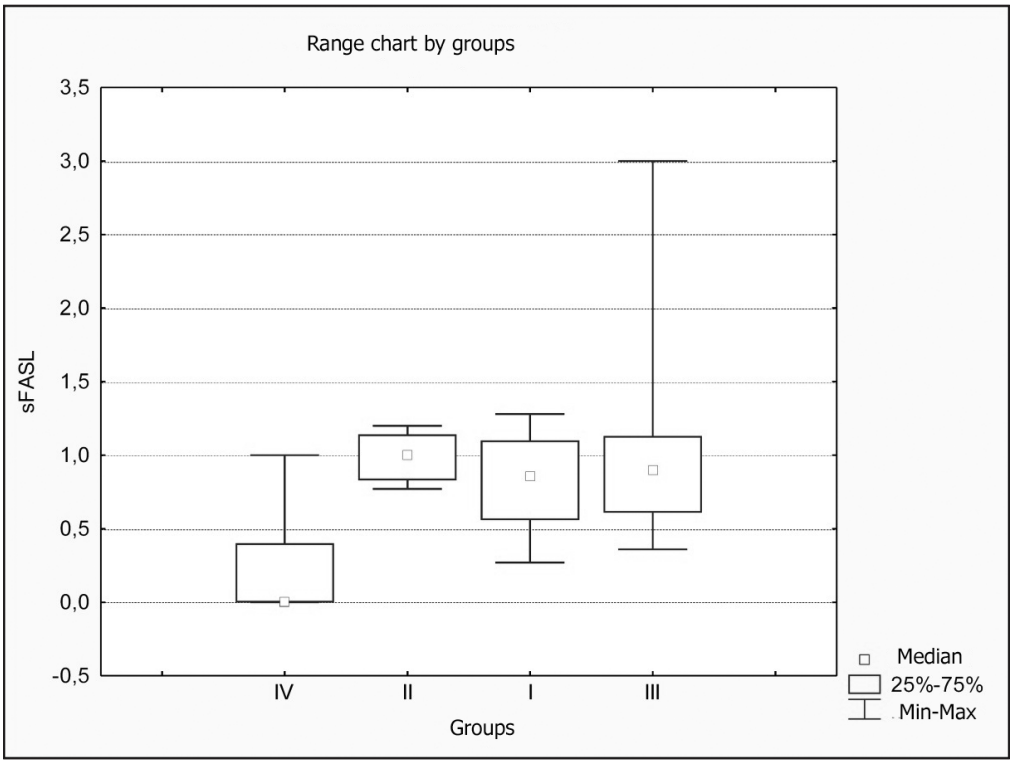


Fig. 1. The level of sFasL (ng / ml) in the peripheral blood of pregnant women in the examined groups.

Table I. Concentration of MMP-2 in serum of healthy pregnant women (ng/ml)

Clinical groups	Statistical indices						
	Average	Median	Min	Max	LQ	UQ	Standard error
Control group (IV)	11.88	12.79	0.000	18.56	10.72	15.67	5.16

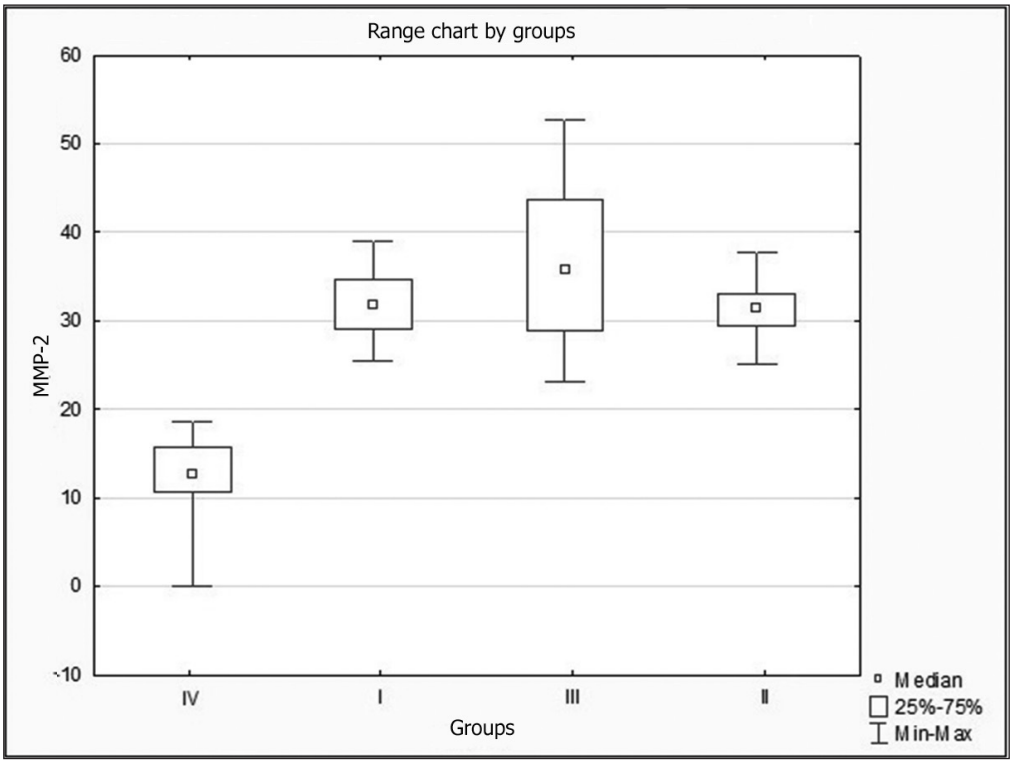


Fig. 2. Level of MMP-2 (ng / ml) in peripheral blood of pregnant in clinical groups under study.

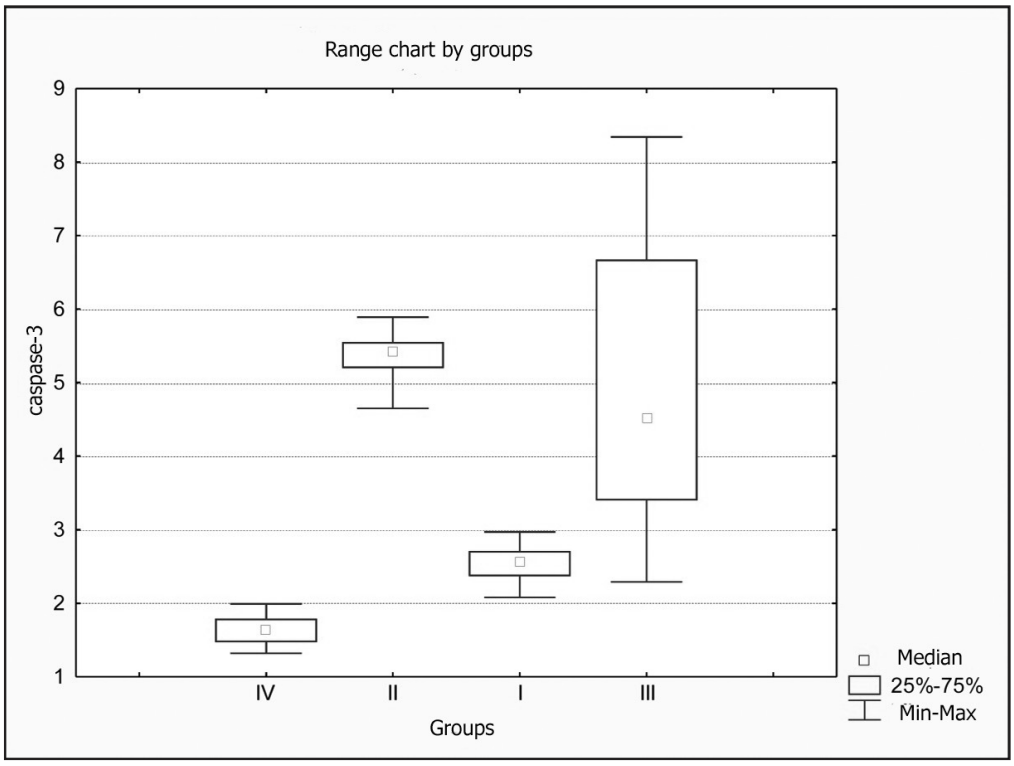


Fig. 3. The level of caspase-3 (ng / ml) in the placenta of pregnant women in the groups under investigation.

II and III. In this case, the median was 2.50 ng / ml, the interquartile range was 2.22 ÷ 2.78 ng / ml. On the one hand, we assume that in the case of viral infection not only caspase-dependent but also other signaling pathways of apoptosis are activated in the placenta, and on the other hand we cannot exclude simultaneous development of complex regulatory processes that block the apoptosis in the cells infected with the viruses.

Assessment of ultrasound findings on the condition of the placenta in the examined patients showed the following results: placental hypoplasia occurred in (46.6 ± 6.4)% of cases in Group I and in (30 ± 6.4)% in Group III. At the same time, in the Groups II and III, placental hyperplasia was detected in (20 ± 5.2)% and (26 ± 5.7)% of cases, respectively. These data suggest that perinatal viral infections cause a shift of cellular processes in the placenta toward apoptosis, whereas perinatal bacterial infections activate not only apoptosis, but also processes of cell proliferation in the placenta, manifested in the development of hyperplasia of the latter in Groups II and III.

CONCLUSIONS

The findings showed that secondary to the impact of perinatal infection among a number of indices in the peripheral blood of pregnant women, a significant role is played by an increase in levels of MMP-2 and sFasL. These indices reflect and determine the induction of apoptotic response from the placenta in this category of pregnant women. The revealed increase in caspase-3 level means that pregnant women with perinatal infections undergo caspase cascade of apoptosis in placental cells. Thus, the obtained results indicate that pregnant women with perinatal infections develop an increase in the level of MMP-2 and activation of the Fas-dependent signaling pathway of programmed cell death, which leads to the launch of a caspase cascade of apoptosis in placental cells. An increase in the loss of placental cells due to apoptosis may be one of the key factors triggering the development of placental dysfunction [10,11] and, as a consequence, unfavorable conditions for fetal development.

REFERENCES

1. Byikov AV. Kompleksnyiy podhod k diagnostike platsentarnoy nedostatochnosti s uchetom markerov apoptoza i kletochnoy proliferatsii [avtoreferat]. Samara: SGMU; 2008. 25s.
2. Lipatov IS, Tezikov YuV. Prognozirovanie i diagnostika platsentarnoy nedostatochnosti na osnove markerov endotelialnoy disfunktsii, detsidualizatsii, apoptoza i kletochnoy proliferatsii. Saratovskiy nauchno-meditsinskiy zhurnal. 2011; 1: 52-59.
3. Kurnosenko IV, Dolgushina VF, Sipok AP. Kliniko-immunologicheskie osobennosti hronicheskoy platsentarnoy nedostatochnosti u zhenschin s genitalnoy infektsiey. Vestnik Uralskoy meditsinskoy akademicheskoy nauk. 2011; 2: 165-66.
4. Soboleva GM, Suhih GT. Semeystvo matriksnyih metalloproteinaz: obschaya harakteristika i fiziologicheskaya rol. Akusherstvo i ginekologiya. 2007; 1: 5-8.
5. Yarmolinskaya MI, Molotkov AS, Denisova VM. Matriksnyie metalloproteinazyi i ingibitoryi: klassifikatsiya, mehanizm deystviya. Zhurnal akusherskih i zhenskih bolezney. 2012; 1: 113-25.
6. Poteryaeva ON. Matriksnyie metalloproteinazyi: stroenie, regulyatsiya, rol v razvitii patologicheskih sostoyaniy. Meditsina i obrazovanie v Sibiri. 2010; 5: 7-17.

7. Musiał K, Zwolińska D. Matrix metalloproteinases and soluble Fas/FasL system as novel regulators of apoptosis in children and young adults on chronic dialysis. Apoptosis. 2011; 16(7): 653-59.
8. Vigivska LA. Perinatalni infektsii u vagitnih grupi visokogo riziku diagnostika, profilaktika ta likuvannya [avtoreferat]. Harkiv: Harkivskiy nats. med. universitet; 2018. 38 s.
9. Scherbina NA, Vygovskaya LA, Kapustnik NV. Harakter ekspressii i rol markerov apoptoza v razvitii platsentarnoy disfunktsii u beremennyih s urogenitalnyimi infektsiyami. Georgian medical news. 2016; 2(251): 12-16.
10. Kudryashova AV, Sotnikova NYu, Posiseeva LV i dr. Rol immunnoy sistemyi v formirovanii zaderzhki vnutriutrobnogo razvitiya ploda. Ivanovo: OAO Izdatelstvo «Ivanovo»; 2009. 240 s.
11. Tyukavkina SYu. Rol apoptoza v formirovanii immunopatologicheskih protsessov, sposobstvuyuschih razvitiyu infektsionnyih zabolevaniy. Immunologiya. 2013; 1: 52-57.

Conflict of interest:
The Author declare no conflict of interest.

CORRESPONDING AUTHOR
Lyudmila A. Vygivska
Kharkiv National Medical University
4 Nauki av. 61000, Kharkiv, Ukraine
tel: +380509675487
e-mail: liudmilavygovskaya@gmail.com

Received: 15.03.2019
Accepted: 18.06.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

ASSOCIATION OF PLATELET-DERIVED MICROVESICLES WITH HIGH ON-TREATMENT PLATELET REACTIVITY IN CONVALESCENT ISCHEMIC STROKE PATIENTS TREATED WITH ACETYLSALICYLIC ACID

ZWIĄZEK MIKROCZĄSTEK POCHODZENIA PŁYTKOWEGO Z RESZTKOWĄ REAKTYWNOŚCIĄ PŁYTEK KRWI U PACJENTÓW PO UDARZE NIEDOKRWIENNYM MÓZGU LECZONYCH KWASEM ACETYLOSALICYLOWYM

Justyna Rosińska[1], Joanna Maciejewska[2], Robert Narożny[1], Wojciech Kozubski[1], Maria Łukasik[2]
[1]DEPARTMENT OF NEUROLOGY, POZNAN UNIVERSITY OF MEDICAL SCIENCES, POZNAN, POLAND
[2]LABORATORY OF FLOW CYTOMETRY AND VASCULAR BIOLOGY, DEPARTMENT OF NEUROLOGY, POZNAN UNIVERSITY OF MEDICAL SCIENCES, POZNAN, POLAND

ABSTRACT

Introduction: Elevated concentrations of platelet-derived microvesicles are found in cerebrovascular diseases. The impact of acetylsalicylic acid on these microvesicles remains inconsistent, despite its well-established effect on platelet aggregation. High residual platelet aggregation is defined as high on-treatment platelet reactivity, while "treatment failure" is the occurrence of vascular events despite antiplatelet treatment.

The aim of this study was to determine whether the antiaggregatory effect of acetylsalicylic acid correlates with platelet-derived microvesicles in convalescent ischaemic stroke patients and cardiovascular risk factor controls as well as to evaluate the association between high on-treatment platelet reactivity and recurrent vascular events with the studied platelet-derived microvesicle parameters.

Materials and methods: The study groups consisted of 76 convalescent stroke patients and 74 controls. Total platelet-derived microvesicles, annexino-positive microvesicles number, and platelet-derived microvesicles with surface expression of proinflammatory (CD40L, CD62P, CD31) and procoagulant (PS, GPIIb/IIIa) markers were characterized and quantified using flow cytometry. Cyclooxygenase-1-specific platelet responsiveness, with whole blood impedance platelet aggregation under arachidonic acid stimulation and the serum concentration of thromboxane B2, were evaluated.

Results: Neither acetylsalicylic acid intake nor modification of its daily dose caused statistically significant differences in the studied microvesicle parameters. Additionally, no statistically significant differences in the studied microvesicle parameters were revealed between high on-treatment platelet reactivity and non-high on-treatment platelet reactivity subjects in either study subgroup. However, elevated concentrations of PAC-1$^+$/CD61$^+$, CD62P$^+$/CD61$^+$ and CD31$^+$/CD61$^+$ microvesicles were found in stroke patients with treatment failure, defined in this study as a recurrent vascular events in a one-year follow-up period.

Conclusions: This study revealed no relationship between circulating microvesicle number and platelet aggregation. The procoagulant and proinflammatory phenotype of circulating platelet-derived microvesicles might contribute to acetylsalicylic acid treatment failure.

KEY WORDS: platelet-derived microvesicles, ischaemic stroke, acetylsalicylic acid, treatment failure, high on-treatment platelet reactivity, recurrent vascular event

STRESZCZENIE

Wstęp: Zwiększone stężenia mikrocząstek pochodzenia płytkowego występują w chorobach naczyń mózgowych. Wpływ kwasu acetylosalicylowego na te mikrocząstki wciąż pozostaje niejasny, pomimo jego udowodnionego wpływu na agregację płytek. Wysoka resztkowa agregacja płytek jest definiowana jako wysoka reaktywność płytek krwi podczas leczenia natomiast „niepowodzenie leczenia" to występowanie zdarzeń naczyniowych pomimo leczenia przeciwpłytkowego.

Cel pracy: Celem badania było ustalenie, czy antyagregacyjny efekt kwasu acetylosalicylowego koreluje z mikrocząstkami pochodzenia płytkowego w grupie pacjentów po przebytym udarze niedokrwiennym mózgu oraz w grupie kontrolnej obciążonej czynnikami ryzyka sercowo-naczyniowego, jak również ocena związku między wysoką reaktywnością płytek krwi podczas leczenia oraz nawracającymi zdarzeniami naczyniowymi z badanymi parametrami mikrocząstek pochodzenia płytkowego.

Materiał i metody: Grupy badane stanowiło 76 pacjentów po przebytym udarze niedokrwiennym mózgu oraz 74 osoby kontrolnych obciążonych czynnikami ryzyka sercowo-naczyniowego. Za pomocą cytometrii przepływowej oznaczono ilościowo mikrocząstki pochodzenia płytkowego oraz ekspresję powierzchniową markerów prozapalnych (CD40L, CD62P, CD31) i prokoagulacyjnych (PS, GPIIb / IIIa). Oceniono również agregacją płytek krwi przy stymulacji kwasem arachidonowym za pomocą agregometru impedancyjnego oraz stężenie tromboksanu B2 w surowicy.

Wyniki: Zarówno przyjmowanie kwasu acetylosalicylowego, jak również modyfikacja jego dziennej dawki nie powodowały statystycznie istotnych różnic w badanych parametrach mikrocząstek. Ponadto nie wykazano różnic w badanych parametrach mikrocząstek między pacjentami z i bez wysokiej reaktywności płytek krwi podczas leczenia w żadnej z badanych podgrup. Natomiast zwiększone stężenia mikrocząstek PAC-1 + / CD61 +, CD62P + / CD61 + i CD31 + / CD61 + stwierdzono u pacjentów po przebytym udarze niedokrwiennym, u których wystąpił kolejny epizod naczyniowy w rocznym okresie obserwacji.

Wnioski: Badanie nie wykazało związku między liczbą krążących mikrocząstek a agregacją płytek krwi. Fenotyp prokoagulacyjny i prozapalny krążących mikrocząstek pochodzenia płytkowego może przyczyniać się do niepowodzenia leczenia za pomocą kwasu acetylosalicylowego.

SŁOWA KLUCZOWE: mikrocząstki pochodzenia płytkowego, udar niedokrwienny, kwas acetylosalicylowy, niepowodzenie leczenia, wysoka reaktywność płytek w trakcie leczenia, nawracające zdarzenia naczyniowe

Wiad Lek 2019, 72, 8, 1426-1436

INTRODUCTION

Platelet-derived microvesicles (pMVs) are small (100 to 1000 nm in diameter) membrane-derived vesicles released from activated platelets. They expose receptors that are also found on the surfaces of platelets, such as GPIIb/IIIa (CD41/61), GPIX (CD42a), GP Ibá (CD42b), as well as molecules promoting coagulation and fibrin formation (phosphatidylserine, PS), inflammation [P-selectin (CD62P) and CD40L (CD154)] or adhesion to endothelial cells [platelet/endothelial cell adhesion molecule, PECAM-1 (CD31)] [1]. The microvesicles (MVs) participate in fact, in both physiological and pathological phenomena, including procoagulant, anticoagulant, proinflammatory, and proatherosclerotic processes. The pathophysiological effects of pMV lead to the development of atherosclerosis, that predisposes patients to cardiovascular disease. Elevated pMV concentrations were observed in cerebrovascular diseases such as multi-infarct dementia, transient ischaemic attack (TIA), and both the acute and chronic phases of ischaemic stroke (IS) [2-6].

Acetylsalicylic acid (ASA), an irreversible inhibitor of the cyclooxygenase isoenzymes COX-1 and COX-2, affects the production of thromboxane A2 (TXA2) from arachidonic acid (AA), thereby limiting platelet activation and aggregation, and is widely used in the prevention and treatment of cardiovascular diseases. Despite its well-established effect on platelet aggregation, the effect of ASA on pMV release, especially on their prothrombotic or proinflammatory properties, is still poorly understood and inconsistent. Even with ASA secondary prevention, approximately 10-12% of patients suffer another stroke within one year after IS, and the risk is 5-8% in each subsequent year, with the cumulative risk of recurrent stroke in a period of 5 years reaching 30-40%. In addition, 15% of stroke patients also experience myocardial infarction [7-9]. This phenomenon, termed "treatment failure", can occur with any antithrombotic treatment. Additionally, vascular disease prevention is accompanied by high on-treatment platelet reactivity (HTPR), defined as high residual platelet aggregation in vitro despite antiplatelet treatment. A meta-analysis of patients with cerebrovascular events detected HTPR in 23% (95% CI: 20-28%) of patients treated with ASA [10]. This prevalence is highly diverse, ranging from 3 to as high as 85% depending on the method, the agonist used, the studied population or the evaluated dose of ASA as well as the operational definition of HTPR [8, 11]. Platelet function tests used to define HTPR, such as light transmission aggregometry (LTA) or multiple electrode platelet aggregometry (MEA), are useful for confirming the pharmacodynamic effect of drugs; however, they are not appropriate for indicating which patients will experience vascular event (VE) [12]. Therefore, HTPR should not be interpreted as a lack of therapeutic effect. The serum concentration of thromboxane B2 (TXB2), a stable metabolite of TXA2, seems to be the most specific test for measuring the pharmacological effect of ASA and is associated with an increased risk of major adverse cardiovascular events [13].

THE AIM

The aim of this study was to determine whether the number and phenotypes of pMVs correlate with HTPR under ASA treatment as well as with treatment failure, defined as the occurrence of a VE within the one-year period of observation despite appropriate prevention, in convalescent IS patients and cardiovascular risk factor (RF) controls.

MATERIALS AND METHODS

Recruitment was conducted from July 2015 to September 2017. Based on the collected medical data at the Department of Neurology, we reviewed the available medical records of 261 subjects who had suffered from first-ever IS in the period from 3 to 12 months before the medical data collection. Then, using the inclusion and exclusion criteria, we selected 129 subjects potentially appropriate for participation in our study. Of these patients, 92 agreed to be involved in our study, 12 were excluded due to recurrent VEs before study enrolment, 15 did not agree to participate in the study and 10 could not participate because of disability. Finally, based on the patients' medical history and the results of the examinations, 76 patients were included.

The following inclusion criteria were used: age over 45 years; first-ever IS in the period from 3 to 12 months before the medical data collection as a result of large-artery atherosclerosis (LAA) or small-artery occlusion (SAO) based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification criteria and confirmed on computed tomography (CT) or magnetic resonance (MR) examination, along with at least two concomitant vascular

disease RFs, including hypertension (systolic blood pressure ≥ 140 mmHg and diastolic pressure ≥ 90 mmHg or hypertensive treatment) and/or hypercholesterolemia (blood low-density lipoprotein (*LDL*) *cholesterol* level ≥ 115 mg/dl on a diet or treatment with statins) and/or current smoking and/or diabetes (managed with diet and/or hypoglycaemic treatment) and/or obesity (defined as body mass index (BMI) ≥ 30 kg/m¸). The exclusion criteria included stroke of potential cardioembolic or undetermined aetiology, treated with thrombolysis in stroke subjects, haemorrhagic stroke, VEs in the period between stroke and blood sampling, malignancies, autoimmune diseases, inflammatory disorders, infections, heart diseases (acute coronary syndrome (ACS), atrial fibrillation (AF), valvular heart diseases), liver and kidney failure, haematological disorders, abnormal platelet count, alcohol or drug abuse and treatment with antithrombotic or antiplatelet drugs other than ASA and/or non-steroidal anti-inflammatory drugs (NSAIDs) during the previous 3 months. The concurrent control group consisted of 74 individuals matched by age, sex and vascular disease RFs who were recruited from the Department of Neurology in Poznan, Poland and had never experienced any VE (a TIA/IS/ACS). They met the same inclusion (except IS) and exclusion criteria as stroke patients and were examined simultaneously and according to the same protocol as the stroke subjects. The study protocol was approved by the local ethics committee, and informed written consent was obtained from all study subjects.

CLINICAL ASSESSMENT

IS was identified based on available inpatient history and discharge files and confirmed based on the clinical examination as well as angio-CT or angio-MR results. Data on vascular RFs and medication were collected. Physical and neurological examinations were performed, including height and weight assessments (to calculate BMI) and blood pressure measurements. The clinical evaluation was supplemented with laboratory investigations, including blood counts, biochemical tests and electrocardiography. The disability of the IS subjects was assessed with the modified Rankin scale (mRS). All study subjects were followed up for 1 year using telephone interviews to establish the occurrence of VEs.

ACETYLSALICYLIC ACID TREATMENT

All included stroke subjects reported chronic treatment with ASA as their secondary stroke prevention, taken in a daily dose of 75 mg (n=38) or 150 mg (n=38) before study enrolment. Some subjects with cardiovascular disease RFs in the control group reported receiving ASA at a daily dose of 75 mg (n=43) before study enrolment, while the others (n=31) were ASA naive. To avoid the impact of ASA non-compliance, all study subjects received ASA at a unified daily dose of 150 mg after baseline blood sampling (D0) and continued for 3 consecutive days (D3). The subjects were informed of the importance of drug compliance at the baseline visit, and drug adherence was assessed by history taking. The effect of ASA was confirmed based on the results of serum TXB2 concentrations and MEA. After blood samples were taken at D3, both convalescent stroke subjects and controls returned to the daily ASA dose used prior to study inclusion according to previous recommendations and were followed up for one year using telephone interviews to collect information on ASA compliance, doses or changes in antiplatelet treatment.

BLOOD SAMPLES

Blood samples were collected twice, at baseline (D0) and after a 3-day period of ASA intake in a dose of 150 mg/day (D3), in all studied subjects according to the protocol described previously [14,15]

EVALUATION OF COX-1-SPECIFIC PLATELET RESPONSIVENESS

COX-1-specific platelet responsiveness was evaluated by whole blood impedance platelet aggregation under AA stimulation and by the serum concentration of TXB2.

MULTIPLE ELECTRODE PLATELET AGGREGOMETRY

MEA in whole blood was performed using a Multiplate® analyser (Roche Diagnostics) according to the protocol described previously [15]. The coefficient of variation for MEA measurements was 8.9% for stroke subjects and 9.1% for controls, and it was calculated based on 10 measurements of different samples from one donor. The cut-off value for MEA data defined for HTPR during ASA treatment was defined as an aggregation value of more than 75 AU, established as the upper quintile by measuring residual platelet aggregation by the in vitro action of AA in the impedance aggregometry study in age- and sex-matched controls [16].

EVALUATION OF THROMBOXANE B2 SERUM CONCENTRATION

Serum concentrations of TXB2 were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences GmbH, Germany) according to the manufacturer's instructions.

PLATELET-DERIVED MICROVESICLE PREPARATION AND LABELLING. FLOW CYTOMETRIC ANALYSIS.

The previously frozen PFP samples were thawed at room temperature (RT), and the MV fraction was isolated from the PFP by two-step high speed centrifugation for 30 min at 18890 x g and washed twice with 0.2 μm filtered citrate/phosphate-buffered saline (PBS) solution

after each centrifugation. Then, 7.5 µl of MV suspension was stained according to the three-colour protocol and incubated (30 min, RT, in the dark) with 3.75 µl of the following antibodies (Ab): phycoerythrin (PE)-conjugated anti-CD61 (platelet-gating Ab) (Becton Dickinson), allophycocyanin (APC)-conjugated Annexin V (Ab against superficial PS) (BioLegend), and fluorescein isothiocyanate (FITC)-conjugated PAC-1 (Ab against the active forms of GPIIb/IIIa) (BioLegend), FITC-conjugated CD62P (Ab against P-selectin, BioLegend) or CD154/FITC (Ab against CD40L, BioLegend) or CD31/FITC (Ab against PECAM-1, BioLegend) and 15 µl of 25 mM $CaCl_2$. At the end of incubation, samples were diluted with 450 µl of 0.22 µm filtered 0.9% NaCl before being analysed on a flow cytometer (FC). Analyses of labelled samples were performed on an Apogee A-50 Micro FC (Apogee Flow Systems, UK) equipped with 488-nm (blue) and 638-nm (red) lasers and dedicated to the detection of MVs according to the protocol described previously [14]. The numbers of positive events per 1 µL of studied sample for $CD61^+$, AnV^+, $CD62P^+/CD61^+$, $PAC\text{-}1^+/CD61^+$, $CD40L^+/CD61^+$, $CD31^+/CD61^+$ MVs as well as the percentage of $CD61^+$ in the AnV^+ population and the percentage of AnV^+ in the $CD61^+$ population were evaluated.

STATISTICAL ANALYSIS

All data were statistically analysed with PQStat software v.1.6.6. The normality of the distribution of each tested parameter was verified using the Shapiro-Wilk test. Data that followed the normal distribution were expressed as the means ± SD, while data that did not fit the normal distribution were expressed as median and interquartile range values. Comparisons of data within groups between D0 and D3 were performed with the nonparametric paired Wilcoxon signed-rank test or parametric Student's *t*-test, and the significance of intergroup differences was evaluated using the nonparametric Mann-Whitney U test or the parametric Student's *t*-test. Student's t-test, ANOVA and a post hoc Tukey test or nonparametric Kruskal-Wallis ANOVA and a post hoc Dunn-Bonferroni test were used for multiple comparisons. Categorical data were compared by the chi-squared test. Possible relationships between studied parameters were evaluated using the Spearman rank correlation test. Univariate logistic regression analysis was performed to determine the predictive factors of VE occurrence and odds ratio (OR) and 95% confidence interval (CI) were reported. The differences were assumed to be significant at $p<0{,}05$ and $p<0.01$ for multiple comparisons. For correlations, a p-value $<0{,}05$ was considered statistically significant.

RESULTS

BASELINE GROUP CHARACTERISTICS

The basic demographic and clinical characteristics are given in Table 1. Seventy-six patients were assessed in the convalescent phase [median 9, IQR (6 -11) months] of IS, and the results were compared with those of the control group with cardiovascular disease RFs (n=74). There were no significant differences between stroke patients and controls in any of the main clinical parameters except for glucose concentration, baseline ASA intake and TXB2 concentration at D3. There were no significant differences in the concentrations of total pMV (defined as $CD61^+$ MVs), total AnV^+ MVs or any single pMV phenotype between stroke subjects and controls after 3 days of ASA intervention, except for a lower concentration of $CD62P^+/CD61^+$ MV in stroke subjects than in controls.

PMV FORMATION VS PLATELET AGGREGATION UNDER AA STIMULATION AS A MARKER OF HTPR DURING TREATMENT WITH ASA

When we compared HTPR stroke subjects (n=17; 22.4%) with non-HTPR stroke subjects (n=59; 77.6%) and HTPR control subjects (n=14; 18.9%) with non-HTPR controls (n=60; 81.1%), no statistically significant difference was found in the concentration of any of the studied MV parameters (Table 2). Similarly, when all study subjects were divided into tertiles with low, medium and high values of platelet aggregation after unification of their daily ASA dose (D3), there were also no statistically significant differences in the studied MV parameters among these three subgroups in convalescent stroke patients or control subjects. There were also no significant correlations between any of the studied pMV parameters and maximum aggregation as measured by MEA in stroke subjects or controls.

PMV FORMATION VS RECURRENT VASCULAR EVENTS AS A MARKER OF "TREATMENT FAILURE" WITH ASA

The one-year follow-up period was completed in 73 (96%) stroke patients and 68 (91.9%) controls. Recurrent VEs, defined as recurrent IS (n=8), TIA (n=5) or ACS (n=7), were experienced by a total of 27.4% of stroke subjects (n = 20) and in a total of 17.6% (n =12) of control subjects [IS (n=3), TIA (n=4), ACS (n=5)]. Although no statistically significant differences in the total number of pMVs, AnV^+ MVs or $AnV^+/CD61^+$ MVs were found between subjects with and without recurrent VEs in either of the studied groups, stroke patients and controls (Table 3), significantly elevated concentrations of pMV with surface expression of selectin P, active-form GPIIb/IIIa and PECAM-1 were observed only in IS subjects who experienced recurrent VEs (Figure 1). In univariate analyses, concentration of PAC-1+/CD61+ MVs at D0 (OR 1.13, 95% CI 1.01-1.25, p = 0.03), as well as at D3 (OR 1.11, 95% CI 1.01–1.24, p = 0.03) and CD31+/CD61+ at D0 (OR 1.07, 95% CI 1.01 -1.13, p = 0.03) as well as at D3 (OR 1.05, 95% CI 1-1.1, p = 0.04) in convalescent stroke subjects as the predictive factors for recurrent VE were found.

There were no statistically significant differences in platelet aggregation under AA stimulation between subjects who suffered from VE and those without VE in

Table 1. Characteristics of the studied population.

Parameters	Stroke subjects (n=76)	Controls (n=74)	p
Age, years	66 (62-77)	64 (60-69)	0.07
Male sex, n (%)	45 (59.2)	38 (51.4)	0.33
mRS	1 (1-2)	n/a	
BMI, kg/m^2	28.2 ± 4.8	27.4 ± 4.6	0.31
Platelets, T/ěl	240 ± 53	234 ± 52	0.52
PDW, fl	13.3 (12-14.5)	13.5 (12.1-14.8)	0.69
MPV, fl	10.7 (10-11.3)	10.9 (10.3-11.4)	0.46
Total cholesterol, mmol/l	175 (155-210)	193 (154-230)	0.27
LDL, mmol/l	90 (72-127)	110 (74-146)	0.16
HDL, mmol/l	54 (47-65)	53 (45-64)	0.45
Triglycerides, mmol/l	103 (87-134)	110 (84-160)	0.40
Glucose, mg/dl	109 (98-130)	101 (92-109)	0.003
Hb1AC	6.1 (5.8-6.9)	6 (5.7-6.9)	0.72
Hypertension, n (%)	72 (94.7)	67 (90.5)	0.32
Diabetes, n (%)	28 (36.8)	21 (28.3)	0.27
Dyslipidemia, n (%)	70 (92.1)	67 (90.5)	0.73
Smoking, n (%)	25 (32.9)	28 (37.8)	0.53
Obesity, n (%)	26 (34.2)	24 (32.4)	0.81
Medications, n (%):			
ASA D1	76 (100)	43 (58.1)	p < 0.001
75mg D1	38 (50)	43 (58.1)	p < 0.001
150mg D1	38 (50)	n/a	
ACE-I	52 (68.4)	48 (64.9)	0.42
Sartans	11 (14.5)	18 (24.3)	0.12
Diuretics	31 (40.8)	27 (36.5)	0.59
Ca-channel blockers	31 (40.8)	26 (35.1)	0.08
â-blockers	25 (32.9)	30 (40.5)	0.33
Statins	59 (77.6)	52 (70.3)	0.3
Oral hypoglycemic	24 (31.6)	18 (24.3)	0.17
Insulin treatment	7 (9.2)	7 (9.5)	0.96
MV parameters at D3, n/µl			
CD61+	1313 (831-1593)	1406 (911-1937)	0.27
AnV+	326 (227-521)	354 (254-470)	0.74
CD61+/AnV+	167 (110-292)	205 (119-268)	0.64
AnV+ %	15.5 ± 3.9	14.6 ±4	0.27
CD61+ %	49.8 ±9.4	52.5 ±10.4	0.19
CD62P+/CD61+	8 (5-12)	14 (6-21)	0.03
PAC-1+/CD61+	8 (5-14)	8 (5-17)	0.7
CD154+/CD61+	5 (4-11)	7 (5-13)	0.12
CD31+/CD61+	18 (9-30)	22 (14-32)	0.33
Parameters of platelet responsiveness at D3			
AUC (AU*min)	962 (795-1210)	1054 (836-1185)	0.55
Aggregation (AU)	55.8 (46.2-69.6)	63.3 (47.6-72.9)	0.39
TXB2, ng/ml	1.16 (0.5-2.24)	1.87 (0.89-4.0)	0.03

mRS, modified Rankin scale;
BMI, body mass index;
PDW, platelet distribution width;
MPV, mean platelet volume;
LDL, low-density lipoprotein;
HDL, high-density lipoprotein;
ASA, acetylsalicylic acid;
ACE-I, angiotensin-converting enzyme inhibitor;
CD61+, MVs of platelet-origin;
AnV+, the number of MVs with surface expression of phosphatidylserine;
CD61+%, CD61+ MV percentage in the population of all AnV+ MVs; AnV+%, AnV+ MV percentage in the population of all CD61+ MVs;
CD62P+/CD61+, the number of pMVs with the surface expression of P-selectin;
PAC-1+/CD61+, the number of pMVs with the surface expression of GP IIb/IIIa active;
CD154+/CD61+, the number of pMVs with the surface expression of CD40L;
CD31+/CD61+, the number of pMVs with the surface expression of PECAM-1;
AUC, area under the curve; AU, aggregation units;
Aggregation, maximum platelet aggregation under AA stimulation using whole blood impedance aggregometry; TXB2, serum level of thromboxane B2;

Table 2. High on treatment platelet reactivity and MV parameters in both studied groups after ASA at daily dose of 150mg

PARAMETERS	STROKE SUBJECTS		p value	CONTROLS		p value
	HTPR (n=17)	non-HTPR (n=59)	Stroke HTPR vs non-HTPR	HTPR (n=14)	non-HTPR (n=60)	controls HTPR vs non-HTPR
Max Agg [AU]	122 (86-138)	53 (40.8-61.9)	< 0.001	85.1 (81.4-151.1)	59.6 (40.9-67.6)	< 0.001
AUC	2068 (1426-2303)	886 (693-1032)	< 0.001	1404 (1334-2256)	996 (665-1129)	<0.001
CD61+ MV, /µl	980 (872-1556)	1352 (787-1604)	0.63	1285 (1081-1493)	1543 (1013-2052)	0.53
AnV+ MV, n/µl	380 (304-464)	310 (205-531)	0.43	314 (268-427)	386 (258-491)	0.54
CD61+/AnV+ MV, n/µl	179 (147-242)	157 (104-292)	0.7	165 (141-243)	213 (122-280)	0.65
AnV+ %	17.4 ± 3.2	15 ± 3.9	0.08	14.4 ± 5	15 ± 3.9	0.73
CD61+ %	48.5 ± 5.3	50.2 ± 10.2	0.6	52.7 ± 8.3	52.2 ± 11.6	0.9
CD62P+/CD61+ MV, n/µl	7 (5-12)	8 (5-13)	0.44	14 (7-16)	14 (9-23)	0.3
PAC-1+/CD61+ MV, n/µl	7 (6-18)	8 (4-13)	0.9	7 (4-9)	13 (7-20)	0.07
CD154/CD61+ MV, n/µl	5 (4-8)	5 (4-11)	0.84	6 (5-9)	8 (6-14)	0.22
CD31+/CD61+ MV, n/µl	21 (16-24)	18 (6-35)	0.58	19 (12-25)	22 (16-35)	0.28

CD61+, MVs of platelet-origin; AnV+, the number of MVs with surface expression of phosphatidylserine;
CD61+%, CD61+ MV percentage in the population of all AnV+ MVs; AnV+%, AnV+ MV percentage in the population of all CD61+ MVs;
CD62P+/CD61+, the number of pMVs with the surface expression of P-selectin;
PAC-1+/CD61+, the number of pMVs with the surface expression of GP IIb/IIIa active;
CD154+/CD61+, the number of pMVs with the surface expression of CD40L;
CD31+/CD61+, the number of pMVs with the surface expression of PECAM-1;
HTPR, high-on treatment platelet reactivity

either subgroup (post-stroke or control), but there were differences in TXB2 concentrations only at D0 (Table 3).

DOSE OF ACETYLSALICYLIC ACID AND PLATELET-DERIVED MICROVESICLE FORMATION

ASA compliance at D3 was verified based on serum TXB2 concentrations. The concentration of TXB2 at D3 compared to D0 indicated that the studied subjects followed the study protocol and took a unified ASA dose of 150 mg/d (Table 4). The concentrations of PAC-1$^+$/CD61$^+$ MVs were significantly higher in stroke subjects who reported taking ASA in a daily dose of 75 mg than in those who reported taking 150 mg per day before study enrolment (D0). Interestingly, this intergroup difference disappeared after unification of the daily ASA dose to 150 mg (D3); however, the modification of the ASA daily dose (from 75 mg to 150 mg) did not cause statistically significant differences in the studied MV parameters at D3 compared to D0 in either stroke or control subjects. There were no statistically significant differences in any of the other studied MV parameters depending on the dose of ASA taken before study enrolment in stroke subjects (Table 4). Moreover, in ASA-naive control subjects (n=31), introducing 150 mg ASA daily for 3 days did not produce a significant difference in any of the studied MVs between D0 and D3, whereas serum TXB2 concentrations and platelet aggregation under AA stimulation were significantly decreased after ASA intake in these subjects, which confirmed the antiaggregatory efficacy of the drug (Table 4).

DISCUSSION

Our study revealed no differences in the total number or phenotypes pMV between convalescent stroke subjects and matched cardiovascular RF controls treated with 150 mg ASA daily. Even lower concentrations of CD62P$^+$ pMV **were observed in stroke subjects.** It is in line with previous observations that in the convalescent phase of IS, the

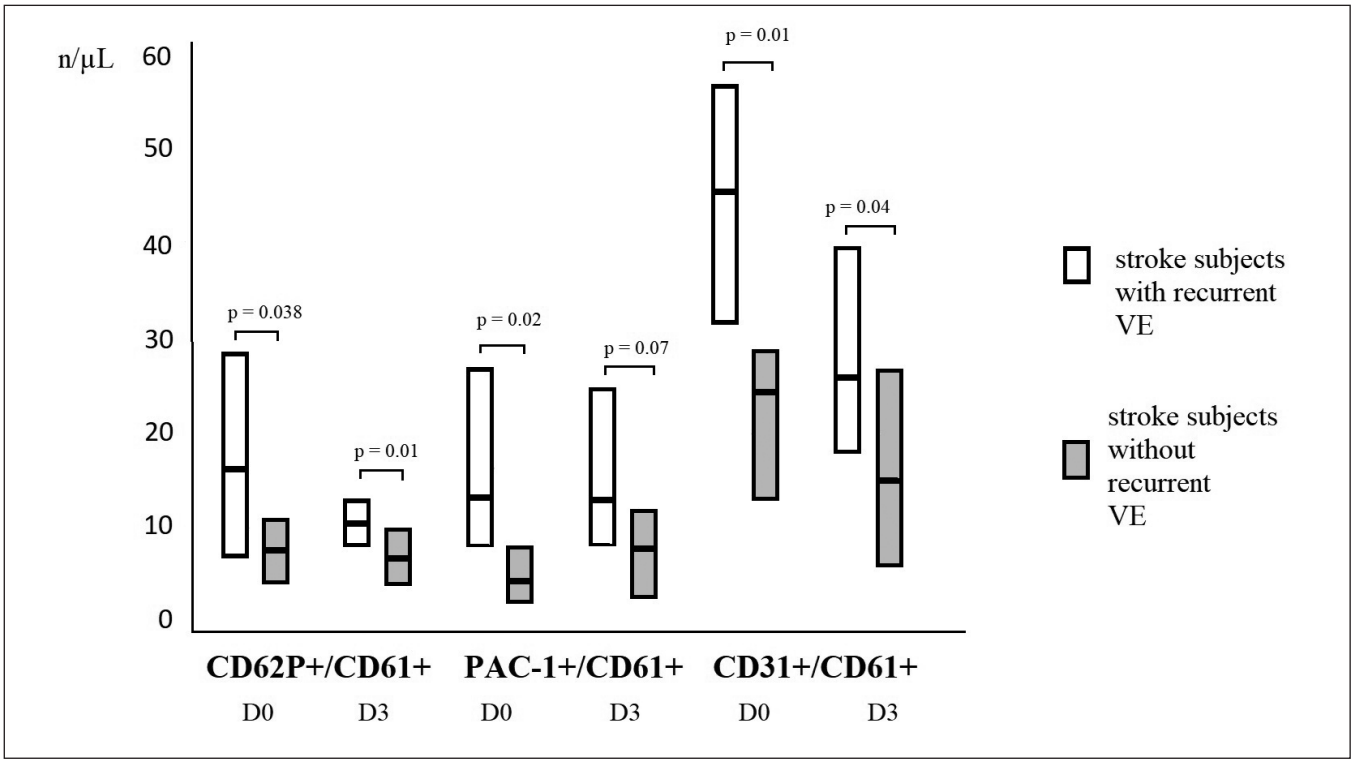


Fig. 1. Differences in concentrations of CD62P+/CD61+, PAC-1+/CD61+ and CD31+/CD61+MVs in stroke patients with and without recurrent vascular events (VEs) at D0 and D3.
The data are presented as medians with the upper and lower quartiles.

increases in the surface expression of CD62P and active-form GPIIb/IIIa receptors after *ex vivo* stimulation with TRAP or ADP are diminished, and a reduced percentage of CD62P-positive platelets is circulating, probably due to the exhaustion of platelets [17].

In the context of the effect of ASA on MVs, the interactions via the AA pathway appear to be the most important [18]. Thus, we decided to evaluate COX-1-specific platelet responsiveness as measured by MEA after AA stimulation as well as serum concentrations of TXB2. It was found that pMVs transfer AA, a strong platelet agonist, between endothelial cells, monocytes and platelets, affecting their functions, inducing the expression of COX-2 in endothelial cells (ECs) *de novo* and generating PGI2 in an AA-dependent manner, resulting in platelet activation [19, 20]. pMVs also metabolize endothelially derived AA to TXA2 and then to its stable form, TXB2 [21]. pMV-derived AA stimulates transcriptional activity of COX-2 as well as c-Jun and Elk-1 proteins, induces monocyte chemotaxis, upregulates intracellular cell adhesion molecule-1 (ICAM-1) and enhances adhesion of monocytes to ECs in vitro [20, 22]. Despite such a prominent association between pMV and AA, we did not find any correlations between the antiaggregatory effect of ASA as measured by MEA (with AA as an agonist) and the total number of pMVs, the number of AnV^+ MVs, or any of the pMV phenotypes in either group (convalescent stroke patients or controls). This is consistent with a previous study in which no correlation between the percentage of pMV and the plasma level of the TXB2 metabolite 2,3-dinor-TXB2 was confirmed [23]. Moreover, in cardiovascular RF controls, despite the effective inhibition of COX-1-dependent platelet aggregation with ASA, pMV release seemed not to be reduced by this antiplatelet agent, since we found that ASA intake (150 mg per day) did not reduce the formation of pMVs or AnV^+ MVs or the phenotypes of pMVs in ASA-naive cardiovascular RF controls. Moreover, the modification of the ASA daily dose (from 75 mg to 150 mg) in both stroke subjects and controls also did not significantly affect the pMV parameters. These findings confirm most previous studies showing the limited impact of ASA intake on pMV concentration in healthy subjects [24] as well as in acute- and chronic-phase IS [2, 3], acute and stable coronary artery disease [25, 26], diabetes [23, 27], non-valvular AF [28] and peripheral arterial occlusive disease [29]. The elevated concentrations of $PAC^+/CD61^+$ MVs in stroke subjects who reported taking a lower daily dose of ASA (75 mg) compared with those reporting a higher daily dose (150 mg) before study enrolment and the disappearance of such an intergroup difference after unification of the ASA daily dose to 150 mg suggest that a higher ASA dose affects the surface expression of the active forms of GPIIb/IIIa on pMVs without affecting the overall quantity. Furthermore, subjects with HTPR had similar pMV parameters to those without HTPR during ASA treatment in both studied subgroups, indicating ongoing platelet activation and formation of pMVs despite ASA

Table 3. Parameters of platelet responsiveness and platelet-derived microvesicle data in all study subjects with (VE+) and without (VE-) vascular events in a one-year follow-up period.

Parameters	VE + stroke subjects (n=20)	VE – stroke subjects (n=53)	P VE + stroke vs VE – stroke	VE + controls (n=12)	VE – controls (n=56)	P VE + control vs VE- controls
Max Agg, AU D0	59 (47.4-81.4)	66.8 (51.3-99)	0.6	115.6 (65-139)	134.6 (65.6-159)	0.61
Max Agg, AU D3	56.3 (44.6-74.3)	57 (49.7-69.2)	0.77	67 (49-71.4)	61.7 (42.3-72.3)	0.84
AUC, AU*min D0	1036 (855-1383)	1080 (843-1969)	0.79	2108 (1138-2580)	2352 (1020-3046)	0.78
AUC, AU*min D3	962 (773-1245)	1005 (810-1217)	0.63	1143 (793-1271)	1049 (712-1186)	0.67
TXB2, ng/ml D0	1.3 (0.6-2.6)	4.5 (1.6-7.8)	0.007	5.2 (4.3-7.1)	11.7 (3-41)	0.67
TXB2, ng/ml D3	0.7 (0.6-1.4)	1.1 (0.5-2.4)	0.43	1.8 (0.9-9.4)	1.7 (0.8-3.2)	0.32
CD61+ MV, n/µl D0	966 (799-1807)	972 (695-1476)	0.77	2149 (1734-2539)	1186 (932-2144)	0.1
CD61+ MV, n/µl D3	1373 (1007-1820)	1096 (748-1544)	0.32	1676 (1526-1904)	1318 (805-1880)	0.13
AnV+ MV, n/µl D0	367 (281-648)	344 (277-468)	0.46	554 (404-729)	385 (272-680)	0.35
AnV+ MV, n/µl D3	388 (299-695)	305 (205-498)	0.12	420 (372-457)	324 (226-470)	0.16
CD61+/AnV+ MV, n/µl D0	174 (147-345)	157 (92-233)	0.2	288 (241-446)	194 (131-389)	0.3
CD61+/AnV+ MV, n/µl D3	228 (152-361)	149 (103-251)	0.1	244 (206-252)	173 (111-168)	0.2
CD62P+/CD61+ MV, n/µl D0	16 (7-30)	8 (4-11)	0.038	10 (9-23)	17 (8-27)	0.81
CD62P+/CD61+ MV, n/µl D3	11 (8-13)	7 (4-10)	0.01	14 (7-15)	15 (5-23)	0.8
PAC-1+/CD61+ MV, n/µl D0	13 (8-27)	4 (2-8)	0.02	9 (8-35)	14 (6-23)	0.56
PAC-1+/CD61+ MV, n/µl D3	13 (8-25)	7 (3-12)	0.007	10 (6-19)	8 (5-17)	0.8
CD154/CD61+ MV, n/µl D0	11 (7-26)	4 (4-7)	0.01	22 (10-35)	9 (5-15)	0.23
CD154/CD61+ MV, n/µl D3	6 (5-14)	5 (3-8)	0.3	10 (5-12)	7 (5-14)	0.65
CD31+/CD61+ MV, n/µl D0	46 (32-57)	23 (13-29)	0.01	15 (11-21)	30 (13-45)	0.3
CD31+/CD61+ MV, n/µl D3	24 (18-40)	15 (6-27)	0.038	22 (16-35)	22 (15-31)	0.65

CD61+, MVs of platelet-origin;
AnV+, the number of MVs with surface expression of phosphatidylserine;
CD61+%, CD61+ MV percentage in the population of all AnV+ MVs;
AnV+%, AnV+ MV percentage in the population of all CD61+ MVs;
CD62P+/CD61+, the number of pMVs with the surface expression of P-selectin;
PAC-1+/CD61+, the number of pMVs with the surface expression of GP IIb/IIIa active;
CD154+/CD61+, the number of pMVs with the surface expression of CD40L;
CD31+/CD61+, the number of pMVs with the surface expression of PECAM-1;
AUC, area under the curve;
AU, aggregation units;
Max Agg, maximum platelet aggregation under AA stimulation using whole blood impedance aggregometry;
TXB2, serum concentration of thromboxane B2;
VE, vascular event

intake. To the best of our knowledge, a possible association among circulating pMV, AnV^+ MVs, their phenotypes and HTPR in convalescent stroke patients and cardiovascular RF controls treated with ASA has not been elucidated thus far. Only the association between platelet aggregation measured with MEA and circulating pMVs in patients

Table 4. Microvesicle and platelet responsiveness parameters in stroke subjects and controls depending on dose of chronic ASA treatment at baseline (D0)

Parameter, n/µl	**Stroke subjects with 75mg/d of ASA at D0 (n=38) A**	**Stroke subjects with 150 mg/d of ASA at D0 (n=38) B**	**p A vs B**	**Controls with 75mg/d of ASA at D0 (n=43) C**	**Controls ASA-naďve at D0 (n=31) D**	**p C vs D**	**p A vsC**
CD61+ D0	1140 (963-1822)	840 (608-1437)	0.1	2086 (1184-3033)	1176 (589-1297)	0.006	0.02
CD61 + D3	1247 (941-1520)	1312 (830-1742)	0.9	1584 (1060-2252)	1229 (788-1524)	0.05	0.07
AnV+ D0	344 (277-583)	367 (277-497)	0.6	596 (415-950)	286 (220-373)	0.02	0.04
AnV+ D3	316 (249-425)	375 (227-550)	0.5	396 (296-512)	268 (223-394)	0.06	0.1
CD61+/AnV+ D0	170 (118-344)	163 (85-227)	0.4	331 (221-602)	135 (103-156)	0.001	0.02
CD61+/AnV+ D3	180 (109-292)	157 (118-253)	0.9	211 (137-314)	148 (99-237)	0.06	0.3
AnV+ % D0	15.4 ± 3.7	16.7 ±3.2	0.3	16.3 ± 5.6	15.8 ± 3.8	0.1	0.09
AnV+ % D3	14.7 ± 3.6	16.2 ± 4.1	0.2	14.7 ± 3.6	14.3 ± 4	0.7	0.9
CD61+ % D0	48.3 ± 11.9	41.2 ± 10.4	0.1	56 ± 7.3	48.9 ± 14.4	0.09	0.03
CD61+ % D3	52.4 ± 7	47.4 ± 10.7	0.06	53.1 ± 10.5	51.6 ± 10.5	0.6	0.8
CD62P+/CD61+ D0	9 (7-20)	6 (4-14)	0.08	14 (8-28)	17 (8-23)	0.9	0.6
CD62P+/CD61+ D3	7 (5-10)	8 (5-12)	0.7	16 (9-24)	11 (4-15)	0.1	0.02
PAC-1+/CD61+ D0	16 (12-27)	2 (2-5)	< 0.001	16 (8-26)	7 (4-21)	0.1	0.9
PAC-1+/CD61+ D3	11 (5-12)	7 (5-14)	0.6	13 (6-20)	7 (5-11)	0.06	0.6
CD154/CD61+ D0	8 (4-22)	5 (4-7)	0.2	9 (6-15)	13 (6-26)	0.6	0.8
CD154/CD61+ D3	5 (5-8)	5 (3-11)	0.6	7 (5-13)	7 (5-13)	0.7	0.3
CD31+/CD61+ D0	28 (15-48)	30 (22-46)	0.7	16 (14-40)	27 (5-43)	0.9	0.5
CD31+/CD61+ D3	19 (16-27)	16 (6-36)	0.7	24 (17-36)	17 (11-22)	0.06	0.2
AUC (AU*min) D0	1038 (823-1214)	1110 (957-1769)	0.39	1129 (929-1654)	2990 (2771-3247) **	<0.001	0.48
AUC (AU*min) D3	909 (798-1176)	1003 (804-1354)	0.42	1058 (879-1267)	1053 (782-1146) **	0.58	0.28
Aggregation (AU) D0	60.6 (47.8-74.6)	66.8 (55.8-97.9)	0.42	70.2 (55.7-103.7)	154 (139.4-162.3) **	<0.001	0.4
Aggregation (AU) D3	54.5 (49-67.2)	58 (45-84)	0.48	64.4 (50.1-74.4)	63.3 (45.3-70.3) **	0.63	0.2
TXB2, ng/ml D0	1.89 (0.72-4.59)	4.1 (2.9-12.4)	0.002	4.4 (0.65-10.7)	38.9 (12.7-76.8) **	< 0.001	0.2
TXB2, ng/ml D3	1.05 (0.5-2.04)	1.3 (0.5-2.8)	0.48	1.8 (0.7-4.1)	1.9 (0.96-2.9) **	0.96	0.06

AUC, area under the curve;
AU, aggregation units;
Aggregation, maximum platelet aggregation under AA stimulation using whole blood impedance aggregometry;
TXB2, serum concentration of thromboxane B2;
** $p < 0.001$ for comparison between parameters at D0 and D3

with ACS during antiplatelet therapy with clopidogrel [30, 31] and with other P2Y12 adenosine diphosphate receptor antagonists such as ticagrelor and prasugrel [32] have been investigated before, and it was found that ACS patients with HTPR to clopidogrel had significantly higher numbers of pMV and pMV exposing CD62P than patients without HTPR, but there was no difference in CD154 expression [30]. Reduced pMV generation was also revealed in vitro in ACS patients who were clopidogrel responders compared to non-responders [31]. In contrast to ASA, data on the effect of ADP receptor inhibitors on the reduction in pMV concentration in healthy people [33], subjects with stable coronary artery disease [34] and ACS patients [26, 30-32] are unambiguous in confirming the inhibitory effect of these drugs on MV release.

Another important finding of this study is an association between higher concentrations of pMV with surface expression of P-selectin, active GPIIb/IIIa and PECAM-1 and "treatment failure" evaluated as recurrent VE during a one-year follow-up period in stroke subjects despite the lack of a relationship between pMV number and platelet reactivity under AA stimulation. This means that in post-stroke subjects, even if ASA effectively limits platelet aggregation, it may not affect the procoagulant and proinflammatory phenotype of pMV that appears to play a role in recurrent VE. In addition, because no differences

in platelet aggregation under AA stimulation measured with MEA between subjects who suffered from VE and those without VE were found, platelet aggregation do not appear to be good indicator of the risk of recurrent VE [35]. Whereas the differences in TXB2 concentrations measured at D0 between subjects who experienced VE and those without VE result from different daily doses of ASA before study enrollment. It should also be emphasized that, regarding the impact of ASA on pMVs, the results obtained at D3 are more reliable than those obtained at D0 because the patients were more closely monitored at D3 to avoid ASA non-compliance, while data obtained at D0 should be taken into account more in terms of treatment failure because they more closely reflect the reality and actual status of ASA use by patients.

There are some limitations of our study that must be addressed. Due to the lack of standardized and unified protocols for the determination of MVs, our study differs methodologically from previous studies, rendering it difficult to compare the results. It should also be noted that MEA as used in the present study measures the effect of only one of the platelet agonists, AA; thus, only one pathway of platelet activation was assessed.

CONCLUSIONS

In conclusion, this study revealed no relationship between circulating MV count and platelet aggregation; however, it seems that ASA may affect the functions of pMVs rather than their numbers. The procoagulant phenotype of circulating pMVs might contribute to treatment failure, and the concentration of circulating pMVs correlates with carotid atherosclerotic lesions [5, 14]. For that reason, pMVs might emerge as a factor favouring recurrent VE and poor response to pharmacologic interventions.

REFERENCES

1. Piccin A, Murphy WG, Smith OP. Circulating microparticles: pathophysiology and clinical implications. Blood Reviews 2007;21(3):157–71.
2. Lee YJ, Jy W, Horstman LL et al. Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. Thrombosis Research 1993;72(4):295–304.
3. Shirafuji T, Hamaguchi H, Kanda F. Measurement of platelet-derived microparticle level in the chronic phase of cerebral infarction using an enzyme-linked immunosorbent assay. The Kobe Journal of Medical Sciences 2008;54(1):E55–61.
4. Cherian P, Graeme JH, John WE et al. Endothelial and platelet activation in acute ischemic stroke and its etiological subtypes. Stroke 2003;34(9):2132–7.
5. Lukasik M, Rozalski M, Luzak B et al. Enhanced platelet-derived microparticle formation is associated with carotid atherosclerosis in convalescent stroke patients. Platelets 2013;24(1):63–70.
6. Pawelczyk M, Chmielewski H, Kaczorowska B et al. The influence of statin therapy on platelet activity markers in hyperlipidemic patients after ischemic stroke. Archives of Medical Science 2015;11(1):115–21.
7. Wilterdink JL, Easton JD. Vascular event rates in patients with atherosclerotic cerebrovascular disease. Archives of Neurology 1992;49(8):857–63.
8. Topcuoglu MA, Arsava EM, Ay H. Antiplatelet resistance in stroke. Expert Review of Neurotherapeutics 2011;11(2):251–63.
9. Sacco RL, Wolf PA, Kannel WB et al. Survival and recurrence following stroke. The Framingham Study. Stroke 1982;13(3):290–5.
10. Fiolaki A, Katsanos AH, Kyritsis AP et al. High on treatment platelet reactivity to aspirin and clopidogrel in ischemic stroke: a systematic review and meta-analysis. Journal of the Neurological Sciences 2017;376:112–6.
11. Lim ST, Coughlan CA, Murphy SJ et al. Platelet function testing in transient ischaemic attack and ischaemic stroke: a comprehensive systematic review of the literature. Platelets 2015;26(5):402–12.
12. Dahlen JR, Price MJ, Parise H et al. Evaluating the clinical usefulness of platelet function testing: considerations for the proper application and interpretation of performance measures. Thrombosis and Haemostasis 2013;109(5):808–16.
13. Frelinger AL, Li Y, Linden MD et al. Association of cyclooxygenase-1-dependent and -independent platelet function assays with adverse clinical outcomes in aspirin-treated patients presenting for cardiac catheterization. Circulation 2009;120(25):2586–96.
14. Rosinska J, Ambrosius W, Maciejewska J et al. Association of platelet-derived microvesicles and their phenotypes with carotid atherosclerosis and recurrent vascular events in patients after ischemic stroke. Thrombosis Research 2019;176:18–26.
15. Rosińska J, Maciejewska J, Narożny R et al. Effect of acetylsalicylic acid intake on platelet derived microvesicles in healthy subjects. Platelets 2019; DOI: 10.1080/09537104.2019.1588242;
16. Sibbing D, Braun S, Morath T et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. Journal of the American College of Cardiology 2009;53(10):849–56.
17. Lukasik M, Rozalski M, Luzak B et al. Platelet activation and reactivity in the convalescent phase of ischaemic stroke. Thrombosis and Haemostasis 2010;103(3):644–50.
18. Rosinska J, Lukasik M, Kozubski W. The impact of vascular disease treatment on platelet-derived microvesicles. Cardiovascular Drugs and Therapy 2017;31(5-6):627–44.
19. Barry OP, Pratico D, Lawson JA et al. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. The Journal of Clinical Investigation 1997;99(9):2118–27.
20. Barry OP, Kazanietz MG, Pratico D et al. Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. The Journal of Biological Chemistry 1999;274(11):7545–56.
21. Pfister SL. Role of platelet microparticles in the production of thromboxane by rabbit pulmonary artery. Hypertension 2004;43(2):428–33.
22. Barry OP, Pratico D, Savani RC et al. Modulation of monocyte-endothelial cell interactions by platelet microparticles. The Journal of Clinical Investigation 1998;102(1):136–44.
23. Duarte RC, Goncalves LH, Campos FM et al. Effect of acetylsalicylic acid on platelet activation and oxidative profile in a set of Brazilian patients with type 2 diabetes mellitus. Blood Coagulation & Fibrinolysis 2015;26(2):123–30.
24. Lubsczyk B, Kollars M, Hron G et al. Low dose acetylsalicylic acid and shedding of microparticles in vivo in humans. European Journal of Clinical Investigation 2010;40(6):477–82.
25. Camargo LM, Franca CN, Izar MC et al. Effects of simvastatin/ezetimibe on microparticles, endothelial progenitor cells and platelet aggregation in subjects with coronary heart disease under antiplatelet therapy. Brazilian Journal of Medical and Biological Research 2014;47(5):432–7.

26. Skeppholm M, Mobarrez F, Malmqvist K et al. Platelet-derived microparticles during and after acute coronary syndrome. Thrombosis and Haemostasis 2012;107(6):1122–9.
27. Chiva-Blanch G, Suades R, Padro T et al. Microparticle shedding by erythrocytes, monocytes and vascular smooth muscular cells is reduced by aspirin in diabetic patients. Revista EspanŢola de Cardiologiĕa 2016;69(7):672–80.
28. Choudhury A, Chung I, Blann AD et al. Elevated platelet microparticle levels in nonvalvular atrial fibrillation: relationship to p-selectin and antithrombotic therapy. Chest 2007;131(3):809–15.
29. Mobarrez F, He S, Broijersen A et al. Atorvastatin reduces thrombin generation and expression of tissue factor, P-selectin and GPIIIa on platelet-derived microparticles in patients with peripheral arterial occlusive disease. Thrombosis and Haemostasis 2011;106(2):344–52.
30. Kafian S, Mobarrez F, Wallen H et al. Association between platelet reactivity and circulating platelet-derived microvesicles in patients with acute coronary syndrome. Platelets 2015;26(5):467–73.
31. Kalantzi KI, Dimitriou AA, Goudevenos JA et al. The platelet hyporesponsiveness to clopidogrel in acute coronary syndrome patients treated with 75 mg/day clopidogrel may be overcome within 1 month of treatment. Platelets 2012;23(2):121–31.
32. Chyrchel B, Drożdż A, Długosz D et al. Platelet reactivity and circulating platelet-derived microvesicles are differently affected by P2Y(12) receptor antagonists. International Journal of Medical Sciences 2019;16(2):264–75.
33. Zhang Y, Liu X, Liu L et al. Contact- and agonist-regulated microvesiculation of human platelets. Thrombosis and Haemostasis 2013;110(2):331–9.
34. Franca CN, Pinheiro LF, Izar MC et al. Endothelial progenitor cell mobilization and platelet microparticle release are influenced by clopidogrel plasma levels in stable coronary artery disease. Circulation Journal 2012;76(3):729–36.
35. Sztriha LK, Sas K, Seres E et al. Optical platelet aggregomet ry does not appear useful as a means of assessing the risk of recurrent vascular events in aspirin-treated patients. Acta Neurol Scand 2008: 117: 250–254

Funding

This study was supported financially by a governmental research grant from the National Science Centre (2014/15/B/NZ4/00736).

Conflict of interest

All authors declare no conflict of interest

CORRESPONDING AUTHOR

Justyna Rosińska
Department of Neurology,
Poznan University of Medical Sciences,
Przybyszewskiego 49, 60-355 Poznan, Poland
tel.: +48 61 8691535; +48 61 8691456
fax: +48 61 8691697
e-mail: justynarosinska@interia.pl

Received: 12.04.2019
Accepted: 07.06.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

DIAGNOSTIC VALUE OF SPECIFIC ANTIBODY SYNTHESIS IN BRAIN OF PATIENTS WITH NEUROINFECTIONS

Pavel Dyachenko[1], Igor Filchakov[1], Anatoly Dyachenko[2], Valeria Shahinian[1], Victoria Kurhanskaya[2]
[1]CENTER OF INFECTIOUS DISORDERS OF THE NERVOUS SYSTEM, SI "L.V. GROMASHEVSKY INSTITUTE OF EPIDEMIOLOGY AND INFECTION DISEASES OF NAMS OF UKRAINE", KYIV, UKRAINE
[2]SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

Introduction: Viral encephalitis accounts for 40-70% of all cases worldwide, central nervous system infections pose a diagnostic challenge because clinical manifestations are not typically pathognomonic for specific pathogens, and a wide range of agents can be causative.

The aim: To assess the diagnostic value of intrathecal synthesis of specific antibodies in patients with inflammatory lesions of the central nervous system.

Materials and methods: Within the framework of the study, two groups of 90 people in each were formed from the patients with neuroinfections admitted to our Center. Intrathecal synthesis (ITS) of total (unspecific) IgG in members of one of group (group of compare) was determined. Brain synthesis of specific antibodies (Ab) to some neurotropic pathogens (herpes simplex virus 1/2, cytomegalovirus, Epstein-Barr virus, varicella zoster virus, rubella virus, Borrelies) was studied in the second group of patients (group of interest). There were no statistically significant differences between groups by gender and age. Encephalitis and encephalomyelitis prevailed among patients of both groups.

Results: ITS of total IgG was established in 30 (33.3 ± 6.1 %) patients of the first group with IgG index more than 0.6 indicating on inflammatory process in CNS and no marked changes of CSF. ITS of specific Ab was determined in 23 of 90 (25.6 ± 4.6 %) patients included into group of interest. In more than half of cases Ab to several infectious agents were detected simultaneously. ITS of various specificity, in particular, to measles and rubella viruses, and VZV, known as MRZ-reaction, is characteristic of some autoimmune lesions of CNS, multiple sclerosis first of all. In fact, further research of 5 patients with MRZ-reaction confirmed their autoimmune failure of CNS. Detection of ITS in the CSF samples didn't depend on concentration of specific Ab in serum and CSF and wasn't followed by HEB dysfunctions which were observed with the same frequency in patients with or without ITS (13.0 % and 13.6 % respectively).

Conclusion: Specific Ab synthesis to several neurotropic pathogens in the CSF of significant part of examined patients was established. Thus, diagnostic value of ITS of specific immunoglobulins seems to be limited to cases in which autoimmune damage of the CNS is suspected.

KEY WORDS: neuroinfections, intrathecal synthesis, specific antibodies

Wiad Lek 2019, 72, 8, 1437-1441

INTRODUCTION

Central nervous system (CNS) infections pose a diagnostic challenge because clinical manifestations are not typically pathognomonic for specific pathogens, and a wide range of agents can be causative. Smear staining and culture growth have of limited value, particularly for viral infections. Meanwhile, viral encephalitis accounts for 40-70% of all cases worldwide [1-5].

Recently diagnosis of CNS viral disease was mainly based on serologic investigations, including detection of immunoglobulin (IgG) intrathecal synthesis and specific antibodies index. It is known that specific IgG circulated in the blood can be sometimes detected in CSF, but in smaller titers. Therefore, persistence of the antibodies in CSF reflects rather the general immune response, not allowing to judge the presence and activity of the infectious process in the CNS. 'Early antibodies', immunoglobulin M or A (IgM/IgA), are not adequate indicator of acute brain infection also. In addition, IgA easily overcomes the blood-brain barrier (BBB).

Molecular methods nowadays have become the gold standard for the diagnosis of neuroinfections, however, cases of negative PCR are not uncommon, even when encephalitis was confirmed by clinical and visual signs. The reason of that may be, for example, short time (less than four days) after disease onset. Besides, viral propagation at lapse occurs in fact in brain parenchyma cells and only a few viral particles may release in the liquor [6-8]. In this case, the detection of ITS may be useful in making the correct diagnosis. The immunoglobulin synthesis in brain is provided by memory B lymphocytes which immigrate into the brain after the primary infection and settle in the brain tissue. The immigrated B-lymphocytes are antigen primed, affinity maturated and isotype specific. They synthesize immunoglobulins locally in the perivascular lymphocyte cuffs [9] and their pattern is locally varying but rather stable over the initial diagnostic phase of the diseases [10]. During flair ups priming B cells multiply, forming small (up to 1 mm in diameter) aggregates in different brain compartments [11]. B cell aggregates appear already a few

days after disease onset and increasingly display the phenotype of tertiary lymphoid organs (TLOs). Initially it was assumed that ITS is a sign of a specific immune response, but further research has shown that ITS is determined not only in the case of an acute infectious process in the CNS, when the pathogen is in the brain parenchyma and the immune response effectors migrate into the brain tissue, causing damage (encephalitis, myelitis, meningoencephalitis). The antigen-independent activation of B cells with T-lymphocytes and Toll-receptors on B-lymphocytes is also possible. As a result, a polyspecific immune response develops from ITS of varying specificity at the same time. In other words, ITS is not necessarily an indicator of an acute infectious process, but may be a part of the polyspecific immune response, which is due to antigen-independent B cells activation [12]. Below we present the data allowing to estimate the diagnostic value of intrathecal antibody synthesis in the patients with several neuroinfections.

THE AIM

To study frequencies of intrathecal synthesis of specific antibodies in patients with inflammatory disorders of the CNS.

MATERIALS AND METHODS

This prospective study to estimate the role of intrathecal specific Ab synthesis in making correct diagnosis, took place from January 2016 until the end of December 2016 at The Center of Infectious Disorders of the Nervous System (Kyiv, Ukraine). CIDNS is an adult tertiary referral, infectious diseases hospital covering all country territory. It has 20 beds with approximately 200-250 admissions annually. Patients were enrolled in the study if they have clinical evidence of a CNS infection: fever >38°C, or febrile episode reported within the previous month; CSF abnormalities (>four white blood cells per mm3 or CSF proteins >0.4 g/L); at least one of the neurological signs (confusion, altered mental status, seizures, focal deficiency). Exclusion criteria included noninfectious CNS diseases (cerebral tumor, cerebral abscess, and neurosurgery within the previous two-four months), and meningitis without clinical manifestation of brain involvement. Patients below 18 years were not included in the study, since children with CNS infections are managed in other specialized centers. Two groups were formed from our patients: first one included 90 patients with 30 (33.3 %) males and 60 (66.6 %) females from 21 to 70 years old age (average 38.9 ± 9.8). Intrathecal synthesis of total (unspecific) IgG was determined in members of the group (group of compare). Brain synthesis of specific antibodies to some neurotropic pathogens (herpes simplex virus 1/2, cytomegalovirus, Epstein-Barr virus, varicella zoster virus, rubella virus, Borrelies) was studied in patients of the second group (group of interest). There were no statistically significant differences between groups by gender and age. Encephalitis and encephalomyelitis prevailed among patients of both groups.

Quantification of total IgG in serum (S) and CSF was carried out by immunoturbidimetry method on automatic biochemical analyzer Cobas 311 (Roche, Swiss). Intervals between blood and CSF sampling did not exceed one hour. IgG index (I_{IgG}) was calculated by formula: I_{IgG} = tot. IgG_{CSF} x alb_S / tot. IgG_S x alb_{CSF}.

Quantitative study of specific Ab in serum and CSF was provided by standard IFA method with Euroimmun ELISA-test systems to Herpes simplex virus type 1/2 (Ab HSV 1/2), cytomegalovirus (Ab CMV), Epstein-Bark Virus (Ab EBV), Varicella zoster virus (Ab VZV), measles virus (Ab Msls), Rubella virus (Ab Rub), Borrelia (Ab Bor) [13,14]. The level of ITS was calculated accordingly to *H. Reiber* [12].

Permeability of BBB and blood-lymphatic barrier was evaluated upon ratio of albumin concentration in serum and CSF (albumin coefficient, Q_{alb}), taking into account age ratios [15]. The formula used in this study to compare levels of specific antibodies in the CSF and in serum sampled at the same time was that developed by *Tibbling and Link* [16]. This formula takes into account the level of specific IgG and albumin in the CSF and comparatively in serum, to verify that the IgG measured are only of intrathecal origin. If the index obtained by the following formula: IgG index = (IgG_{CSF} / $Albumin_{CSF}$) / (IgG_{serum} / $Albumin_{serum}$) is over 0.7, it means that specific IgG originate in CNS. If the result is less than 0.7, the IgG measured in the CSF crossed from the blood [12].

The degree of BBB dysfunction was determined on the basis of *Schliep* and *Felgenhauer* criteria [17].

RESULTS AND DISCUSSION

The prevalence of specific antibodies to some neurotropic pathogens in patients of studied group was very high and ranged from 100% (Ab-EBV) to 25.6% (Ab-Bor). Presence of antibodies in the blood was accompanied by their appearance in the CSF, which is obviously explained by their diffusion and penetration through the BB barrier (fig. 1). It should be noted that specific antibodies were usually found in the same patient simultaneously in the blood and CSF. The only difference between the frequency of Ab in serum and CSF related of measles virus (88,9 ± 3,3% in sera and 73,3 ± 4,7% in CSF, p <0,05). The prevalence of antibodies to herpesviruses (HSV, CMV, EBV, VZV) in the sera and CSF in the group of interest were very close. This fact may be explained by a lower concentration of anamnestic post-mortem or vaccine-derived antibodies against measles and rubella, in contrast to herpesviruses, which periodically stimulate immune response during relapse. The relatively low rate of detection of Ab-Bor is resulted the relatively low incidence of the infection in this group. As was to be expected, the prevalence of antibodies to herpesviruses and measles virus in first group were close to the second one, while Ab to borrelia were not found.

As can be seen from the data presented, Ab to several pathogens including various herpesviruses was established in both the blood and CSF of virtually all patients examined. Thus, serology makes it impossible to clearly differentiate the etiological factor of the disease. Moreover, even

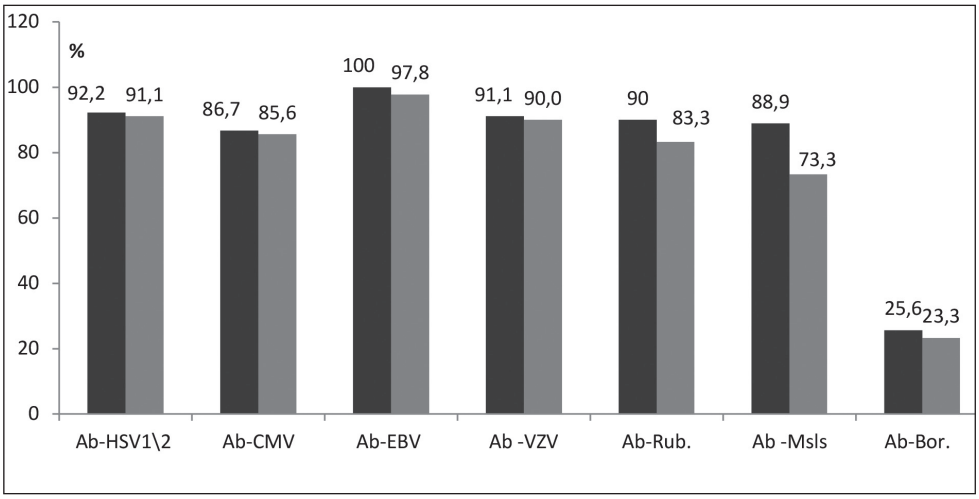


Figure 1. The prevalence of specific antibodies in the blood (black) and CSF (gray).

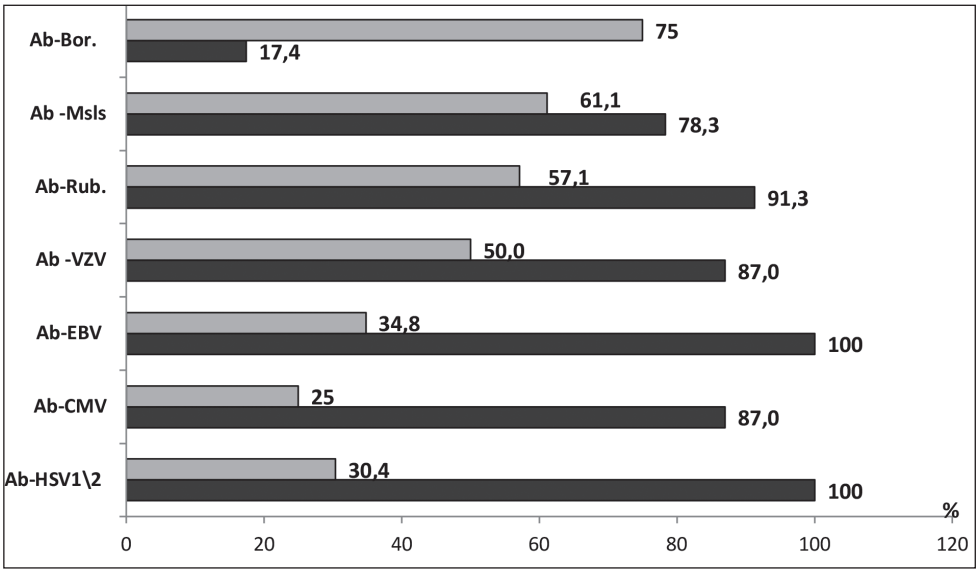


Figure 2. The prevalence of specific antibodies in CSF (black) and the proportion of their intrathecal synthesis (gray).

PCR cannot always help in solving problem: CSF samples obtained from almost 90% of patients at admission were negative for all pathogens of interest.

In 30 (33.3 ± 6.1%) patients of the first group, ITS of total IgG was identified with an index higher than 0.6, which is evidence of the inflammatory process in the CNS. The average age of patients with total IgG ITS did not differ from the average age of patients in the group and was 38.1 ± 9.9 years. At the same time, females prevailed (22 out of 30 - 73.3%) among patients with ITS IgG. It should be emphasized that in most patients with ITS IgG, there were no pronounced changes in CSF indices: the cytosis was less than 10 cells, the total protein was within the normal range. Most of them did not reveal dysfunction of the BBB / HLB.

ITS of specific antibodies, in the second group, was detected in 23 of 90 (25.6 ± 4.6%) patients examined. Ab to one pathogen were revealed in 11 (47.8%) patients, one patient (4.4%) has ITS to two, and 11 (47.8%) - to three and more pathogens. The latter to be considered as a polyspecific immune response of unclear origin. In one of the patient with a multiple sclerosis we found ITS against all of the pathogens examined, that confirmed autoimmune nature of the disorder. We compared the presence of ITS antibodies with the frequency of detection of antibodies in CSF (fig. 2). Antibodies to the herpes simplex virus and Epstein-Barr in CSF were present in all examined patients, but the frequency of intrathecal synthesis of antibodies to these pathogens when they were present in CSF was relatively low (30.4% and 34.8%, respectively). When antibodies to the measles virus were detected in the CSF, in most cases ITS antibodies to this virus were detected. At the same time, antibodies to CMV in CSF were present in 87% of those examined, and ITC to this virus was found only in 25% of patients in whom antibodies were detected in CSF. It should be noted that in 75% of patients in whom antibodies to borrelia were detected in CSF, their intrathecal synthesis was established.

These data clearly demonstrate that the detection of specific antibodies in the CSF cannot be considered as a tool useful in making correct diagnosis of CNS damage. At the same time, the diagnostic value of the method for determining ITS antibodies is also shown.

When ITS to only one of the pathogens is detected, the etiology of the CNS lesion becomes more apparent. It

was found, for example that ITS to borrelia is a specific test, confirming neuroborreliosis. According to our data, all patients with the ITS to borrelia had no intrathecal production of antibodies to other pathogens. At the same time, detection of antibodies to borrelia in the serum is not always sufficient for establishing the diagnosis of neuroborreliosis, since borreliosis is an endemic infection in some regions of Ukraine, therefore, the number of virus carriers, and recovered is quite high.

In our study, there were clinical cases where ITS assay results pointed on activation of the infectious. For example, intrathecal synthesis of Abs to VZV accompanied by highly positive PCR allowed to confirm the activation of VZV-infection in the patient. In two cases, the detection of ITS to borellia allowed to confirm the clinical diagnosis of neuroborelliosis.

However, as noted above, in more than half of the patients ITS against two or three pathogens was detected. In some cases, ITS only to the measles virus was found. We believed the autoimmune conflict in the CNS developed in these cases. A number of studies from other authors have shown that ITS of varying specificity, in particular to measles, rubella, VZV ("MRZ-reaction") characteristic for a number of autoimmune lesions of the CNS, in particular for multiple sclerosis (MS) [18]. In MS, the cause of ITS is the formation of ectopic lymphoid tissue in the brain parenchyma and its subsequent activation by immunopathological mechanisms at which differentiation of the memory B-lymphocytes is stimulated in the absence of antigens, due to the so-called unspecific activation. In this case, the immune response can reflect the individual history of previous infections and immunizations and depends on the regional prevalence of certain infections [10]. In our study "MRZ-reaction" was found in two patients. In one case, ITS was detected for measles and VZV, in other one, to measles and rubella viruses. Subsequently, oligoclonal antibodies, which are characteristic of MS were detected in one of them.

A separate discussion deserves the role of the Epstein-Barr virus in the CNS pathology. There is evidence in the literature that Ab-EBV is often found both in the serum and CSF of patients with MS [18]. According to the authors, the Epstein-Barr virus acts as a trigger signal, getting into the central nervous system and persisting in glial cells long before intrathecal inflammation and the formation of the TLO [11]. Other authors believe that the activation of naive B-cells penetrating into the central nervous system is due mainly to primary acute EBV infection [19]. Because at the time of acute infection, the production of specific antibodies has not yet started, this hypothesis can explain the high (almost 100%) incidence of Ab-EBV in patients with MS and the low frequency of ITS Ab-EBV. The data we obtained also indicate 100% of the detection rate of antibodies in CSF to EBV in the patients examined, with the frequency of ITS being relatively low - 34.8% (Fig. 2). It is possible that EBV was a trigger for chronic CNS lesions, which developed on the underlying immunopathological process.

It should be noted that there were no significant violations of the functions of the GEB / HLB in the examined patients. The minor violations, according to the Schliepand-Felgenhauer criteria, were established only in 13.6% of patients without ITS and 13.0% with intrathecal antibody synthesis. That is, relatively high concentrations of specific antibodies in CSF were not associated with increased permeability of GEB / HLB, but due to their direct production in brain tissues.

The conducted research has outlined the possible directions of using the ITS method for etiological and differential diagnostics of infectious, inflammatory, demyelinating and vascular diseases of the central nervous system.

CONCLUSIONS

1. Intrathecal synthesis of specific immunoglobulins was established in 23 out of 90 (25,6 ± 4,6%) patients with CNS lesions. Ab to several neurotropic agents were simultaneously present in 52,2% of cases.
2. Detection of ITS did not depend on the concentration of specific antibodies both in the serum and CSF and was not accompanied by a violation of the GEB / HLB function. The incidence of GEB / HLB dysfunction in patients with, and without ITS was found to be the same (13.6% and 13.0% respectively).
3. In the absence of ITS, the presence of specific Abs in CSF depended on their concentration in the blood and the permeability of GEB / HLB.
4. Although ITS assay has significant limitations on specificity, it can be useful especially at the onset of the disease and a negative PCR result.

REFERENCES

1. Khetsuriani N, Holman RC, Anderson LJ. Burden of encephalitis-associated hospitalizations in the United States, 1988–1997. Clin Infect Dis. 2002;35:175–82 10.1086/341301[PubMed] [Cross Ref]
2. Davison KL, Crowcroft NS, Ramsay ME, Brown DWG, Andrews NJ. Viral encephalitis in England, 1989–1998: what did we miss? Emerg Infect Dis. 2003;9:234–40 . 10.3201/eid0902.020218 [PMC free article] [PubMed][Cross Ref]
3. Koskiniemi M, Rantalaiho T, Piiparinen H, von Bonsdorff CH, Färkkilä M, Järvinen A, et al. Infections of the central nervous system of suspected viral origin: a collaborative study from Finland. J Neurovirol. 2001;7:400–8 . 10.1080/135502801753170255 [PubMed] [Cross Ref]
4. Huppatz C, Durrheim DN, Levi C, Dalton C, Williams D, Clements MS, et al. Etiology of encephalitis in Australia, 1990–2007. Emerg Infect Dis. 2009;15:1359–65 10.3201/eid1509.081540 [PMC free article] [PubMed][Cross Ref]
5. Mailles A, Stahl JP. Infectious encephalitis in France in 2007: a national prospective study. Clin Infect Dis. 2009;49:1838–47 10.1086/648419 [PubMed] [Cross Ref]
6. De Tiege X, Heron B, Lebon P, Ponsot G, Rozenberg F. Limits of early diagnosis of herpes simplex encephalitis in children: a retrospective study of 38 cases. Clin Infect Dis. 2003;36(10):1335–9.
7. Puchhammer-Stockl E, Presterl E, Croy C, Aberle S, Popow-Kraupp T, Kundi M, et al. Screening for possible failure of herpes simplex virus PCR in cerebrospinal fluid for the diagnosis of herpes simplex encephalitis. J Med Virol. 2001;64(4):531–6.

8. Weil AA, Glaser CA, Amad Z, Forghani B. Patients with suspected herpes simplex encephalitis: rethinking an initial negative polymerase chain reaction result. Clin Infect Dis. 2002;34(8):1154–7
9. Reiber H, Kruse-Sauter H, Quentin CD. Antibody patterns vary arbitrarily between cerebrospinal fluid and aqueous humor of the individual multiple sclerosis patient: specificity-independent pathological B cell function. J Neuroimmunol. 2015;278:247-54. doi:10.1016/j.jneuroim.2014.11.013
10. Reiber H. Cerebrospinal fluid data compilation and knowledge-based interpretation of bacterial, viral, parasitic, oncological, chronic inflammatory and demyelinating diseases: diagnostic patterns not to be missed in Neurology and Psychiatry. Arq Neuropsiquiatr. 2016;74(5):336-49. doi:10.1590 /0004-282X201600441
11. Kuerten S, Schickel A, Kerkloh C, Recks MS, Addicks K, Ruddle NH, et al. Tertiary lymphoid organ development coincides with determinant spreading of the myelin-specific T cell response. Acta Neuropathol (2012) 124(6):861–73.doi:10.1007/s00401-012-1023-3
12. Reiber H. Knowledge-base for interpretation of cerebrospinal fluid data patterns. Essentials in neurology and psychiatry. Arq.Neuropsiquiatr. 2016;74(6):501-512.
13. Reiber H, Lange P. Quantification of virus-specific antibodies in cerebrospinal fluid and serum: Sensitive and specific detection of antibody synthesis in brain. Clin Chem. 1991;37(7):1153-1160.
14. Schliep G., Felgenhauer K. Serum-CSF protein gradients, the blood-CSF barrier and local immune response. J.Neulor. 1978;218(2):77-96.
15. Denes E, Labach C, Duroxa H, et al. Intrathecal synthesis of specific antibodies as a marker of herpes simplex encephalitis in patients with negative PCR. Swiss Med Wkly. 2010;140:w13107.
16. Reiber H., Peter J. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J NeurolSci. 2001;184(2):101-122.
17. Link H, Tibbling G. Principles of albumin and IgG analyses in neurological disorders. III. Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. Scand J Clin Lab Invest. 1977;37(5):397–401.
18. Olival G.S., Lima B.M., Sumita L.M., et al. Multiple sclerosis and herpesvirus interaction. Arq Neuropsiquiatr 2013;71(9-B):727-730.
19. Odumade O.A., Hogquist K.A., Balfour H.H., et al. Progress and Problems in Understanding and Managing Primary Epstein-Barr Virus Infections. Clin. Microbiol. 2011;24(1):193-209. doi:10.1128/CMR.00044-10.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Anatoly Dyachenko
Sumy State University
1 Sanatorna Str., 40018, Sumy, Ukraine
tel: +380507713008
e-mail: vladlor60@ukr.net

Received: 05.03.2019
Accepted: 19.06.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

CLINICAL EVALUATION OF THE EFFECTIVENESS OF THE DEVELOPED METHOD OF CONDUCTIVE ANESTHESIA OF THE FACIAL BRANCHES OF GREAT AURICULAR NERVE

Oleg Mokryk, Svitlana Ushtan, Yuliya Izhytska
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

Introduction: The most common method of local anesthesia of maxillofacial region in the modern surgical practice is conductive anesthesia of the peripheral branches of trigeminal nerve. In order to reach the total anesthesia of the lateral facial region it is necessary to block not only auriculo-temporal and buccal nerves, but also facial branches of great auricular nerve taking part in the innervation of parotid-masticatory area and the part of the cheek. Topographic-anatomical investigations of corpses revealed the anatomical variability of the branching of great auricular nerve on the neck and the head. Taking into account the topographic-anatomical aspects of variability of innervation of the lateral facial region, we developed the method of conductive anesthesia of the facial branches of great auricular nerve.

The aim: To evaluate the clinical effectiveness of the developed method of conductive anesthesia of the facial branches of great auricular nerve taking into account individual anatomical peculiarities of its branching in patients with the different forms of the skull.

Materials and methods: Clinical observations were conducted on 69 patients of different age (from 18 to 70) and sex (43 males and 26 females). Under the local anesthesia we conducted surgery in the parotid-masticatory region including: disclosure of the abscesses, excision of migrating granulomas or lymph nodes (in the cases of chronic hyperplastic lymphadenitis); excision of the benign tumors of the soft tissues (atheromas, lipomas, fibromas and keratoacanthomas), excision of the salivary fistulas and keloid scars. Depending on the used methods of local anesthesia of the soft tissues of the parotid-masticatory region the patients were divided into two clinical groups. The first group (30 patients) was exposed to conductive anesthesia of great auricular nerve by the method of P. Raj (2002). according to which the blockade of the nerve is conducted ahead the apex of mastoid process of the temporal bone. 39 patients after the signing of the written agreement were exposed to the developed method of conductive anesthesia of the facial branches of great auricular nerve. In order to detect the individual anatomical features of the facial part of the head in patients, the facial index was determined by the Garson`s formula. Pain sensitivity and perception in patients were studied using subjective and objective methods. The data were analysed by means of the Pearson's chi–square tests.

Results: It is revealed that total anesthesia of the soft tissues of the parotid-masticatory region in all cases was reached in patients with euriprosopic face shape (broad-faced) – in 8 patients of the first clinical group and 10 patients of the second. The least effective was the anesthesia of the anterior branch of great auricular nerve conducted according to P. Raj's method (2002) in patients with leptoprosopic face shape. In patients with leptoprosopic face shape of the second clinical group after administering anesthesia according to the developed method in 9 cases total anesthesia was reached, in 2 cases pain sensitivity in the inferior-anterior quadrant remained ($\chi 2 = 5{,}70$; $p < 0{,}05$). Generally, in patients of the first clinical group the method of conducted anesthesia by P. Raj was effective in 19 cases (63,3 %), and the developed method of conductive anesthesia of the facial branches of great auricular nerve – in 36 cases (92,3 %) – $\chi 2 = 8{,}85$, $p < 0{,}01$.

Conclusions: The results of the research confirm that the developed method of conductive anesthesia of the facial branches of great auricular nerve is more effective in comparison to methods of anesthesia commonly used in today dentistry surgical practice. It allows to reach the total anesthesia of the soft tissues of the parotid-masticatory region in 92,3 % patients with different face shapes.

KEY WORDS: great auricular nerve, face shape, conductive anesthesia, pain perception

Wiad Lek 2019, 72, 8, 1442-1446

INTRODUCTION

Soft tissues of the face are innervated by the branches of trigeminal nerve [1 - 4], as well as by the branches of superficial cervical plexus – great auricular nerve and transverse cervical nerve [5 - 7]. These nerves closely connect with each other by the anastomoses, so there are no distinct borders in the partition of innervation zones on the face. The branching of these nerves varies in every patient [8, 9]. The most common method of local anesthesia of maxillofacial region in the modern surgical practice is conductive anesthesia of the peripheral branches of trigeminal nerve [10, 11]. In order to reach the total anesthesia of the lateral facial region it is necessary to block not only auriculo-temporal and buccal nerves, but also facial branches of great auricular nerve taking part in the innervation of parotid-masticatory area and the part of the cheek [12]. Great auricular nerve crosses the superficial leaf of cervical fascia along the posterior edge of sternocleidomastoid muscle at the level of thyroid cartilage, penetrates into the subcutaneous tissue and then circumflexes sternocleidomastoid muscle in the transverse direction [5, 13]. Reaching the posterior edge of the mandibular ramus this nerve divides into two or three branches [14, 15]. Topographic-anatomical investigations of corpses revealed the anatomical variability of the branching of great

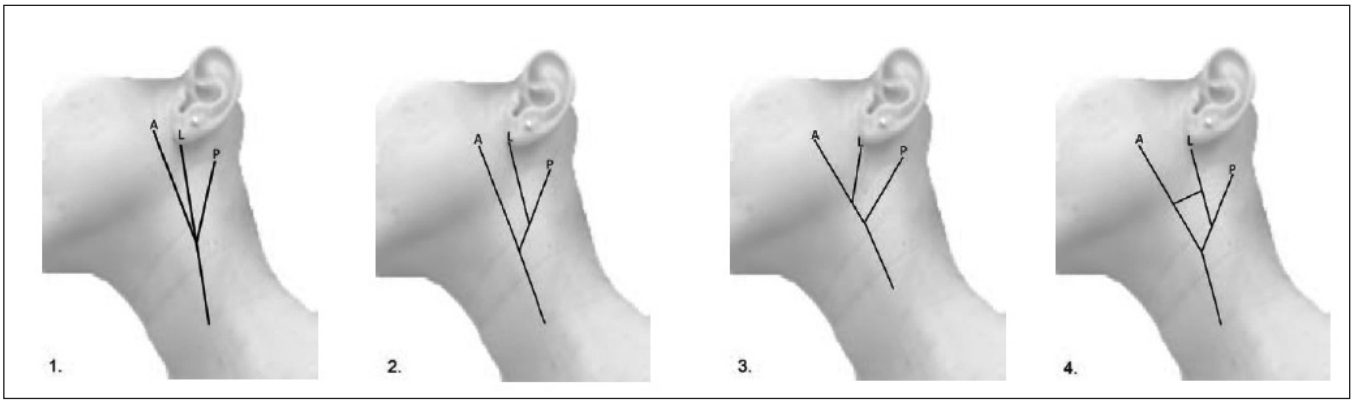


Figure 1. Anatomical variability of branching of great auricular nerve on the neck and the head (according to V. Sharma et al., 2017).
A – anterior branch, L – lobular branch, P – posterior branch.

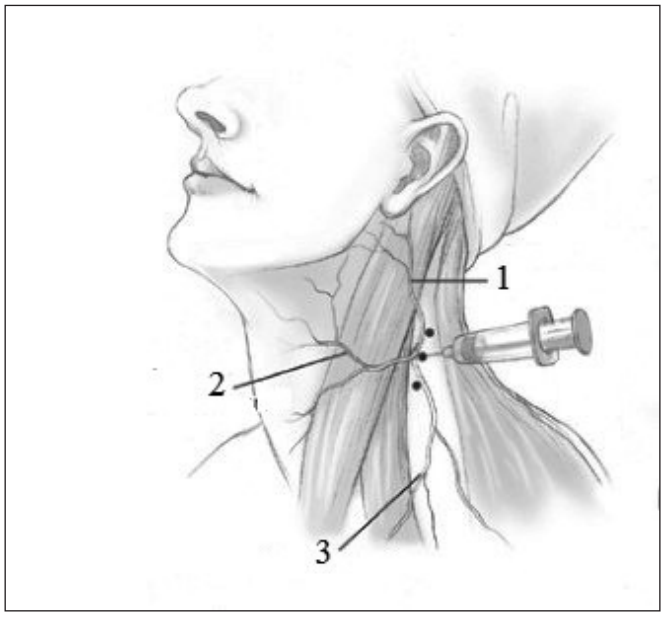


Figure 2. Blockade of the sensitive branches of superficial cervical plexus at Erb's point on the neck (Brown's method).
1 – great auricular nerve, 2 – transverse cervical nerve, 3 – supraclavicular nerve

auricular nerve on the neck and the head (fig. 1) [16]. Its anterior ramus branches in the skin of parotid-masticatory region, lobular ramus – in the lap and auricle (on its concave surface). The posterior ramus of great auricular nerve innervates the skin of the convex surface of auricle and a small area of skin behind the auricle [18]. The anterior ramus of the nerve crosses the anterior edge of the sternocleidomastoid muscle at the distance of 29,1 ± 3,4 mm from the apex of mastoid process of the temporal bone and 27,5 ± 4,5 mm to the angle of the mandible. It is detected that great auricular nerve in the majority of cases is localized in the middle third of the imaginary line connecting the noted topographic anatomical points on the bones [17].

Infiltration anesthesia is the most common method of blockade of the branches of great auricular nerve in the modern surgical practice. However, this method has a number of disadvantages: the introduction of local anesthetic causes the deformation of soft tissues that complicates the carrying out of local plastic surgery; repeated injections into the soft tissues intensify the pain in the emotionally labile patients causing stress reactions [18, 19]. According to classic Brown's method great auricular nerve is blocked at Erb's point on the neck [20] (fig. 2). It is also blocked when the whole superficial cervical plexus is blocked along the posterior edge of the sternocleidomastoid muscle [21, 22]. These methods are risky to damage external jugular vein passing through the area of injection [15]. Due to the risks of local complications the noted methods of local anesthesia are not currently used in the surgical dentistry practice. P. Raj (2002) proposed to block the anterior branch of great auricular nerve ahead the apex of mastoid process of the temporal bone [23].

We used this method to anesthetise the soft tissues of the parotid-masticatory region during surgical procedures. Total anesthesia was reached in 63 % of the patients. In the rest occurred only partial anesthesia of the lap, auricle (concave surface) and the upper part of the parotid-masticatory region. In our opinion, insufficient effectiveness of the method is associated with the anatomical variability of the localization of great auricular nerve on the face in patients with different forms of the facial part of the skull. According to classification by V. Sharma et al. [16], in patients with the 3rd or 4th type of branching of great auricular nerve its anterior branch is much remote from the lap, that's why it can't be blocked by the anesthetic, depot of which is localized ahead the apex of mastoid process of the temporal bone. Taking into account the topographic-anatomical aspects of variability of innervation of the lateral facial region, we developed the method of conductive anesthesia of the facial branches of great auricular nerve.

THE AIM

The purpose of the study: to evaluate the clinical effectiveness of the developed method of conductive anesthesia of the facial branches of great auricular nerve taking into account individual anatomical peculiarities of its branching in patients with the different forms of the skull.

MATERIALS AND METHODS

Clinical observations were conducted on 69 patients of different age (from 18 to 70) and sex (43 males and 26 females). This number consisted of the patients hospitalized in the department of surgical dentistry and maxillofacial surgery of Lviv Regional Hospital and the patients treated at the Dental Medical Centre of Danylo Halytsky Lviv National Medical University. Under the local anesthesia we conducted surgery in the parotid-masticatory region including: disclosure of the abscesses, excision of migrating granulomas or lymph nodes (in the cases of chronic hyperplastic lymphadenitis); excision of the benign tumors of the soft tissues (atheromas, lipomas, fibromas and keratoacanthomas), excision of the salivary fistulas and keloid scars. Depending on the used methods of local anesthesia of the soft tissues of the parotid-masticatory region the patients were divided into two clinical groups. The first group (30 patients) was exposed to conductive anesthesia of great auricular nerve by the method of P. Raj (2002), according to which the blockade of the nerve is conducted ahead the apex of mastoid process of the temporal bone [23]. 39 patients after the signing of the written agreement were exposed to the developed method of conductive anesthesia of the facial branches of great auricular nerve: at the beginning the skin of the parotid-masticatory region is covered with antiseptic. After that the doctor should palpate the posterior edge of the mandibular ramus: the thumb of the left hand should be placed at the projection of the mandibular angle, the forefinger – at the projection of the base of collum of the articular process located 1 cm lower the lap. The skin between two fingers gets pulled in order to better see the contours of the posterior edge of mandibular ramus. The syringe with needle is held in the right hand. The injection is done into the subcutaneous tissue next to the mandibular angle. The direction of needle goes from the bottom to the top, in parallel to the posterior edge of mandibular ramus, and then it ascends from the mandibular angle to the base of collum of the articular process. On this way the local anesthetic infiltrates the subcutaneous tissue and the superficial fascia of the face where the facial branches of great auricular nerve are localized (fig. 3). In cases when the pain sensitivity after the conducted methods of anesthesia didn't disappear patients were additionally exposed to infiltration anesthesia or the blockade of buccal nerve next to the anterior edge of the base of coronary process of the mandible according to the method of J. Iwanaga and R. Tubbs (2019) [24].

In order to detect the individual anatomical features of the facial part of the head (skull) in patients, the facial index (FI) was determined by the Garson's formula – the relation between the morphological height of the face and its width multiplied by 100 %. The FI range for euriprosops (broad-faced) is less than 79,9 %, mesoprosops (medium-headed) – from 80,0 to 89,9 %, leptoprosops (elongated face shape) – from 90,0 to 94,9 % [25]. The parotid-masticatory region was conditionally divided into four quadrants (superior-anterior, superior-posterior, inferior-anterior and inferior-posterior) with the imaginary mutually perpendicular lines going through the center of this topographic anatomical region of the face.

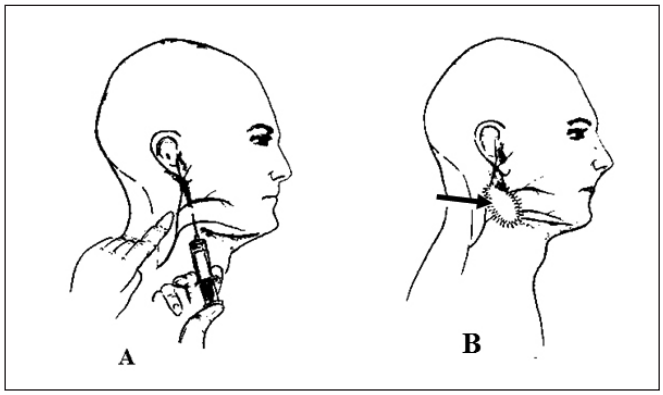


Figure 3. The developed method of conductive anesthesia of the facial branches of great auricular nerve
A – infiltration of the subcutaneous tissue and the superficial fascia of the face along the posterior edge of the mandibular ramus
B – depot of the local anesthetic along the posterior edge of the mandibular ramus where the facial branches of great auricular nerve are blocked (the area is shown by the arrow)

Pain sensitivity and perception in patients were studied using subjective and objective methods. Pain sensitivity was determined by injection of a needle (pinprick) into the epidermis. The assessment of pain sensitivity was performed on a four-point scale in each quadrant: 0 points – no sensitivity, 1 point – sensitivity is sharply reduced, 2 points – sensitivity is moderately reduced, 3 points – tactile and pain sensitivity is completely preserved [26]. Pain perception during local anesthesia administration was evaluated by the Sounds, Eyes and Motor (SEM) scale [27]. SEM scale was used to assess the observed pain. It is divided into two categories of comfort and discomfort. The discomfort response is further divided into three subscales: mild pain, moderate pain and severe pain (Table I).

Autonomic reaction of the cardiovascular system to pain was evaluated by the perfusion index (PI) monitored with the pulse oximeter ELERA SH-K3 (Hong Kong). The normal PI range is 5–6 %. It decreases with the narrowing of the lumen of the peripheral blood vessels caused by the pain triggers [28].

The protocol used in this study conformed to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Danylo Halytsky Lviv National Medical University.

The reliability of the obtained results was evaluated according to the reliability criterion of the Student by statistically computing the data using the commonly used methods of variation statistics from the package «Statistica-7». The data were analysed by means of the Pearson's chi–square tests.

RESULTS AND DISCUSSION

It is revealed that total anesthesia of the soft tissues of the parotid-masticatory region in all cases was reached in patients with euriprosopic face shape (broad-faced) – in 8 patients of the

Table I. The Sounds, Eyes, and Motor (SEM) scale for measuring the comfort or discomfort

Observations	Comfort	Discomfort		
		Mild pain	Moderate pain	Severe pain
Sounds	No sounds indicating pain	Nonspecific sounds; possible pain indication	Specific verbal complaints, e.g., "Ouch", raises Voice	Verbal complaint indicates intense pain, e.g., scream, sobbing
Eyes	No eye signs indicating discomfort	Eyes wide, show of concern, no tears	Watery eyes, eyes flinching	Crying, tears running down face
Motor	Hands relaxed; no apparent body tension	Hands show some distress or tension, grasps chair due to discomfort, muscular tension	Random movement of arms or body without aggressive intention of physical contact, grimace, twitch	Movement of hands to make aggressive physical contact, pulling head away

Table II. The clinical effectiveness of used methods of conductive anesthesia of the facial branches of great auricular nerve

Form of the face (facial index)	Method of conductive anesthesia of the facial branches of great auricular nerve (according to P. Raj, 2002) (n = 30)		Developed method of conductive anesthesia of the facial branches of great auricular nerve (n = 39)		Pearson's correlation coefficient χ^2 (p)
	Total anesthesia of the parotid-masticatory area	Partial anesthesia of the parotid-masticatory area	Total anesthesia of the parotid-masticatory area	Partial anesthesia of the parotid-masticatory area	
Euriprosopic (< 79,9 %)	8 cases	0 cases	10 cases	0 cases	not determined
Mesoprosopic (80,0 - 89,9 %)	8 cases	4 cases	17 cases	1 cases	3,97 (p < 0,05)
Leptoprosopic (90,0 - 94,9 %)	3 cases	7 cases	9 cases	2 cases	5,70 (p < 0,05)
Totally n - cases (%)	19 cases (63,3%)	11 cases (36,7%)	36 cases (92,3 %)	3 cases (7,7 %)	8,85 (p < 0,01)

first clinical group and 10 patients of the second. Pain sensitivity was absent (0 points). During surgery the stress reactions of the autonomic nervous system did not occur (PI = 5,5 ± 0,5 %) as well as the behavioral disorders according to SEM scale were not noticed. In patients with mesoprosopic face shape of the first group the total anesthesia of the parotid-masticatory region was reached in 8 cases, and in 4 cases only a partial loss of the tactile and pain sensitivity in the parotid-masticatory region was noticed. Total anesthesia occurred only in the upper quadrants of the parotid-masticatory region, the lap and auricle. This meant that only the lobular branches of great auricular nerve and auriculotemporal nerve were blocked. The pain sensitivity in the lower quadrants was evaluated in 2,5 ± 0,5 points. With the attempt to begin the surgery in such patients manifested pronounced motor (discomfort according to SEM scale) and autonomic (PI = 2,5 ± 0,5 %) reactions to pain triggers. After additional infiltration anesthesia the total anesthesia was reached. In patients with the mesoprosopic face shape (medium-headed) of the second clinical group total anesthesia of the parotid-masticatory region was reached in 17 cases (χ^2 = 3, 97; p < 0,05). Only in 1 patient the developed method of anesthesia had an unsufficient effect – pain sensitivity in the superior-anterior quadrant of the parotid-masticatory region didn't decrease. The least effective was the anesthesia of the anterior branch of great auricular nerve conducted according to P. Raj's method (2002) in patients with leptoprosopic face shape. Total anesthesia of the parotid-masticatory region occurred in 3 patients, in 7 patients the method was not effective: the tactile and pain sensitivity were not lost (3 points) in the lower quadrants of the parotid-masticatory region. In patients with leptoprosopic face shape of the second clinical group after administering anesthesia according to the developed method in 9 cases total anesthesia was reached, in 2 cases pain sensitivity in the inferior-anterior quadrant remained (χ^2 = 5,70; p < 0,05). We noticed that in patients with leptoprosopic face shape in the sensitive innervation of inferior-anterior quadrants of the parotid-masticatory region can take part buccal nerve. The confirmation of this is the loss of pain sensitivity in the region of innervation after the conductive anesthesia of this nerve. Generally, in patients of the first clinical group the method of conducted anesthesia by P. Raj was effective in 19 cases (63,3 %), and the developed method of conductive anesthesia of the facial branches of great auricular nerve – in 36 cases (92,3 %) – $\chi 2$ = 8,85, p < 0,01 (Table II).

CONCLUSIONS

The results of the research confirm that the developed method of conductive anesthesia of the facial branches of great auricular nerve is more effective in comparison to methods of anesthesia commonly used in today dentistry surgical practice. It allows to reach the total anesthesia of the soft tissues of the parotid-masticatory region in 92,3 % patients with different face shapes.

REFERENCES

1. Hwang K., Suh M.S., Chung I.H. Cutaneous distribution of infraorbital nerve. J. Craniofac Surg. 2004 Jan; 15(1): 3 - 5.
2. Hu KS, Kwak J, Koh KS, Abe S, Fontaine C, Kim HJ. Topographic distribution area of the infraorbital nerve. Surg Radiol Anat. 2007 Jul;29(5):383-8.
3. Yang HM, Won SY, Lee JG, Han SH, Kim HJ, Hu KS. Sihler-stain study of buccal nerve distribution and its clinical implications. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Jul;113 (7):334-9.
4. Takezawa K, Ghabriel M, Townsend G. The course and distribution of the buccal nerve: clinical relevance in dentistry. Australian Dental Journal. 2018 Dec; 63 (12): 66-71.
5. Murphy R, Dziegielewski P, O'Connell D, Seikaly H, Ansari K. The great auricular nerve: an anatomic and surgical study. J. Otolaryngol Head Neck Surg. 2012 Apr;41 Suppl 1:S75-7.
6. Ella B, Langbour N, Caix P, Midy D, Deliac P, Burbaud P. Transverse cervical and great auricular nerve distribution in the mandibular area: a study in human cadavers. Clin Anat. 2015 Jan;28(1):109-17. doi: 10.1002/ca.22369.
7. Kim S, Feldman DU, Yang J. A Systematic Review of the Cervical Plexus Accessory Innervation and Its Role in Dental Anesthesia. J Anesth Hist. 2016 Jul;2(3):79-84. doi: 10.1016/j.janh.2016.04.010.
8. Siemionow M, Gharb BB, Rampazzo A. The face as a sensory organ. Plast Reconstr Surg. 2011 Feb;127(2):652 - 662.
9. Thomas von Arx, Ahmed Z Abdelkarim, Scott Lozanoff. The Face – A Neurosensory Perspective. A literature review. Swiss Dental Journal SSO. 2017 May; 127 (5):1066–1075.
10. Joe Niamtu. Cosmetic Facial Surgery. 25th ed. Louis: Mosby; 2010. 784 p.
11. Dhepe V. Niteen. Local Anesthesia for Cosmetic Procedures, Clinical Use of Local Anesthetics. Rijeka: InTech; 2012. 102 p.
12. William R. Ryan, Willard E. Fee Jr. Great Auricular Nerve Morbidity After Nerve Sacrifice During Parotidectomy. Arch Otolaryngol Head Neck Surg. 2006;132(6):642-649. doi:10.1001/archotol.132.6.642.
13. Thallaj A, Marhofer P, Moriggl B, Delvi BM, Kettner SC, Almajed M. Great auricular nerve blockade using high resolution ultrasound: a volunteer study. Anaesthesia. 2010 Aug;65(8):836-40. doi: 10.1111/j.1365-2044.2010.06443.
14. Yang HM, Kim HJ, Hu KS. Anatomic and histological study of great auricular nerve and its clinical implication. J Plast Reconstr Aesthet Surg. 2015 Feb;68(2):230-6. doi: 10.1016/j.bjps.2014.10.030.
15. Altafulla J, Iwanaga J, Lachkar S, Prickett J, Dupont G, Yilmaz E, Ishak B, Litvack Z, Tubbs RS. The Great Auricular Nerve: Anatomical Study with Application to Nerve Grafting Procedures. World Neurosurg. 2019 Jan 28. pii: S1878-8750(19)30185-8. doi: 10.1016/j.wneu.2019.01.087.
16. Sharma VS, Stephens RE, Wright BW, Surek CC. What Is the Lobular Branch of the Great Auricular Nerve? Anatomical Description and Significance in Rhytidectomy. Plast Reconstr Surg. 2017 Feb;139(2):371e-378e. doi: 10.1097/PRS.0000000000002980.
17. Tayebi Meybodi A, Gandhi S, Lawton MT, Preul MC. Anterior Greater Auricular Point: Novel Anatomic Landmark to Facilitate Harvesting of the Greater Auricular Nerve. World Neurosurg. 2018 Nov;119:e64-e70. doi: 10.1016/j.wneu.2018.07.001.
18. Kuscu OO, Akyuz S. Is it the injection device or the anxiety experienced that causes pain during dental local anaesthesia? Int J Paediatr Dent 2008;18:139-145.
19. Caddick J, Jawad S, Southern S, Majumder S. The power of words: sources of anxiety in patients undergoing local anaesthetic plastic surgery. Ann R Coll Surg Engl. 2012 Mar; 94(2): 94–98. doi: 10.1308/003588412X13171221501267.
20. Thallaj A, Marhofer P, Moriggl B, Delvi BM, Kettner SC, Almajed M. Great auricular nerve blockade using high resolution ultrasound: a volunteer study. Anaesthesia. 2010 Aug;65(8):836-40. doi: 10.1111/j.1365-2044.2010.06443.
21. Kanthan R K. The use of superficial cervical plexus block in oral and maxillofacial surgical practice as an alternative to general anesthesia in selective cases. Ann Maxillofac Surg 2016;6:4-8.
22. Herring AA, Stone MB, Frenkel O, Chipman A, Nagdev AD. The ultrasound-guided superficial cervical plexus block for anesthesia and analgesia in emergency care settings. Am J Emerg Med. 2012 Sep;30(7):1263-7. doi: 10.1016/j.ajem.2011.06.023.
23. Raj PP. Textbook of regional anesthesia. New York: Churchill Livingstone; 2002. 1265 p.
24. Iwanaga J, Tubbs RS. Buccal Nerve Dissection Via an Intraoral Approach: Correcting an Error Regarding Buccal Nerve Blockade. J Oral Maxillofac Surg. 2019 Jun;77(6):1154.e1-1154.e4. doi: 10.1016/j.joms.2019.01.038.
25. Trivedi H, Azam A, Tandon R, Chandra P, Kulshrestha R, Gupta A. Correlation between morphological facial index and canine relationship in adults – An anthropometric study. J Orofac Sci. 2017 January-June; 9 (1):16-21.
26. Miller H. Smith, Kevin E. Lung. Nerve Injuries after Dental Injection: A Review of the Literature. J. Can Dent Assoc. 2006; 72(6):559–64.
27. Wright GZ, Weinberger SJ, Marti R, Plotzke O. The effectiveness of infiltration anesthesia in the mandibular primary molar region. Pediatr Dent. 1991;13:278–83.
28. Raouf Mohameda S. A., Mohameda N. N., Rashwanb D. Pulse co-oximetry perfusion index as a tool for acute postoperative pain assessment and its correlation to visual analogue pain score. Research and Opinion in Anesthesia & Intensive Care. 2015 January; 2(3): 62–67. doi: 10.4103/2356-9115.172783.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Yuliya Izhytska
Danylo Halytsky Lviv National Medical University
Lviv, Ukraine
e-mail: nataizhytska@gmail.com

Received: 10.04.2019
Accepted: 09.07.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

DEVELOPMENT OF OBLITERATION IN THE GREAT SAPHENOUS VEIN AFTER INFLUENCE OF HIGH-FREQUENCY ENDOVENOUS WELDING BY DATA OF MORPHOLOGICAL AND ULTRASONIC STUDIES

Olena O. Dyadyk, Serhiy I. Savolyuk, Roman A. Herashchenko, Valentyn A. Khodos, Vladyslav S. Horbovets
SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KYIV, UKRAINE

ABSTRACT

Introduction: Acute ascending thrombophlebitis (AAT) of the great saphenous vein (GSV) is one of the problems in modern surgery.

The aim: Study of morphological and ultrasonic changes in a thrombosed vein following the influence of high-frequency endovenous electrical welding and assessment of the efficacy of application of this method to treatment of acute ascending thrombophlebitis.

Materials and methods: During the period of 2016-2018, surgery departments of Kyiv City Hospital No. 8 provided surgical treatment for 52 male and female patients with AAT of the GSV, who underwent high-frequency endovenous electrical welding (EVEW) with help of an EK300M Svarmed apparatus. The patients' age ranged from 19 to 78 years (their mean age was 51±2.63 years).

According to the international CEAP (clinical-etiological-anatomical-pathophysiological) classification, C2 was revealed in 4 cases, C3 in 19, C4 in 13, C5 in 9 and C6 in 7. Sections, prepared in compliance with standard methods, were morphologically examined. Photo archiving was made with use of a ZEISS light optical microscope (Germany) and «Axio Imager. A2» data processing system. Duplex ultrasonography (DUS) was performed with help of a TOSHIBA Nemio XG (Japan) device, equipped with a convex transducer having the working frequency of 3.5-5 MC and a linear transducer at a range of 7.5-12 MC. Ultrasonic studies were carried out 2-7 days after the operation. Remote results were assessed after 3, 6 and 12 months.

Results: Morphological examinations of vein preparations after EVEW with standard haematoxylin-eosin staining revealed homogenization of thrombotic masses in the venous lumen and a close relationship of the veins with the treated vascular wall; in the majority of examined cases, thrombotic masses totally obliterated the venous lumen. All the layers of the venous wall – internal, middle and external – coalesced into a single homogenous complex. Specific staining on elastic fibers revealed their total destruction in the internal and middle coats, thinning and fragmentation of elastic fibers in the adventitia. Total occlusion of the GSV was observed sonographically in 49 of 52 cases (94.24%) on days 2-7 after the influence of EVEW. Within 3-6 months, 3 cases (5.76%) developed some partial recanalization. During 9-12 months, colour mapping revealed preservation of some partial and haemodynamically insignificant recanalization in 2 cases (3.84%). One case (1.92%) developed the complete and haemodynamically significant recanalization of the whole welded segment of GSV.

Conclusion: Analysis of ultrasonic studies showed that despite the total destruction of the whole venous wall during the first days after the influence of EVEW there were areas of parietal blood flow in 5.76% of cases. During a remote period of follow-up from 3 to 6 months the above changes could develop partial and haemodynamically insignificant recanalization in 3.84% of cases. Within the period of follow-up from 9 to 12 months those areas of blood flow could lead to the complete and haemodynamically significant recanalization of the welded GSV in 1.92% of cases. High-frequency EVEW of the thrombosed GSV in AAT caused the total destruction of thrombotic masses and all layers of the venous wall and made it possible to achieve the total obliteration of the welded vein in 94.24 % of cases.

KEY WORDS: thrombophlebitis, morphology of veins, electrical welding of live tissues, ultrasonic study

Wiad Lek 2019, 72, 8, 1447-1452

INTRODUCTION

AAT of the GSV is one of the problems in modern surgery [1]. It is not in all cases that conservative methods of treatment prevent development of complications of AAT [2]. Extraction of the thrombosed trunk of GSV on a probe or with separate cuts is the most widespread surgical method for treating this disease [3, 4]. But due to a high injury rate of these methods most phlebologists prefer use of mini-invasive methods of thermal ablation [5, 6]. A widespread use among modern innovative technologies in different fields of surgery is got by the method of high-frequency electrical welding of live tissues (HFEWLT) [7–10]. The mechanism of HFEWLT consists in the fact that passage of high-frequency electrical current through soft tissues causes some partial destruction of cells, denaturation and coagulation of protein molecules with the resultant connection (welding) of the tissues, approximated after their cutting [9, 11, 12].

The method has become widely used in surgery of abdominal wall herniae, proctology, emergency surgery, biliary surgery, thoracic surgery [13–15]. Recent years have witnessed publications on application of high-frequency electrical welding for eliminating vertical reflux in varicose veins of the lower extremities, i.e. endovenous

electrical welding (EVEW) [16]. On the basis of morphological studies the authors showed that the influence of EVEW on a vein, changed by varicosity, causes structural changes in the venous wall in the form of collagen disorganization, dystrophic changes of cells with different extents of membrane destruction and endothelial necrosis with a resultant occlusion of the vein and its subsequent fibrous transformation [16]. In order to improve results of treatment of AAT of GSV, we used the method of EVEW of the thrombosed GSV [17]. Our previous morphological study demonstrated that a 15-second exposure in the «manual mode» was the optimum mode of high-frequency electrical welding for coagulation of the thrombosed GSV [18]. Those findings served for us as the basis when using the above mode in our subsequent clinical work.

THE AIM

Study of morphological and ultrasonic changes in a thrombosed vein following the influence of high-frequency EVEW and assessment of the efficacy of application of this method to treatment of AAT.

MATERIALS AND METHODS

During the period of 2016-2018, surgery departments of Kyiv City Hospital No. 8 treated 52 male and female patients with AAT of GSV, who received treatment by means of EVEW with help of an EK300M Svarmed apparatus. The patients' age ranged from 19 to 78 years (their mean age was 51±2.63 years). The exposure to the energy effect on the thrombosed GSP lasted 15 seconds in the «manual mode». The efficacy of EVEW of the thrombosed GSV was assessed on the basis of morphological studies. Fragments of the operated vein were taken during its surgical treatment and fixed in 10% solution of neutral buffered formalin. Serial histological sections were prepared according to standard methods and stained with haematoxylin and eosin, van Gieson's picrofuchsin (for revealing collagen and smooth muscular fibers, and verification of processes of extreme fibrillogenesis) and Weigert's iron haematoxylin (for verification of elastic fibers) [19].

Microscopic examinations of the stained sections and their photo archiving were carried out with use of a ZEISS light optical microscope (Germany) and Axio Imager. A2 data processing system with lens magnifications 5x, 10x, 20x, 40x, a 1.5 binocular adjustment and an eye lens 10 with ERc 5s. and ZEISS (Germany) cameras having the Primo Star data processing system with Plan-Achromat planar achromatic ZEISS lenses 4x, 10x, 40x and an AxioCam 105color camera. The efficacy of EVEW of the thrombosed GSV was assessed on the basis of ultrasonic data. All the patients underwent DUS with help of a TOSHIBA Nemio XG (Japan) device, equipped with a convex transducer having the working frequency of 3.5-5 MC and a linear transducer at a range of 7.5-12 MC. Ultrasonic control was performed 2-7 days after the operation. Remote results were assessed after 3, 6 and 12 months.

RESULTS

Morphological examinations of vein preparations after EVEW with standard haematoxylin-eosin staining revealed homogenization of thrombotic masses in the venous lumen and a close relationship of the veins with the treated vascular wall; in the majority of examined cases, thrombotic masses totally obliterated the venous lumen. All the layers of the venous wall – internal, middle and external – coalesced into a single homogenous complex (fig. 1).

The venous wall was oedematous. Both fine bullous structures, located in all layers of the wall, and large confluent «bullae», which caused an uneven dissection of the vascular wall with formation of aneurysms, were observed. Tissues around the bullous structures were homogenized (figs. 2–4).

Specific staining on elastic fibers revealed their total destruction in the internal and middle coats, thinning and fragmentation of elastic fibers in the adventitia (fig. 5).

The soft tissues, adjacent to the vessel, and underlying trophic structures were mainly preserved (fig. 6).

Owing to an oedema of the venous wall, ulrasonography on days 2–7 after the operation revealed a larger diameter of GSV in the whole area of EVEW influence. In 49 of 52 cases (94.24%) the venous lumen along its entire length was filled with hypoechogenic thrombotic masses without any signs of blood flow, thereby demonstrating the complete occlusion of GSV. In 3 of 52 cases (5.76%) the lumen of the welded vein revealed areas of parietal blood flow 1–2 cm long: in 1 case in the upper third of the thigh in the confluence of GSV flow, in 1 case in the place of localization of Hunter's perforator, and in 1 case in the place of localization of Dodd's perforator.

During a remote period of 3-6 months the colour flow mapping (CFM) mode in the above 3 cases (5.76%) revealed partial canalization. The lumen of the vein with a smaller diameter contained areas of some haemodynamically insignificant blood flow 1–5 cm long. The blood flow was found in the volume of up to two-thirds of the venous lumen with hyperechogenic and parietally fixed thrombus remnants. In one case the recanalized segment was connected with the functioning Hunter's perforator, in another one with Dodd's perforator, and in the third one with the functioning GSV flow in the upper third of the thigh (fig. 7).

Within the term of 9-12 months, colour mapping revealed in 2 cases (3.84%) preservation of some partial and haemodynamically insignificant recanalization 1–3 cm long. The blood flow was found in the upper and medial thirds of the thigh: in 1 case in the place of confluence of GSV flow, in another one in the place of localization of Dodd's perforator. One case (1.92%) revealed the complete and haemodynamically significant recanalization of the whole welded GSV segment from the inguinal area to the knee level. The recanalized segment of GSV was connected with the functioning Hunter's perforator (fig. 8).

On longitudinal scanning, the venous wall in the recanalized GSV was thickened; B mode revealed heterogeneous echoicity of the wall; CDM mode demonstrated flow past

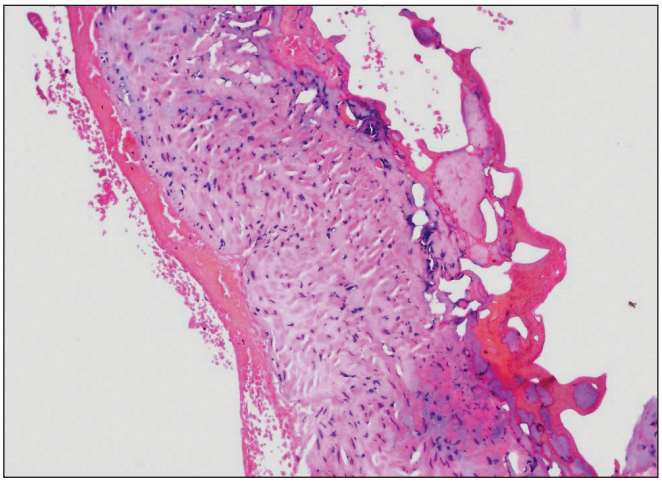


Fig. 1. The internal layer of the venous wall is coagulated with thrombotic masses. All the layers of the venous wall are homogenized and form a single conglomerate. Haematoxylin and eosin staining, × 50.

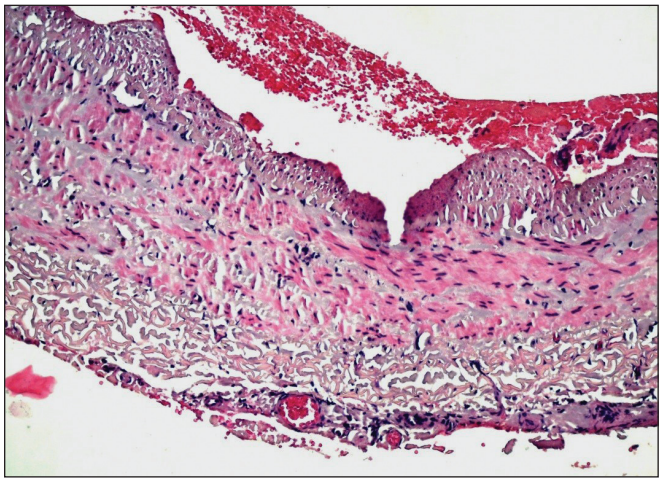


Fig. 2. Thrombotic masses are coagulated and have a close relationship with the vascular wall treated. All the layers of the vascular wall contain fine «bullae». Structures around the «bullae» are homogenized. Haematoxylin and eosin staining, × 100.

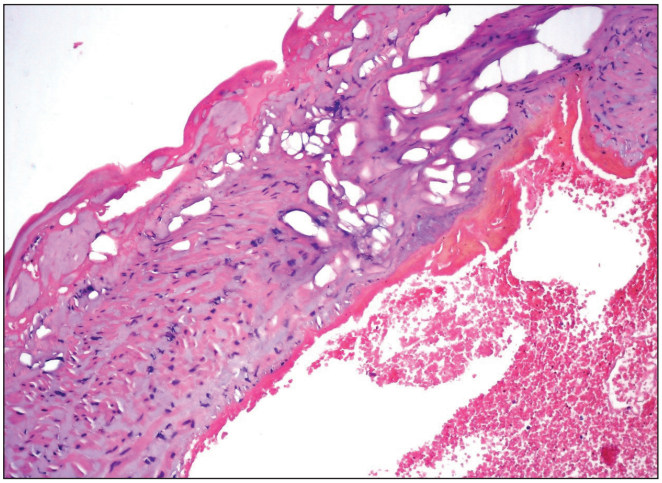


Fig. 3. Thrombotic masses are coagulated and have a close relationship with the vascular wall treated; there are large confluent «bullae» in all layers of the venous wall. Structures around the «bullae» are homogenized. Haematoxylin and eosin staining, × 100.

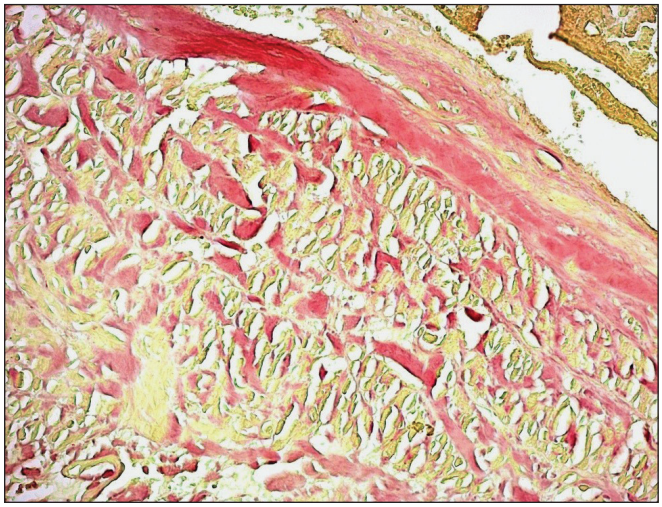


Fig. 4. Layers of the venous wall coalesce into a single homogenous complex; fine bullous structures are located around fibers; collagen and smooth muscular fibers are homogenized. Van Gieson staining, × 200.

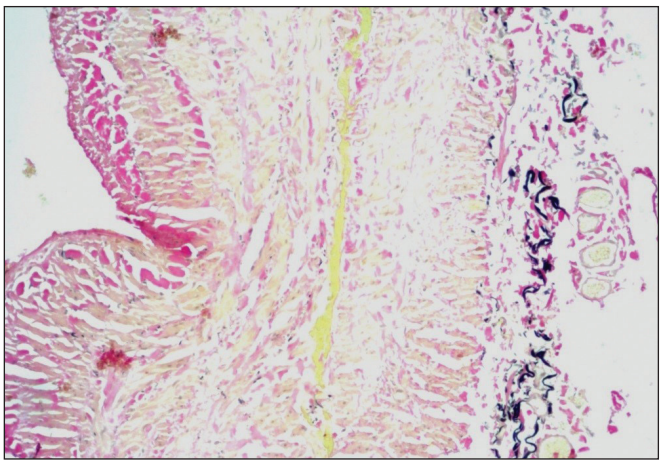


Fig. 5. The internal, middle and external layers coalesce into a single homogenous complex. Fine and confluent bullous structures are located in all layers of the venous wall. Dissection of the vascular wall and formation of an aneurysm are observed. Elastic fibers in the internal and middle coats of the vein are destroyed (the fibers are not visualized). Fragmentation of elastic fibers in the adventitia is seen. Staining on elastic fibers with «Elastic Stain Kit», × 50.

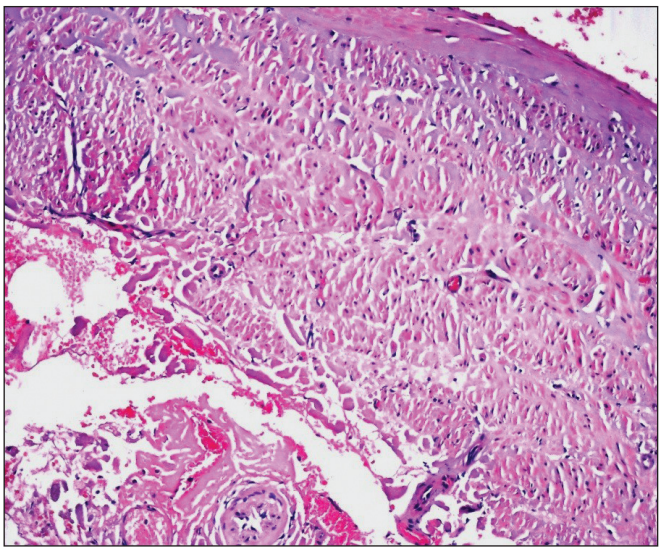


Fig. 6. The soft tissues, adjacent to the vessels, and trophic structures (vasa vasorum, nerve trunks) are preserved. Staining with haematoxylin and eosin. Magnification x100.

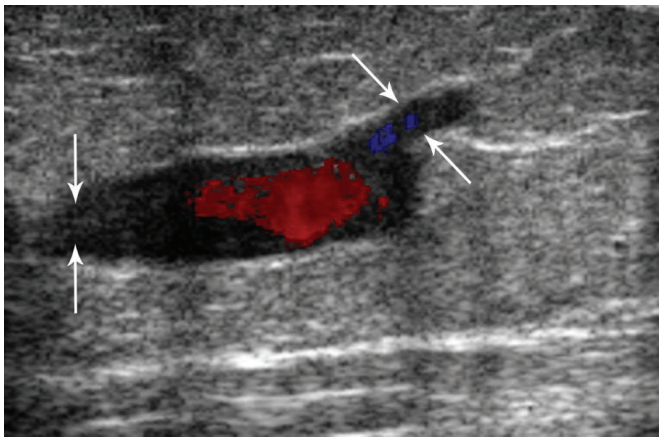


Fig. 7. CFM mode. A partially recanalized GSV segment after the influence of EVEW 3-6 months before; the blood flow up to 3 cm long, hyperechogenic thrombus remnants (↓↑), the functioning flow connected with the recanalized segment (↓↑). The longitudinal scan.

parietal formations, which protruded into the vein lumen, and the recanalized area was visualized in the centre of the vessel (fig. 9).

DISCUSSION

The morphological study of extracted veins in AAT of GSV was carried out after EVEW in the manual mode with a 15-second exposure. Macroscopically, the lumen of veins, sent for the examination, contained thrombotic masses. The venous veins were deformed and unevenly thinned, in some areas the vein lumen was varicose dilated. It should be noted that the influence of EVEW was spread both on the thrombotic masses, which at the moment of the current effect were in the lumen of examined veins, and on the vascular wall. It was macroscopically revealed that the influence of EVEW on the venous wall caused its marked

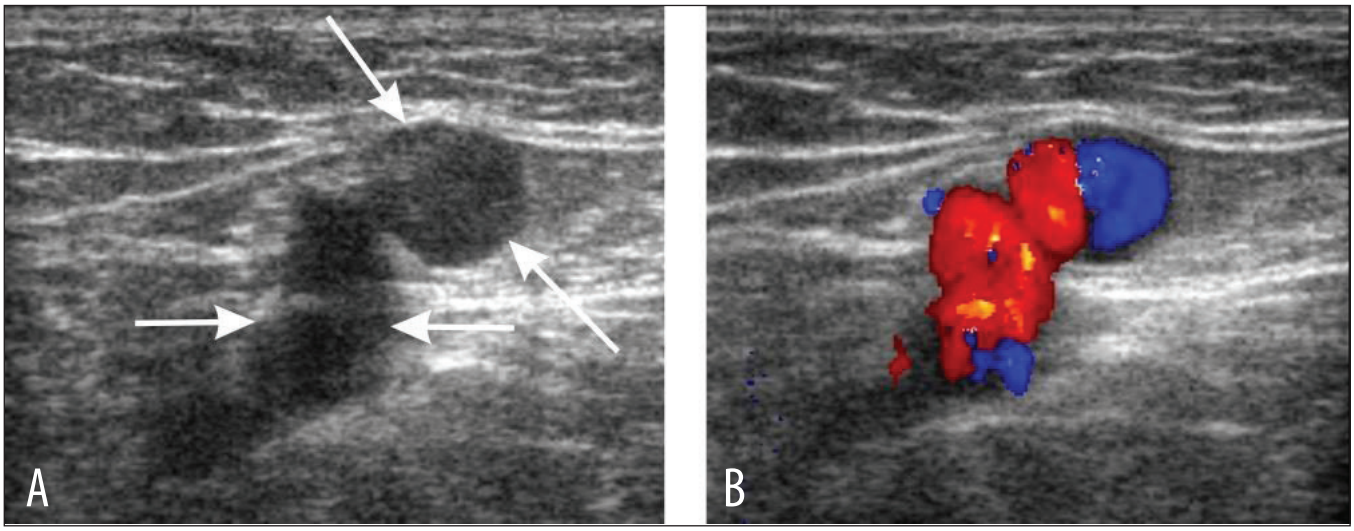


Fig. 8. A. B mode. GSV after 12 months following the influence of EVEW (↓↑), connection with Hunter's perforator (↓↑). B. CDM mode. Reflux in Hunter's perforator connected with the recanalized GSV segment. The transverse scan.

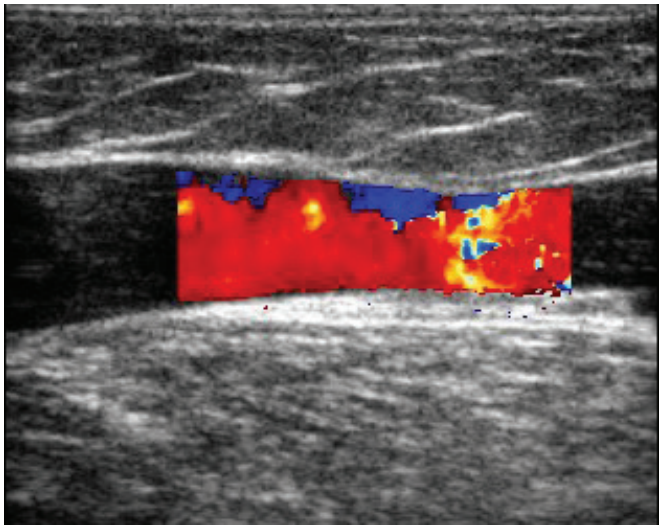


Fig. 9. CDM mode. GSV after 12 months following the influence of EVEW. The recanalized area is in the centre of the vessel (complete recanalization). The longitudinal scan.

reflex spasm that further resulted in the desired effect of occlusion of the vascular lumen: a more close contact of the thrombotic masses with the venous wall endothelium was produced [7, 15, 18]. The above macroscopic changes had their morphological basis in the form of homogenization of the thrombotic masses and their coagulation with the endothelium. Consequently, a single homogenous complex «thrombus-wall of the vein» formed, it causing occlusion of the vascular lumen and being clinically manifested with discontinuation of blood flow. Also, specific Weigert's staining morphologically showed that the influence of EVEW in the manual mode with a 15-second exposure spread on all the layers of the venous wall – internal, middle and external – and was accompanied with destruction of the elastic carcass of the wall, thereby creating conditions for deformation of the venous wall in the outside-inside direction with a reduction in the vascular diameter. Specific van Gieson's picrofuchsin staining morphologically proved that collagen and smooth muscular fibres underwent dys-

trophic changes in the form of dissection, fragmentation and coagulation. Consequently, under the influence of EVEW with a 15-second exposure the venous wall lost its fibrous carcass and, together with a thrombus, formed a single homogenous complex. Study of soft tissues in the adventitia is worth of particular attention, since integrity of the trophic structures (vasa vasorum, nerve trunks, lymph vessels), which are concentrated in the adventitia, has a direct relationship with the development and severity of complications after endovenous interventions [15]. Hence, it was morphologically proved that during the influence of EVEW in the above mode the trophic structures and paravasal fat remained intact.

Consequently, the desired effect of occlusion in EVEW is possible owing to a series combination of the following mechanisms: reflex spasm of the vascular wall, coagulation of thrombotic masses with the endothelium, destruction of the elastic carcass of the venous wall.

Separately, it should be noted that the morphological picture of changes in the venous wall under the influence of EVEW in the manual mode with a 15-second exposure, which we have now described, is close by its character and expression of changes to the morphological picture observed after influence on the venous wall by the technique of radiofrequency ablation [20].

According to ultrasonic studies, the period of follow-up until 12 months revealed 94.24% of cases with a hyperechogenic band in the projection of GSV, thereby demonstrating achievement of the complete obliteration of the vein.

At the same time, analysis of ultrasonic studies during the first days after the influence of EVEW revealed areas of parietal blood flow in the welded GSV in 5.76% of cases. The above areas were localized in the places of confluence of the functioning GSV flow and perforator veins on the thigh and, apparently, caused by arrival of blood by them with a resultant destruction of a still unorganized thrombus in those areas. During a remote period of follow-up from 3 to 6 months those changes in 3.84% of cases could develop some partial and haemodynamically insignificant recanalization.

In a more remote period of follow-up from 9 to 12 months the above areas of blood flow in 1.92% of cases could result in the complete and haemodynamically significant recanalization of the welded GSV.

Ultrasonic changes in a thrombosed vein after the influence of EVEW underwent changes of different character, from anechogenic to hypoechogenic and echogenic, therewith reflecting the processes of occlusion, obliteration and complete ablation of the vessel, as it is confirmed by literature data in obliteration with other thermal techniques of ablation [6].

CONCLUSIONS

1. High-frequency endovenous electrical welding of a thrombosed great saphenous vein in acute ascending thrombophlebitis causes total coagulation of thrombotic masses and homogenization of all layers in the venous wall, making it possible to achieve the total obliteration of the welded vein in 94.24% of cases.
2. The presence of the functioning flow and perforator veins after high-frequency endovenous electrical welding of a thrombosed great saphenous vein during a remote period causes a partial recanalization in 3.84% of cases and the complete one in 1.92% of cases.

REFERENCES

1. Marinsky GS, Chernets AV, Tkachenko VA, Grabovsky DA, Podpryatov CE, Lopatkina EG, et al. Stendovye issledovanija vysokochastotnoj jelektrosvarki biologicheskih tkanej [The poster researches of high-frequency electric welding of biological tissues]. Automatic welding. 2016;12(759):41-45. (Ru)
2. Savoluk SI, Khodos VA, Gerashchenko RA, Gorbovets VS. Pershij dosvid vikoristannja endovenoznogo elektrozvarjuvannja v kompleksnomu hirurgichnomu likuvanni gostrogo vishidnogo tromboflebitu velikoï pidshkirnoï veni [The first experience of using endovascular electric welding in the complex surgical treatment of acute ascending thrombophlebitis of a large subcutaneous vein]. Surgery of Ukraine. 2018;3(67):63-67. DOI: http: //doi.org/10.3978/SU2018-3-63 ISSN 1818-5398. (Ua)
3. He G, Zheng C, Yu MA, Zhang H. Comparison of ultrasound-guided endovenous laser ablation and radiofrequency for the varicose veins treatment: An updated meta-analysis. Int J Surg. 2017;39:267-275. doi: 10.1016/j.ijsu.2017.01.080.
4. van der Velden SK, Biemans AA, De Maeseneer MG, Kockaert MA, Cuypers PW, Hollestein LM, et al. Five-year results of a randomized clinical trial of conventional surgery, endovenous laser ablation and ultrasound-guided foam sclerotherapy in patients with great saphenous varicose veins. Br J Surg. 2015;102(10):1184-1194.
5. Burihan MC. Endovenous ablation (radiofrequency and laser) and foam sclerotherapy versus conventional surgery for great saphenous vein varices. Sao Paulo Med J. 2014;132(1):69. doi: 10.1590/1516-3180.20141321T2.
6. Ivanova ON, Zelnichenko AT, Kunkin DD, Perekrest VV, Todorenko VA. Opyt primenenija VC-elektrosvarochnogo apparata EK-300M1 v hirurgii [Experience in the application of an electromotive waxing apparatus EK-300M1 in surgery]. Automatic welding. 2012;11:58-60. (Ru)
7. Bilyanskyi LS, Zakharash MP, Zakharash YM, Abu Shamsiya RN, Dubenko EM. Experience of application of the electric welding method for biological tissues in urgent and elective surgery. Klinichna khirurhiia. 2017;2:5-6.
8. Kirienko AI, Panchenko EP, Andriyashkin VV. Venoznyj tromboz v praktike terapevta i hirurga [Venous thrombosis in the practice of a therapist and surgeon]. – M.: Planida; 2012. 336 p. (Ru)
9. Paton BE, Krivtsun IV, Marinsky GS, Khudetskiy UY, Lankin YuN, Chernets AV. Svarka, rezka i termicheskaja obrabotka mjagkih tkanej [Welding, cutting and heat treatment of soft tissue]. Automatic welding. 2013;10-11:135-146. (Ru)
10. Rosai J, editor. Rosai and Ackerman's Surgical Pathology. Seven edition. Elsevier Inc. 2011;l1(2, 3). p.25-95.
11. Paton BE. Jelektricheskaja svarka mjagkih tkanej v hirurgii [Electric welding of soft tissue in surgery]. Automatic welding. 2004;9:7-11. (Ru)
12. Podpryatov SS, Marinsky GS, Umanets OI. Elektrozvaryuvannya i nestandartni sytuatsiyi v proktolohiyi [The electric-welding technology and non-standard situations in proctology]. Hospital Surgery. 2014;1:89-90. DOI: https://doi.org/10.11603/1681-2778.2014.1.4382. (Ua)

13. Babiy AM, Shevchenko BF, Ratchik VM, Kunkin DD. Experience of using domestic high-frequency electric welding technology in surgical treatment of patients with abdominal pathology. Gastroenterology. 2014;2(52):61-68.
14. Nychytaylo MYu, Hutsuliak AI, Bulyk II, DybenkoIV. Vykorystannja VCh-elektrozvarjuvannja pry laparoskopichnij holecystektomii z prychyny gostrogo holecystytu, uskladnenogo paravezykalnym infiltratom [Using high-frequency electric welding method during laparoscopic cholecystectomy by reason of acute cholecystitis complicated by paravesical infiltrate]. Hospital surgery. Journal named by L.Ya. Kovalchuk. 2016;4:27-29. (Ua)
15. Savoluk SI, Horbovets VS, Gvozdiak MM, Kunkin DD, Krestianov MYu, Gerashchenko RA. Teoretychni, eksperymentalni ta klinichni aspekty zastosuvannja endovenoznogo elektrozvarjuvannja u likuvanni varykoznoi hvoroby [Theoretical, experimental and clinical aspects of endovenous electric welding in varicose vein treatment]. Endovascular neuroradiology. 2017;1(19):49-65. (Ua)
16. Scott G, Mahdi AJ, Alikhan R. Superficial vein thrombosis: a current approach to management. Br. J. Haematol. 2015;168(5):639-645.
17. Savoliuk SI, Balatskiy RO. Pojednannja laparoskopichnyh ta elektrozvarjuvalnyh tehnologij u likuvanni hvoryh z gostrym apendycytom jak faktor profilaktyky intraabdominalnyh uskladnen [Combination of laparoscopic and electric welding technologies in treatment of patients with acute appendicitis as a preventive factor for intraabdominal complications]. Hospital surgery. Journal named by L.Ya. Kovalchuk. 2016;3:89-93. (Ua)
18. Dyadyk OO, Savolyuk SI, Gerashchenko RA, Khodos VA, Kozlova KS. Morfologicheskoe obosnovanie vybora optimal'nyh parametrov jendovenoznogo jelektrosvarivanija pri lechenii voshodjashhego tromboflebita bol'shoj podkozhnoj veny [Morphological substance of the selection of optimal parameters of endovenous electric welding for treatment of ascending thrombophlebitis of great saphenous vein]. Art of medicine. 2018;3(7):49-53. (Ru)
19. Savelieva VS, Kirienko AI, editors. Vascular surgery. National leadership. M.: GEOTAR-Media; 2014. 464 p. (Ru)
20. Gudz IM. Strategija likuvannja gostrogo tromboflebitu nyzhnih kincivok [Strategy of treatment of acute thrombophlebitis of the lower extremities]. Clinical phlebology. 2009;1:150-151. (Ua)

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Roman A. Gerashchenko
Shupyk National Medical Academy of Postgraduate Education
str. Salyutnaya 19, apt. 19, 04111, Kyiv, Ukraine
tel: +380971090930
e-mail: pathologinew@ukr.net

Received: 12.04.2019
Accepted: 11.07.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

PERFEKCJONIZM DEZADAPTACYJNY A UMIEJĘTNOŚCI AUTENTYCZNEGO PRZYWÓDZTWA U STUDENTÓW RATOWNICTWA MEDYCZNEGO

MALADAPTIVE PERFECTIONISM AND AUTHENTIC LEADERSHIP SKILLS IN EMERGENCY MEDICINE STUDENTS

Mariusz Jaworski[1], Mariusz Panczyk[1], Aneta Binkowska[2], Piotr Leszczyński[3], Robert Gałązkowski[2], Joanna Gotlib[1]
[1]ZAKŁAD EDUKACJI I BADAŃ W NAUKACH O ZDROWIU, WYDZIAŁ NAUKI O ZDROWIU, WARSZAWSKI UNIWERSYTET MEDYCZNY, WARSZAWA, POLSKA
[2]ZAKŁAD RATOWNICTWA MEDYCZNEGO, WYDZIAŁ NAUKI O ZDROWIU, WARSZAWSKI UNIWERSYTET MEDYCZNY, WARSZAWA, POLSKA
[3]INSTYTUT NAUK O ZDROWIU, WYDZIAŁ PRZYRODNICZY, ZAKŁAD PIELĘGNIARSTWA I RATOWNICTWA MEDYCZNEGO, UNIWERSYTET PRZYRODNICZO-HUMANISTYCZNY W SIEDLCACH, SIEDLCE, POLSKA

STRESZCZENIE

Wstęp: Umiejętności przywódcze mogą mieć kluczowe znaczenie w ratownictwie medycznym. Brak jest jednak prac, które dokładniej analizują tę problematykę.

Cel pracy: Analiza nasilenia umiejętności przywódczych u studentów ratownictwa medycznego, a także sprawdzenie, czy perfekcjonizm dezadatacyjny jest zmienną osłabiającą korelację pomiędzy poczuciem własnej skuteczności a umiejętnościami przywódczymi.

Materiał i metody: Analizowana grupa stanowiła 75,76% (n=150; K=74, M=71) wszystkich studentów ratownictwa medycznego podejmujących kształcenie na Warszawskim Uniwersytecie Medycznym w 2018 roku. Średni wiek osób badanych wynosił 23 lata (SD=1,7). Osoby badane podzielono na dwie grupy: Grupa 0 – bez perfekcjnizmu dezadaptacyjnego (n=64) oraz grupa 1– z perfekcjonizmem dezadaptacyjnym (n=79). W badaniu przekrojowym zastosowano trzy wystandaryzowane narzędzia badawcze: Authentic Leadership Questionnaire, Almost Perfect Scale-Revised (APS-R), and General Self-Efficacy Scale (GSES). Przeprowadzono analizę ANCOVA.

Wyniki: Współczynniki kierunkowe prostych regresji dla obu porównywanych grup badanych były istotnie różne (interakcja zmiennych „Perfekcjonizm dezadaptacyjny * poczucie własnej skuteczności": F = 4,841; p = 0,029).Porównując skorygowane wartości średnich dla obu grup (0 *vs.* 1) można stwierdzić, że badani z grupy 0 mieli istotnie wyższe nasilenie umiejętności autentycznego przywództwa w porównaniu z grupą 1 (F = 4,432; p = 0,037).

Wnioski: Należy podjąć badania mające na celu określenie mechanizmów znoszenia pozytywnego związku miedzy poczuciem własnej skuteczności a umiejętnościami przywódczymi u studentów z wysokim perfekcjonizmem dezadaptacyjnym. Pozwoli to na opracowanie efektywnych programów wzmacniania umiejętności przywódczych studentów ratownictwa.

SŁOWA KLUCZOWE: umiejętności przywódcze, perfekcjonizm, poczucie własnej skuteczności, ratownictwo medyczne

ABSTRACT

Introduction: Leadership skills can be critical in emergency medicine. However, there are no works that analyze this issue in much more details.

The aim: To analyze the level of leadership skills in emergency medicine students, and also checking if despondency perfectionism is a variable that reduce the correlation between self-efficacy and leadership skills.

Materials and Methods: The analyzed group consisted of 75.76% of all emergency medicine students taking up education at the Medical University of Warsaw in 2018 (n = 150, W = 74, M = 71). The average age was 23 years (SD = 1.7). All students were divided into two groups: Group 0 - without maladaptive perfectionism (n = 64), and group 1- with maladaptive perfectionism (n = 79). In the cross-sectional study, three standardized research tools were used: Authentic Leadership Questionnaire, Almost Perfect Scale-Revised (APS-R), and General Self-Efficacy Scale (GSES). ANCOVA analysis was used.

Results: The linear regression coefficients for both comparison groups were significantly different (interaction of variables: "maladaptive perfectionism * self-efficacy": F = 4.841, p = .029). Comparing adjusted mean values for both groups (0 vs 1), it can be stated that students from group 0 had a significantly higher level of authentic leadership skills compared to group 1 (F = 4.432, p = .037).

Conclusions: Studies to determine the mechanisms of a positive relationship between the self-efficacy and leadership skills in emergency medicine students with high maladaptive perfectionism are required. This will allow the development of effective programs to strengthen the leadership skills of these students.

KEY WORDS: leadership, self-efficacy, perfectionism, emergency medicine

Wiad Lek 2019, 72, 8, 1453-1459

WSTĘP

Umiejętności przywódcze są szeroko dyskutowane w piśmiennictwie dotyczącym zawodów wymagających współpracy i zarządzania pracą w grupie [1–4]. Aktualnie istnieje wiele typów przywództwa [5]. Jedną z ważnych umiejętności przywódczych są umiejętności autentycznego przywództwa [2–4], które akcentują zdolność prawdziwego budowania relacji, motywowania innych do rozwoju, dostrzegania ich mocnych stron i ich wzmacniania, a także umiejętność adekwatnej oceny sytuacji. Ważną rolę w autentycznym przywództwie pełni nie tylko wiarygodność podejmowanych przez lidera zadań, ale także jego etyczna postawa. Lider poprzez swoje działania staje się etycznym wzorem do naśladowania [2, 4, 6].

W kontekście autentycznego przywództwa najwięcej uwagi poświęca się pielęgniarkom [7, 8] lub lekarzom [9]. Brakuje natomiast prac, które by analizowały ten typ umiejętności u studentów ratownictwa medycznego lub aktywnych zawodowo ratowników medycznych. Warto jednak zaznaczyć, że istnieją pojedyncze prace dotyczących możliwości wykorzystania umiejętności przywódczych na oddziałach ratunkowych, jednakże w dużej mierze skupiają się one bardziej na lekarzach niż ratownikach medycznych [9, 10].

Specyfika zawodu ratownika polega nie tylko na podejmowaniu medycznych czynności ratunkowych, ale także innych czynności związanych między innymi z zabezpieczeniem osób znajdujących się na miejscu zdarzenia, zapobieganiu zwiększeniu liczby ofiar i degradacji środowiska. Dodatkowo, ratownik medyczny bierze udział w zarządzaniu zespołem, a także współpracuje z zespołami ratownictwa medycznego w ramach ustawy o Państwowym Ratownictwie Medycznym. Należy także podkreślić, że ratownik medyczny może być również instruktorem udzielania pierwszej pomocy, kwalifikowanej pierwszej pomocy i medycznych czynności ratunkowych [11]. Takie zadania ratowników medycznych wymagają od nich nie tylko umiejętności szybkiego podejmowania decyzji, ale również efektywnego zarządzania i współdziałania w grupie. Umiejętności przywódczego mogą mieć więc istotne znaczenie w zwiększaniu efektywności pracy i współpracy w zespole medycznym. W piśmiennictwie podkreśla się, że mogą być one pomocne nie tylko podczas szybkiego podejmowania adekwatnych i świadomych decyzji, ale także w kontekście szybkiej adaptacji do nowych szybko zmieniających się warunków [9]. Takie umiejętności połączone z ciągłą samooceną – diagnozą swoich mocnych i słabych stron – mogą zapewnić optymalną i efektywną opiekę medyczną nie tylko na oddziałach ratunkowych [9, 10]. W kontekście pracy na oddziale ratunkowym zwraca się uwagę nie tylko na podstawowe umiejętności przywódcze, ale także na takie umiejętności, które mogą mieć istotne znaczenie na tego typu oddziałach. Mowa wówczas o tzw. umiejętnościach przywódczych w nagłych sytuacjach medycznych (*Emergency applicable leadership skills*) [9]. Wśród tych umiejętności podkreśla się zdolność do szybkiego analizowania złożonych sytuacji, w których miała miejsce nagła sytuacja medyczna [12] oraz szybkie podejmowanie decyzji [13]. Taka szybka analiza otoczenia i wychwycenie istotnych informacji z całego natłoku danych może być kluczowym punktem, w którym ratownik wykorzystuje swoje umiejętności przywódcze. Wstępna ocena i wynikające z niej czynności wykonywane przez ratownika będą determinować efektywność jego działań [12]. Decyzje podejmowane przez ratowników wymagają bazowania na wiedzy oraz doświadczeniu klinicznemu. Jest to szczególnie trudne w sytuacji presji czasu i dylematów etycznych [9]. Uważa się, że wszyscy pracownicy medyczni muszą być przygotowani do stawienia czoła licznym problemom natury etycznej, aby móc podejmować adekwatne decyzje i wynikające z nich działania [13]. Jest to także ważny element umiejętności autentycznego przywództwa [2, 4, 6] nie tylko w warunkach szpitalnych, ale także przedszpitalnych.

W kontekście umiejętności przywódczych duże znaczenie mogą mieć cechy osobowości. Takimi cechami mogą poczucie własnej skuteczności [14] oraz perfekcjonizm [15]. Konstrukt poczucia własnej skuteczności jest szeroko dyskutowany w literaturze przedmiotu jako zmienna warunkująca podejmowanie różnych zachowań oraz określa prawdopodobieństwo realizacji tych działań i ich powielanie [16]. Związek między poczuciem własnej skuteczności a umiejętnościami przywódczymi jest szeroko dyskutowany [7, 17]. Brak jest natomiast prac analizujących tę zależność wśród studentów ratownictwa medycznego. Podobna sytuacja jest w przypadku perfekcjonizmu. Nie jest znana zależność między perfekcjonizmem a umiejętnościami przywódczymi studentów ratownictwa medycznego. W przypadku perfekcjonizmu należy podkreślić złożoność tej cechy. Perfekcjonizm ma dwa podwymiary. Pierwszy z nich może utrudniać prawidłowe funkcjonowanie jednostki. Na przykład osoba może zbytnio skupiać się na szczegółach podejmowanej aktywności, a to całkowicie paraliżuje jej realizację. Mowa wówczas o perfekcjonizmie dezadaptacyjnym. Drugi wymiar – perfekcjonizm adaptacyjny – ma pomagać rzetelnie i skrupulatnie wykonywać podjęte działania [15]. Bazując na danych literaturowych dotyczących innych grup zawodowych [7], można założyć, że poczucie własnej skuteczności wykazuje dodatni związek z umiejętnościami przywódczymi także u studentów ratownictwa medycznego. Natomiast nie jest znana rola perfekcjonizmu, zwłaszcza dezadaptacyjnego, w tej relacji. Na podstawie danych teoretycznych można założyć, że wpływ ten będzie negatywny [15, 18–20]. Wymaga to jednak weryfikacji empirycznej.

CEL PRACY

Celem pracy była analiza nasilenie umiejętności przywódczych, a także ich komponentów, u studentów ratownictwa medycznego. Kluczowe było także sprawdzenie, czy perfekcjonizm dezadatacyjny jest zmienną osłabiającą korelację pomiędzy poczuciem własnej skuteczności a umiejętnościami przywódczymi.

MATERIAŁ I METODY

MATERIAŁ

Badaniami objęto wszystkich studentów ratownictwa medycznego na Warszawskim Uniwersytecie Medycznym. W badaniu brali udział studenci studiów licencjackich. W roku akademickim 2017/2018 na kierunku ratownictwo medyczne studiowało 198 studentów, z czego na pierwszym roku było 86 studentów, na drugim – 51 studentów, a na trzecim – 61. Jednakże ostatecznie w badaniu wzięło udział 150 studentów. Analizowana grupa stanowiła

75,76% wszystkich studentów ratownictwa medycznego podejmujących kształcenie na Warszawskim Uniwersytecie Medycznym w 2018 roku. Wskaźnik zwrotu poprawnie wypełnionych ankiet wynosił 55,81% w przypadku studentów pierwszego roku, 88,24% w przypadku studentów drugiego roku oraz 85,25% w przypadku trzeciego roku.

Kryterium włączenia do badania obejmowały:

1. bycie studentem kierunku ratownictwo medyczne Warszawskiego Uniwersytetu Medycznego,
2. świadoma zgoda na udział w badaniu.

Kryteria wyłączenia obejmowały:

1. bycie studentem innego kierunku niż ratownictwo medyczne Warszawskiego Uniwersytetu Medycznego,
2. brak świadomej zgody na udział w badaniu.

W badanej grupie było 49,3% (n=74) kobiet oraz 47,3% (n=71) mężczyzn. W przypadku 3,3% (n=5) studenci nie podali swojej płci. Wśród studentów studiów licencjackich 48 studentów było z pierwszego roku, 45 z drugiego roku oraz 52 z trzeciego roku. W przypadku 3,3% (n=5) studentów nie wskazało, na którym roku studiów byli podczas prowadzenia tych badań. Średni wiek osób badanych wynosił 23 lata (SD=1,7). Najmłodszy student ratownictwa medycznego biorący udział w badaniu miał 20 lat, a najstarszy – 36.

PROCEDURA ZBIERANIA DANYCH

Przed przystąpieniem do badania uzyskano niezbędne zgody na przeprowadzenie badań wśród studentów ratownictwa medycznego Warszawskiego Uniwersytetu Medycznego. Następnie nawiązano kontakt z osobą odpowiedzialną za dydaktykę na kierunku ratownictwo medyczne (koordynator ratownictwa medycznego) w celu umożliwienia przeprowadzenia badań ankietowych metodą audytoryjną. Koordynator czuwał nad poprawnością przeprowadzenia badań oraz wchodził w skład zespołu badawczego. Został przeszkolony pod względem nadzorowania zbierania danych. Szkolenie przeprowadził psycholog. Było to związane z faktem, że treści, które zawarte były w narzędziu badawczym dotyczyły sfery funkcjonowania psychologicznego. W związku z tym ryzyko potencjalnego wpływu osób zewnętrznych na udzielania odpowiedzi przez respondentów należało zminimalizować. Studenci wypełnili ankietę na początku zajęć i w obecności koordynatora. Podczas zbierania danych nie były obecne osoby trzecie. Studenci zostali poinformowani o celu badań, a także o możliwości odmowy udziału w badaniu. Koordynator badań zapewnił studentów o całkowitej anonimowości. Średni czas zbierania danych wyniósł 10 minut. Badania przeprowadzono w trzech etapach, które podzielone zostały ze względu na rok studiów. Etap pierwszy stanowiły studenci pierwszego roku, drugi etap – studenci drugiego roku, natomiast trzeci etap – studenci trzeciego roku. Prowadzono rejestr studentów, którzy odmówili udziału w badaniu. Jednakże ograniczono się do odnotowania liczby studentów, którzy odmówili udziału w badaniu, ze względu na anonimowość badań nie była możliwa bardziej szczegółowa analiza tej grupy.

NARZĘDZIA BADAWCZE

W pracy wykorzystano trzy wystandaryzowane narzędzia badawcze:

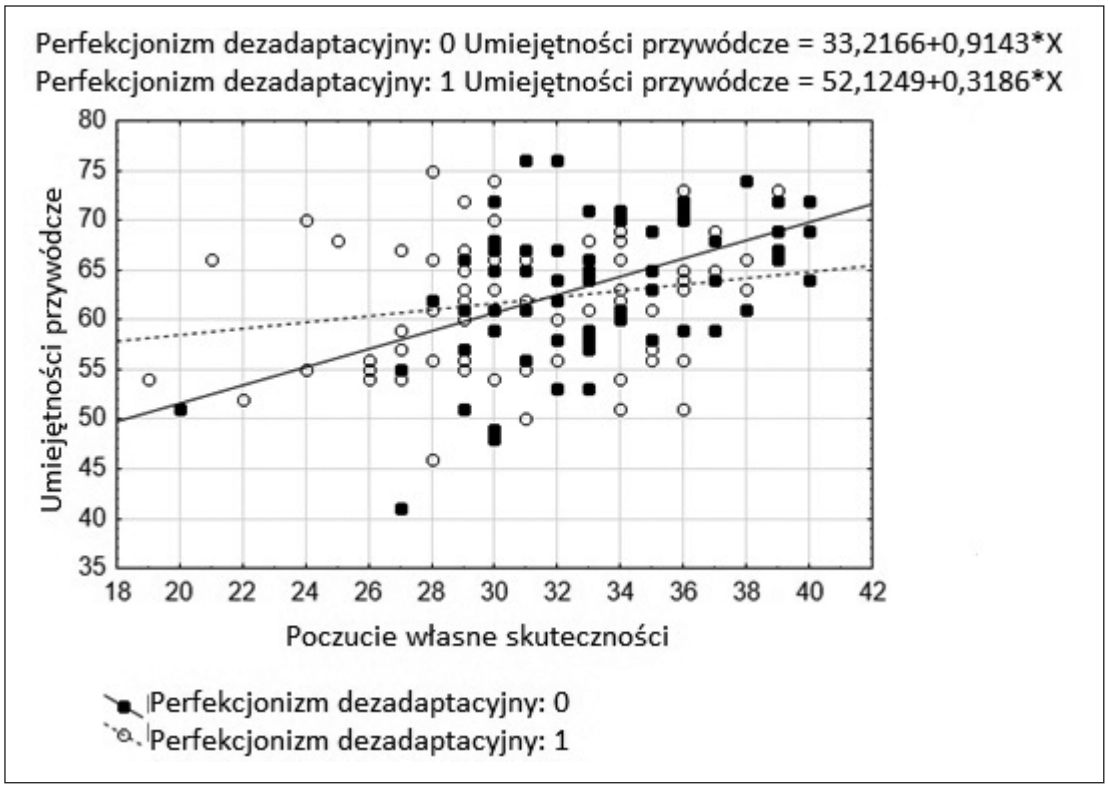
1. A*uthentic Leadership Questionnaire* (ALQ) został opracowany przez Avolio i wsp. ALQ służy do pomiaru podstawowych wymiarów autentycznego przywództwa. Składa się z jednej głównej skali, która określa ogólny poziom umiejętności autentycznego przywództwa oraz czterech podskal: Samoświadomość, Transparentność, Otwartość na informację zwrotną oraz Etyczność/Moralność. Uzyskano zgodę autorów na użycie w badaniach polskiej wersji ALQ. Skala składa się z 16 twierdzeń. Respondent opowiada na każde pytanie, korzystając z 5-punktowej skali (1 – Wcale 5 – Zawsze) [4];
2. *Almost Perfect Scale-Revised* (APS-R) została opracowana przez Slaney'a i wsp. APS-R składa się z 23 twierdzeń, które tworzą trzy podskale: Wysokie Standardy, Porządek oraz Rozbieżność. Respondenci odpowiadają na poszczególne pytania, korzystając z 7-punkotwej skali (1 – Zdecydowanie się nie zgadzam; 7 – Zdecydowanie się zgadzam). Autorzy skali APS-R podkreślają, że podskale Standardy oraz Rozbieżności wchodzą w skład perfekcjonizmu. Wysoki wynik na podskali standardy oznacza wysokie oczekiwania, które wypływają na wysoką potrzebę bycia doskonałym [15]. Te dwie podskale definiują perfekcjonizm, bez względu na jego typ. Wysoki wynik na skali rozbieżności połączony z wysokim wynikiem na skali standardy świadczy o dezadaptacyjnym perfekcjonizmie. Ten typ perfekcjonizmu zasadniczo oznacza mniejszą elastyczność w sytuacji frustracji i niezdolność do osiągnięcia celów lub po prostu ciągłe powtarzanie sobie, że „nie jestem wystarczająco dobry/a" [15, 21];
3. *General Self-Efficacy Scale* (GSES) została opracowana przez Schwarzera i Jerusalem. GSES jest skalą samopisu do pomiaru poczucia własnej skuteczności. Składa się z 10 twierdzeń. Respondent opowiada na każde pytanie, korzystając z 4-punktowej skali (nie, raczej nie, raczej tak, tak). Wynik ogólny jest sumą wszystkich punktów. Im wyższy wynik, tym wyższe nasilenie poczucia własnej skuteczności. Wskaźnik alfa Cronbacha dla GSES wynosi od 0,76 do 0,90 [14]. W prezentowanym badaniu zastosowano polską wersję skali GSES [16].

ZGODA KOMISJI BIOETYCZNEJ

Autorzy niniejszej pracy uzyskali opinię Komisji Bioetycznej Warszawskiego Uniwersytetu Medycznego o braku konieczności uzyskiwania zgody na wykonanie badań w zakresie celu, którego dotyczy niniejsza praca [22].

ANALIZA STATYSTYCZNA

Aby udzielić odpowiedzi na jedno z głównych pytań badawczych: Czy perfekcjonizm dezadatacyjny jest zmienną osłabiającą korelację pomiędzy poczuciem własnej skuteczności a umiejętnościami przywódczymi? – zastosowano analizę kowariancji (ANCOVA). Jednym z istotnych założeń metody



Ryc. 1. Zależność między poczuciem własnej skuteczności a umiejętnościami autentycznego przywództwa w odniesieniu do perfekcjonizmu dezadaptacyjnego.

ANCOVA jest ocena jednorodności współczynników regresji dla wszystkich poziomów zmiennej grupującej [23]. W tym przypadku dotyczy to stwierdzenia, czy linia regresji obliczona dla badanych, którzy zostali zdiagnozowani jako posiadający perfekcjonizm dezadaptacyjny, jest równoległa z linią regresji dla grupy badanych nieposiadających tego typu perfekcjonizmu (dwa poziomy zmiennej grupującej: 0/1). W tym celu wyliczono współczynniki kierunkowe prostych regresji dla obu porównywanych grup badanych. Opierając się na tych ustaleniach, jako właściwe podejście analityczne wybrano metodę ANCOVA w modelu różnych nachyleń. Analizy statystyczne wykonano przy zastosowaniu programu statystycznego STATISTICA 13 na licencji Warszawskiego Uniwersytetu Medycznego.

WYNIKI

OGÓLNA CHARAKTERYSTYKA UMIEJĘTNOŚCI AUTENTYCZNEGO PRZYWÓDZTWA I WYBRANYCH CECH OSOBOWOŚCI U STUDENTÓW RATOWNICTWA MEDYCZNEGO

Charakterystykę umiejętności przywódczych oraz ich podstawowych wymiarów u studentów ratownictwa medycznego zaprezentowano w tabeli I. Średni wyniki na skali GSES w normalizacyjnej polskiej próbie wynosił 27,32 (SD=5,31) i jest on niższy niż w badanej grupie studentów ratownictwa medycznego. Biorąc pod uwagę aktualne normy, analizowana grupę studentów ratownictwa została podzielona na niskie, przeciętne i wysokie nasilenie poczucia własnej skuteczności (Tab. I). W przypadku skali APS-R klucz uwzględnia tylko charakterystykę osób z wysokimi wynikami na poszczególnych skalach. W związku z tym studentów ratownictwa scharakteryzowano pod kątem wysokich wyników uzyskiwanych na skali APS-R, które jednocześnie świadczą o wysokim nasileniu perfekcjonizmu. Szczegółowe dane zaprezentowano w tabeli I. Perfekcjonizm dezadaptacyjny należy identyfikować u osób, które cechują się wysokimi wynikami na skali standardy oraz skali rozbieżności. W związku z tym w badanej grupie studentów ratownictwa medycznego u 79 studentów (52,7%) odnotowano perfekcjonizm dezadaptacyjny. Innymi słowy, co drugi student ratownictwa medycznego cechował się perfekcjonizmem dezadaptacyjnym, który może utrudniać prawidłowe funkcjonowanie i wykonywanie podjętych działań.

ANALIZA KOWARIANCJI

Ocena równości współczynników regresji dla dwóch poziomów zmiennej grupującej (posiadający / nieposiadający perfekcjonizm dezadaptacyjny) wykazała, że współczynniki kierunkowe prostych regresji dla obu porównywanych grup są istotnie różne (F = 4,841, P = 0,029). Graficzną prezentacje prostych regresji dla obu porównywanych grup badanych zaprezentowano na rycinie 1. Współczynniki kierunkowy dla grupy studentów bez perfekcjonizmu dezadaptacyjnego wynosił 0,91, natomiast dla studentów z tym rodzajem perfekcjonizmu współczynnik ten był równy 0,32.

Porównując skorygowane wartości średnich dla obu grup (0: brak perfekcjonizmu dezadaptacyjnego *vs.* 1: obecność

Tabela I. Charakterystyka studentów ratownictwa pod względem analizowanych zmiennych

	Min - max	Średnia	SD
Umiejętności autentycznego przywództwa			
Transparentność	11-24	19,21	2,576
Etyczność/moralność	4-20	16,20	2,632
Otwartość na informację zwrotną	3-15	11,71	2,227
Samoświadomość	6-20	15,01	2,795
Umiejętności autentycznego przywództwa (Wynik ogólny)	24-76	62,12	8,122
Wybrane cechy osobowości			
Poczucie własnej skuteczności (GSES)	19-40	31,79	4,256
Niskie nasilenie GSES (n=5; 3,3%) Przeciętne nasilenie GSES (n=76; 50,7%) Wysokie nasilenie GSES (n=69; 46%)			
Perfekcjonizm – skala Standardy	13-49	39,01	7,037
Wysokie wyniki na skali Standardy (n=140; 93,3%)			
Perfekcjonizm – skala Porządek	0-28	20,91	4,551
Wysokie wyniki na skali Inni (n=137; 91,3%)			
Perfekcjonizm – skala Rozbieżności	13-83	42,77	15,331
Wysokie wyniki na skali rozbieżności (n=80; 53,3%)			

SD- odchylenie standardowe

Tabela II. Wynik analizy kowariancji

Efekt	b	ß	95% Przedział ufności		t	p
			Dolna granica	Górna granica		
Wyraz wolny	52,125	-	-	-	9,410	0,000
Perfekcjonizm dezadaptacyjny (0)* Poczucie własnej skuteczności	0,914	2,215	1,200	3,230	4,314	0,000
Perfekcjonizm dezadaptacyjny (1)* Poczucie własnej skuteczności	0,319	0,722	-0,078	1,521	1,784	0,077
Perfekcjonizm dezadaptacyjny 0: brak 1: obecny	-18,908	-1,366	-2,649	-0,083	-2,105	0,037

b – niewystandaryzowany współczynnik regresji
ß – wystandaryzowany współczynnik regresji
p – poziom istotności statystycznej

perfekcjonizmu dezadaptacyjnego) stwierdzono, że badani z grupy 0 osiągali istotnie wyższy wynik na skali umiejętności autentycznego przywództwa w porównaniu z grupą 1 (63.52 vs 61.92; F = 4.432, P = 0.037). Z modelu ANCOVA wynika, że przewidywany średni wynik na skali umiejętności autentycznego przywództwa w grupie 0 był o blisko 1,60 wyższy niż w grupie 1. Szczegółowe zestawienie wyników ANCOVA różnych nachyleń dla obu grup przedstawiono w tabeli II.

DYSKUSJA

Prezentowane badanie sugeruje, że kształtowanie umiejętności przywódczych u studentów ratownictwa medycznego może być uzależnione nie tylko od nasilenia poczucia własnej skuteczności, które mocno akcentuje się w publikacjach [7, 17]. Kluczowe jest jednak uwzględnienie perfekcjonizmu i jego relacji z poczuciem własnej skuteczności. Wzajemny układ tych cech może mieć ważne znaczenie w znoszeniu korelacji pomiędzy poczuciem własnej skuteczności a umiejętnościami przywódczymi. Dotyczy to zwłaszcza perfekcjonizmu dezadaptacyjnego. Aby lepiej zrozumieć ten mechanizm, należy odwołać się do ogólnej definicji tej zmiennej psychologicznej. Omawiany typ perfekcjonizmu związany jest ze stawianiem sobie nierealnych celów [15], a brak możliwości ich osiągnięcia może stać się źródłem stresu i negatywnych stanów emocjonalnych [24]. Należy podkreślić, że studenci ratownictwo, podobnie jak studenci medycyny, narażeni są na wysokie nasilenie stresorów. Zbyt intensywny stres, a raczej brak efektywnego radzenia sobie z nim, może przyczyniać się do

częstszych problemów psychicznych i złego samopoczucia, a to wpływa na życie osobiste i zawodowe studentów. Takie nasilenie stresu może przekładać się także na słabsze wyniki w nauce, niechęć do nabywania nowych kompetencji, przerywanie studiów, a nawet nadużywania różnych substancji [24].

Według badań Kliajic i wsp. [19] perfekcjonizm wiąże się z częstszym wypaleniem akademickim (*Academic Burnout*). Studenci szybciej zniechęcają się do studiowania, mają obniżoną motywację do nauki i zdobywania nowych kompetencji, a także częściej mogą przerywać studia [19]. Warto podkreślić, że prac analizujących rolę perfekcjonizmu w wypaleniu akademickim jest niewiele i nadal brakuje mocnych dowodów, które wyjaśniłyby ten mechanizm. Uważa się, że ważną rolę w tym kontekście może pełnić negatywne myślenie (*Negative Thinking*) [20]. Negatywne myślenie związane jest z niepomyślnym myśleniem o przeszłych i / lub przyszłych sytuacjach, co prowadzi do negatywnych stanów emocjonalnych [25]. Macedo i wsp. [18] podkreślili, że powtarzające się negatywne myślenie pośredniczy w częstszym doświadczaniu negatywnych stanów psychicznych (lęk, depresja, wrogość, zmęczenie). Co więcej, może pośredniczyć w szybszym wypaleniu akademickim.

Negatywne myślenie towarzyszące perfekcjonizmowi dyspozycyjnemu może częściowo tłumaczyć uzyskane wyniki badań, w których wykazano, że w przypadku studentów z wysokim perfekcjonizmem dezadaptacyjnym odnotowano znoszenie związku między poczuciem własnej skuteczności a umiejętnościami przywódczymi. Warto podkreślić, że znoszenie tego związku może świadczy o negatywnej roli perfekcjonizmu dezadapatcyjnego na kształtowaniem umiejętności przywódczych w tej grupie studentów.

Analizując perfekcjonizm dezadaptacyjny w kontekście umiejętności przywódczych, należy poszukiwać współwystępowania innych czynników lub mechanizmów psychologicznych, które powodować mogą znoszenie dodatniej zależności między poczuciem własnej skuteczności a umiejętnościami przywódczymi. Być może ważne znaczenie mogą mieć stany emocjonalne, zwłaszcza umiejętność radzenia sobie z negatywnymi emocjami i negatywnym myśleniem. Holmes i wsp. [26] podkreślają, że indywidualne różnice w czynnikach takich jak: wyobraźnia, umiejętność radzenia sobie z emocjami oraz stresem mogą mieć w tym kontekście istotne znaczenie. W związku z tym w dalszych badaniach dotyczących roli perfekcjonizmu dezadaptacyjnego w kształtowaniu umiejętności przywódczych należy uwzględnić indywidualne różnice w aspekcie radzenia sobie i kontrolowania negatywnych emocji

Z przeprowadzonych badań wynika, że należy zwrócić uwagę na perfekcjonizm dezadaptacyjny u studentów ratownictwa. Wprowadzenie efektywnym metod radzenia sobie z wysokimi wymaganiami, zwłaszcza ich urealnianiem może być kluczowe w utrzymaniu zdrowia psychicznego studentów tego kierunku. W piśmiennictwie wiele uwagi poświecą się wzmacnianiu poczucia zadowolenia z życia, wzmacniania samoopieki (*self-care*) psychicznej i lepszemu funkcjonowaniu emocjonalnemu. A zatem rozwijania kompetencji psychospołecznych, które będą nie tylko warunkować prawidłowe zdrowie psychiczne i somatyczne studentów, ale także poziom ich zadowolenia z życia. To będzie przekładało się na efektywność nabywania specjalistycznych umiejętności w toku kształcenia oraz lepsze ich wykorzystanie w przyszłej praktyce zawodowej [24].

OGRANICZENIA BADANIA

Prezentowane badanie nie jest pozbawione ograniczeń. W związku z tym powinno być interpretowane z dużą ostrożnością. Po pierwsze, prezentowane badanie ma charakter badań przekrojowych, a to wyklucza jakiekolwiek wnioski dotyczące kierunku zmian w czasie. W związku z tym uzasadnione wydaje się podjęcie badań podłużnych, mogących pomóc rozwiązać ten problem. Prezentowane badanie należy potraktować jako doniesienie wstępne do zaprojektowania badań podłużnych, które pozwolą uwzględnić rolę perfekcjonizmu i poczucia własnej skuteczności w kontekście umiejętności przywódczych. Jest także wysoce prawdopodobne, że ten związek jest bardziej wyraźny w różnych punktach czasowych (np. okres sesji egzaminacyjnej). Dlatego też przyszłe doniesienia powinny dalej badać te czynniki. Po drugie, w badaniu zastosowano próbkę wygodną, co może ograniczać ogólność wyników do szerszej populacji studentów. Kolejnym ograniczeniem jest brak norm dotyczących nasilenia umiejętności przywódczych studentów ratownictwa oraz brak możliwości porównania uzyskanych wyników z innymi pracami. Potrzebna jest dalsza ocena psychometryczna skali oceny umiejętności przywódczych na różnych próbkach uniwersyteckich. Należy wziąć jednak pod uwagę, że liczba studentów ratownictwa jest znacznie mniejsza niż liczba studentów medycyny czy pielęgniarstwa. W prezentowanym badaniu nie uwzględniono żadnych dodatkowych mediatorów lub moderatorów, jak np. negatywne myślenie czy kontrola emocjonalna. Przyszłe badania powinny dotyczyć wpływu tych zmiennych jako potencjalnych mediatorów lub moderatorów.

MOCNE STRONY BADANIA

Mocną stroną badania jest grupa osób badanych (studentów ratownictwa medycznego). Badania przeprowadzono wśród wszystkich studentów tego kierunku na Warszawskim Uniwersytecie Medycznym, który posiadali status studenta w 2018 roku. Ankietę zwrotną otrzymało od 150 studentów, co stanowiło 75,76% wszystkich studentów WUM tego kierunku. Mocną stroną pracy jest uwzględnienie perfekcjonizmu w kontekście umiejętności przywódczych. W piśmiennictwie brakuje takich badań w odniesieniu do studentów ratownictwa medycznego. Kolejną mocną stroną prezentowanych badań jest podjęcie oceny umiejętności przywódczych u studentów ratownictwa medycznego. W piśmennictwie brak jest danych na ten temat, a uzyskane wyniki mogą rzucić nowe światło na programy nauczania na tym kierunku. Ważną obserwacją tego badania jest także podkreślenie potrzeby opracowania odpowiednich programów rozwoju umiejętności psychospołecznych dla studentów ratownictwa medycznego, a także wzmacniana ich zdrowia psychicznego.

WNIOSKI

Umiejętności przywódcze mogą pełnić ważną rolę w pracy zawodowej ratowników medycznych. Ich kształtowanie podczas studiów jest związane z poczuciem własnej skuteczności

oraz odpowiednim nasileniem perfekcjonizmu. Jednakże perfekcjonizm dezadapacyjny może znosić pozytywny związek między poczuciem własnej skuteczności a umiejętnościami przywódczymi. W związku z tym należy podjąć działania mające na celu poznanie mechanizmów potencjalnego wpływu perfekcjonizmu na umiejętności przywódcze. Poznanie tych mechanizmów pozwoli na opracowanie efektywnych programów wzmacniania umiejętności przywódczych studentów ratownictwa, a tym samym może pomóc przeciwdziałać wypaleniu akademickiemu tych studentów.

PODZIĘKOWANIA

Autorzy pragną podziękować Kamili Malesie za pomoc w digitalizacji danych z ankiet.

PISMIENNICTWO

1. Al-Moamary MS, Al-Kadri HM, Tamim HM. Authentic leadership in a health sciences university. Med Teach 2016;38 Suppl 1:S19-25.
2. Avolio BJ, Gardner WL: Authentic leadership development: Getting to the root of positive forms of leadership. Leadership Quart 2005;16(3):315-338.
3. Eigel KM, Kuhnert KW. Authentic development: Leadership development level and executive effectiveness. Authentic leadership theory and practice: origins, effects and development. Monographs in Leadership and Management; 2005;3:357-385.
4. Gardner WL, Avolio BJ, Walumbwa FO. Authentic leadership theory and practice: Origins, effects and development, vol. 3. Bingley: Emerald Group Publishing Limited, 2005.
5. Eagly AH, Johannesen-Schmidt MC, van Engen ML. Transformational, transactional, and laissez-faire leadership styles: a meta-analysis comparing women and men. Psychol Bullet 2003;129(4):569-591.
6. Walumbwa FO, Avolio BJ, Gardner WL, Wernsing TS, Peterson SJ. Authentic leadership: Development and validation of a theory-based measure. J Manag 2008;34(1):89-126.
7. Panczyk M, Jaworski M, Iwanow L, Cieslak I, Gotlib J. Psychometric properties of Authentic Leadership Self-Assessment Questionnaire in a population-based sample of Polish nurses. J Adv Nurs 2019;75(3):692-703.
8. Sharkey S, Lefebre N. Leadership Perspective: Bringing Nursing Back to the Future Through People-Powered Care. Nurs Leadersh (Tor Ont) 2017;30(1):11-22.
9. Hershkovich O, Gilad D, Zimlichman E, Kreiss Y. Effective medical leadership in times of emergency: a perspective. Disaster Milit Med 2016;2:4.
10. Waugh Jr WL, Streib G: Collaboration and leadership for effective emergency management. Publ Admin Rev 2006;66:131-140.
11. Fedorczuk W, Pawlas K. Ryzyko zawodowe w pracy ratownika medycznego. Hygeia 2011;46(4):437-441.
12. Hannah ST, Uhl-Bien M, Avolio BJ, Cavarretta FL. A framework for examining leadership in extreme contexts. Leadership Quar 2009;20(6):897-919.
13. Merin O, Miskin IN, Lin G, Wiser I, Kreiss Y. Triage in mass-casualty events: the Haitian experience. Prehosp Disaster Medi 2011;26(5):386-390.
14. Schwarzer R, Jerusalem M: The general self-efficacy scale (GSE). Anxiety, Stress, and Coping 2010;12(1):329-345.
15. Slaney RB, Rice KG, Mobley M, Trippi J, Ashby JS. The revised almost perfect scale. Measurement and evaluation in counseling and development 2001;34(3):130.
16. Juczyński Z: Narzędzia pomiaru w psychologii zdrowia. Przegl Psychol 1999;42(4):43-56.
17. Murphy SE, Johnson SK. Leadership and Leader Developmental Self-Efficacy: Their Role in Enhancing Leader Development Efforts. New Directions for Student Leadership 2016;2016(149):73-84.
18. Macedo A, Soares M, Amaral A, Nogueira V, Madeira N, Roque C, Marques M, Maia B, Bos S, Valente J: Repetitive negative thinking mediates the association between perfectionism and psychological distress. Personality and Individual Differences 2015;72:220-224.
19. Kljajic K, Gaudreau P, Franche V: An investigation of the 2× 2 model of perfectionism with burnout, engagement, self-regulation, and academic achievement. Learning and Individual Differences 2017;57:103-113.
20. Garratt-Reed D, Howell J, Hayes L, Boyes M. Is perfectionism associated with academic burnout through repetitive negative thinking? Peer J 2018;6:e5004.
21. Bhupathiraju SN, Hu FB. Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. Circ Res 2016;118(11):1723-1735.
22. Komisja Bioetyczna Warszawskiego Uniwersytetu Medycznego [http://komisja-bioetyczna.wum.edu.pl/content/szczeg%C3%B3%C5%82owe-informacje-oraz-wzory-dokument%C3%B3w]
23. Stanisz A: Przystępny kurs statystyki: z zastosowaniem STATISTICA PL na przykładach z medycyny. Statystyki podstawowe: StatSoft; 2006.
24. Slonim J, Kienhuis M, Di Benedetto M, Reece J. The relationships among self-care, dispositional mindfulness, and psychological distress in medical students. Medical Education online 2015;20:27924.
25. McLaughlin KA, Nolen-Hoeksema S: Rumination as a transdiagnostic factor in depression and anxiety. Behaviour research and therapy 2011;49(3):186-193.
26. Holmes EA, Geddes JR, Colom F, Goodwin GM: Mental imagery as an emotional amplifier: Application to bipolar disorder. Behaviour Research and Therapy 2008;46(12):1251-1258.

Źródło finansowania

Pracę wykonano w ramach projektu badawczego zatytułowanego: Umiejętności przywódcze a wybrane cechy osobowości w kontekście pracy zawodowej studentów Wydziału Nauki o Zdrowiu (NZD/PM1/18/18), realizowanego w latach od 2018 do 2019, finansowanego ze środków dotacji statutowej uzyskanej przez Wydział Nauki o Zdrowiu Warszawskiego Uniwersytetu Medycznego.

Konflikt interesów:

Autorzy deklarują brak konfliktu interesów.

AUTOR KORESPONDUJĄCY

Mariusz Panczyk
Zakład Edukacji i Badań w Naukach o Zdrowiu,
Wydział Nauki o Zdrowiu,
Warszawski Uniwersytet Medyczny,
ul. Zwirki i Wigury 61, 02-091 Warszawa
tel +48 22 572 0490, fax +48 22 572 0491,
e-mail: mariusz.panczyk@wum.edu.pl

Nadesłano: 27.04.2019
Zaakceptowano: 14.06.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

ANALYSIS OF PSYCHO-EMOTIONAL STATE AND THE SEVERITY OF ASTHENIC SYNDROME IN CHILDREN WITH ACUTE LEUKEMIA AFTER THE FIRST STAGE OF CHEMOTHERAPY

Nataliia M. Adamchuk[1], Olena Y. Sorokina[2]
[1]UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY, POLTAVA, UKRAINE
[2]STATE INSTITUTION «DNIPROPETROVSC MEDICAL ACADEMY OF THE MINISTRY OF HEALTH OF UKRAINE», DNIPRO, UKRAINE

ABSTRACT

Introduction: Children and adolescents with hematological diseases are constantly in a state of prolonged psychological stress caused by hospitalization, debilitating treatment, lifestyle changes, which ultimately leads to a significant reduction in the quality of life. In this connection it is necessary to study the dynamics of mental and emotional condition of the child at different stages of chemotherapy and the timing of formation of asthenic syndrome.

The aim: Determine the presence of psycho-emotional disorders and the severity of asthenic syndrome in children with acute leukemia after the first stage of chemotherapy in order to further predict the development of this pathology and develop a package of solutions that minimize its manifestations.

Materials and methods: A survey of 36 children was conducted, of them 21 people aged 6–10 years old, 15 people aged 11–16 years old. There are 27 boys (75%) and 9 girls (25%). The survey was conducted on the 78th day of the ALL IC-BFM 2009 protocol, which corresponds to the end of the first phase of chemotherapy. In the survey did not participate children with the syndrome of multiple organ failure, as well as with the presence of background mental disorders. To assess the psycho-emotional state, a questionnaire was used: "state of health, activity, mood", adapted for children. For the study of asthenia was used questionnaire I. K. Schatz.

Results: The findings suggest that absolutely all children receiving chemotherapy at the end of the first phase have been violations of the psycho-emotional background. The index of state of health suffers the most, and to a lesser extent - activity. Indicators of state of health had lower values in the older group - from 11 to 16 years. Thus according to the data in the group of children aged 6 to 10 years being the average was 4.02 ± 0.09 ($p < 0.05$), in children age 11 - 16 years - 3.83 ± 0.11 ($p < 0.05$). The average activity substantially the same in groups and indicates a favorable condition. Mood indicators are also within the framework of a favorable state in both groups. Also during the investigation revealed that the general for all children is a symptom asthenic. According to the data, fatigue reaction (less than 7 points) was present only in 9 children (25%). The most numerous manifestations were moderate asthenia - 21 children (58%). Severe asthenia (13 - 18 points) was observed only in 6 children (17%).

Conclusions: This study will allow to develop a package of solutions for the prevention and minimization of asthenic syndrome in children with acute leukemia receiving chemotherapy.

KEY WORDS: children, acute leukemia, psycho-emotional state, asthenic syndrome

Wiad Lek 2019, 72, 8, 1460-1462

INTRODUCTION

The past decade has seen an increased incidence of leukemia in children [1]. Based on the analysis of databases WHO and the National Cancer Registry in Ukraine, analysis of the literature published from 1997 to 2018, we can conclude about the growth of cancer-incidence in children. According to WHO, by 2016 the percentage of cancers in children in the world was 4.7% of all cases of cancer pathology. [2]. Treatment of acute leukemia in children involves long-term combination therapy. The effectiveness of this treatment has been proven and leads to long-term remission in 80% of cases. [3]. However, apart from the positive effects of chemotherapy leads to a lot of negative consequences in the future reduce the patient's quality of life [4]. Also, children and adolescents with oncohematological diseases are constantly in a state of prolonged psychological stress caused by hospitalization, debilitating treatment, and lifestyle changes. A significant contribution to the development of asthenic component contributes and the presence of acute pain at different stages of the disease, often with pain chronicity [5, 6, 7, 8, 9, 10]. In the world supplied a lot of effort to deal with complications due to chemotherapy, improve the management of pain therapy. However, the determination of the presence of chronic pain syndrome, asthenic syndrome and psycho-emotional state disorders is usually carried out to achieve remission - that is, after the completion of the protocol treatment of leukemia [11,12]. And often, the rehabilitation of such children is quite difficult, requiring considerable effort from not only medical workers, but also psychologists [13]. To date, there are no clear data on changes in psychoemotional status at different stages of chemotherapy in children with acute leukemia, nor is the pattern established between changes in these indicators and correction of psychosomatic disorders caused by treatment of the underlying disease. In this regard, it is necessary to

Table I. Mean values of health indicators, activity, mood in children with acute leukemia in points.

Indicators	Children aged 6-10	Children aged 11 – 16
Health indicators	4,02±0,09	3,83±0,11
Activity	4,0±0,097	4,02±0,11
Mood	4,33±0,13	4,47±0,11

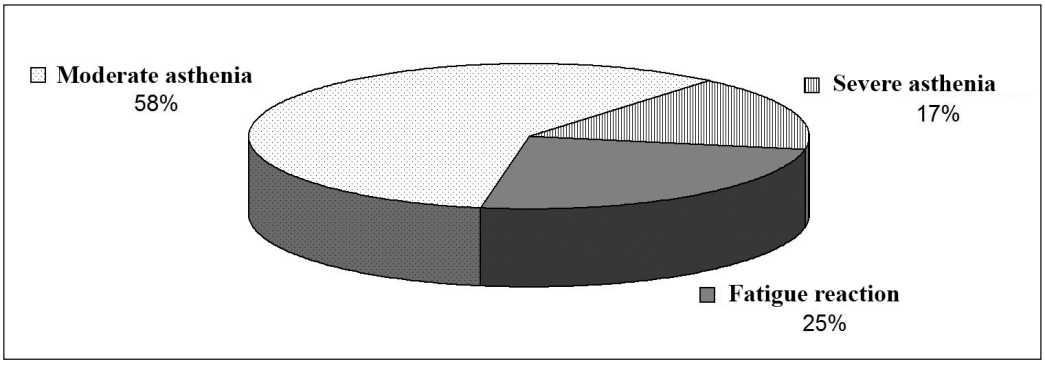


Figure I. Asthenic syndrome in children with acute leukemia on the 78th day of the chemotherapy protocol.

study the dynamics of the psycho-emotional state of the child and the timing of the formation of asthenic syndrome at different stages of chemotherapy. Since the timely correction of violations will improve not only the quality of life in the future, but also the response to the ongoing therapy of the underlying disease.

THE AIM

Determine the presence of psycho-emotional disorders, and severity of asthenic syndrome in children with acute leukemia after the first phase of chemotherapy for the subsequent prediction of this pathology and the development of a package of decisions that minimize its appearance.

MATERIALS AND METHODS

We observed 36 children aged 6 - 16 years of age with a diagnosis of "acute lymphoblastic leukemia", who were treated at the Child Oncology and Hematology Center of the city of Poltava (Ukraine). All parents agreed to the examination, and the design and methods of clinical studies were approved by the bioethical commission.

A survey of 36 children was conducted, of them 21 people aged 6–10 years old, 15 people aged 11–16 years old. There are 27 boys (75%) and 9 girls (25%). All children were treated for acute lymphoblastic leukemia according to the ALL IC-BFM 2009 protocol. The survey was conducted on day 78 of the protocol, which corresponds to the end of the first stage of chemotherapy. In the survey did not participate children with the syndrome of multiple organ failure, as well as with the presence of background mental disorders.

To assess the psycho-emotional state was used questionnaire "state of health, activity, mood," adapted to children's age. For the study of asthenia was used questionnaire I. K. Schatz.

Statistical processing carried out by conventional methods to the calculation of interest, the mean values and their errors using software Microsoft Excel 2007, STATA.

RESULTS AND DISCUSSION

The data obtained indicate that all children receiving chemotherapy, at the end of the first stage of treatment, there are violations of the psycho-emotional background (tab. I). When interpreting the results of a questionnaire assessment of each item (state of health, activity and mood), in excess of 4 points, according to the favorable condition of the test, less than 4 - an unfavorable condition. Normal evaluation condition are in the range of 5.0-5.5 points. In our study, we see that children suffer the most health indicator, to a lesser extent - the activity. Health indicators had lower values in the older group - from 11 to 16 years. So according to the data, in the group of children aged from 6 to 10 years old, the average well-being was 4.02 ± 0.09 ($p < 0.05$), in children aged 11–16 years - 3.83 ± 0.11 ($p < 0.05$). This difference in performance is due to a greater extent the features of the disease in different age groups. The average activity in the groups is almost the same and indicates a favorable condition. The mood indicators are also within the framework of a favorable condition in both groups, with a more pronounced positive value in the older group (Table I).

However, in the course of the survey did not reveal any child with at least one indicator of normal - higher than 5 points. Consequently, already at the end of the first stage of treatment, we have a violation of the psycho-emotional state of children of both age groups. Considering that the protocol treatment of acute leukemia involves several stages with a predominance of invasive diagnostic and therapeutic manipulations, the violations already existing in children will only worsen, ultimately leading to a catastrophic de-

cline in the quality of life, as evidenced by the data [13].

Also during the investigation revealed that the general for all children is a symptom asthenic. According to the data, the fatigue reaction (less than 7 points) was present in 9 children (25%). These children have prevailed physical fatigue, to a minor extent - mood lability. The most numerous manifestations were moderate asthenia - 21 children (58%) (picture I). It is dominated by complaints of sleep disturbance, appetite, physical fatigue and increased irritability. Severe asthenia (13 - 18 points) was observed only in 6 children (17%). The children of this group didn't make contact with the questionnaire; therefore, parents were involved in the survey. Children with severe asthenia had significant sleep disorders, mainly due to prolonged insomnia. Also present were pronounced negativism, aggression, mood lability with sharp drops in the emotional sphere, increased physical fatigue and a complete lack of appetite.

Thus, we can conclude that the adverse physical and stress factors that affect the child during the treatment of acute leukemia, after the first stage of treatment led to the formation patopsihologicheskih reactions. Analysis of the data obtained showed that in the total mass of respondents there were both children with minimal manifestations of disorders, and children with pronounced personality disorders. Considering that the ultimate goal of our study is the correction of anesthetic therapy in children with acute leukemia in order to minimize the chronic pain process and asthenia, the data obtained will help develop an optimal approach to improving the management of analgesia. The dynamics of well-being, activity and mood, as well as the severity of asthenic syndrome manifestations at the stages of treatment can reflect the effectiveness of anesthetic therapy along with the generally accepted scales of pain syndrome characteristics (visual analogue scale, face scale). In this connection, we will continue to study these parameters in children in the stages of chemotherapy in comparison to the treatment of pain.

CONCLUSIONS

This study will help develop a package of solutions for preventing or minimizing the development of asthenic syndrome in children with acute leukemia receiving chemotherapy.

REFERENCES

1. Nacionalniy kancer-reestr. Rezim dostupu http://www.ncru.inf.ua/publications/
2. Adamchuk NM., Sorokina OY. Bolevoj sindrom u detej s gemoblastozami. Sovremennoe sostoyanie problemy. [Pain syndrome in children with gemblastosis. Current state of the problem]. Medicina nevidkladnih staniv. 2018;5(92):80-84.
3. WHO model formulary for children. – Geneva: World Health Organization. 2010
4. Pekpak E., İleri T, Ertem M, Uysal Z. Toxicity of Vincristine Combined With Posaconazole in Children With Acute Lymphoblastic Leukemia. J Pediatr Hematol Oncol. 2018;40 (5):309-310.
5. Schappacher KA., Styczynski L, Baccei ML. Early life vincristine exposure evokes mechanical pain hypersensitivity in the developing rat. Pain. 2017;58(9):1647-1655.
6. Lavoie Smith EM., Li L, Chiang C, Thomas K [et al]. Patterns and severity of vincristine-induced peripheral neuropathy in children with acute lymphoblastic leukemia. J Peripher Nerv Syst. 2015;20 (1):37-46.
7. Nazir HF., AlFutaisi A, Zacharia M [et al]. Vincristine-induced neuropathy in pediatric patients with acute lymphoblastic leukemia in Oman: Frequent autonomic and more severe cranial nerve involvement. Pediatr Blood Cancer. 2017;64 (12).
8. Arcos-Machancoses JV, García-Williams S, Fuentes-Socorro C, Fernández-Navarro JM, Menor-Serrano F, Verdeguer-Miralles. Headache, hypertension and seizures. J Paediatr Child Health. 2015;51(12):1239-1241.
9. Hasan H, Shaikh OM, Rassekh SR, Howard AF, Goddard K. Comparison of hypersensitivity rates to intravenous and intramuscular PEG-asparaginase in children with acute lymphoblastic leukemia: A meta-analysis and systematic review. Pediatr Blood Cancer. 2017;64 (1):81 – 88.
10. Apiliogullari S, Arun O, Celik D. Postdural puncture headache: a neglected complication after lumbar puncture and intrathecal chemotherapy in children with acute lymphoblastic leukemia. J Clin Oncol. 2014;19(4):769–770.
11. Martínez-Cayuelas E, Domingo-Jiménez R, Pascual-Gázquez JF [et al]. Neurological complications in the population of children with leukaemia. Rev Neurol. 2015;60(3): 108-114.
12. Eiser C, Stride CB, Vora A, Goulden N. Prospective evaluation of quality of life in children treated in UKALL 2003 for acute lymphoblastic leukaemia: A cohort study. Pediatr Blood Cancer. 2017;64(11).
13. Armstrong G.T., Kawashima T. [et al]. Aging and risk of severe, disabling, life-threatening, and fatal events in the childhood cancer survivor study. J. Clin. Onkol. 2014;32(12):1218-1227.

The work is a fragment of the research work of the Department of Anesthesiology and Intensive Therapy of the Ukrainian Medical Dental Academy, state registration number: 0114U006326. "Optimization of the quality of anesthesiology and the intensive care of patients taking into account the age and gender dimorphism of clinical and functional, immune and metabolic changes."

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olena Y. Sorokina
Vladimir Vernadsky St., 9, 49000 Dnipro, Ukraine
tel: +380502015520
e-mail: sorokinaeu@ukr.net

Received: 17.03.2019
Accepted: 03.06.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

LUNG VESSELS THROMBOSIS IN HOSPITALIZED PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA: ROLE OF ENDOTHELIAL FUNCTION, HEMOSTASIS, FIBRINOLYSIS AND INFLAMMATION ON DIFFERENT PHASES OF TREATMENT

Mariia A. Krykhtina[1], Kseniia O. Bielosludtseva[1], Larysa A. Botvinikova[1], Nataliia M. Matikina[2]
[1] DEPARTMENT OF INTERNAL MEDICINE №1, STATE INSTITUTION "DNIPROPETROVSK MEDICAL ACADEMY OF MINISTRY OF HEALTH OF UKRAINE", DNIPRO, UKRAINE
[2] STATE ESTABLISHMENT "DNIPROPETROVSK CITY CLINICAL HOSPITAL №6" OF DNIPROPETROVSK REGIONAL COUNCIL, DNIPRO, UKRAINE

ABSTRACT
Introduction: There are limited data on the relationship between the severity of community-acquired pneumonia (CAP), biomarkers of inflammation and coagulation as well.
The aim was to evaluate the association between the severity of CAP and risk of thrombosis in patients with moderate and severe CAP. To estimate the role of parameters of systemic inflammation, endothelial dysfunction, hemostasis, coagulation on different phases of treatment.
Materials and methods: The main group was 75 patients CAP. We divided the main group according severity: subgroup 1 – 41 patients with moderate CAP, subgroup 2 – 34 patients with severe CAP. Blood coagulation test, determination of biomarkers was performed at admission before starting of antibacterial treatment and after clinical stability on 7–10 day after hospitalization.
Results: We found that in both subgroup 1 and subgroup 2 the mean levels of CRP and fibrinogen were higher than in control group. Moreover, the mean level of D-dimer was significantly higher and protein C (PC) was significantly lower in both subgroups in comparison with control group.
Normalization of PC is coming after 7–10 days of antibacterial treatment, vice versa ET-1, which reflects prolong endothelial dysfunction in patients with severe CAP.
Conclusions: patients with severe CAP have the high risk of thrombosis which can be associated with endothelial dysfunction; definition of such parameters as ET-1 and PC can be useful for establishment of different coagulant disorders in patient with mild and moderate CAP, and their dynamic changes could be the initial point of prescribing or cancelling of anticoagulant treatment.

KEY WORDS: CAP, thrombosis, endothelial dysfunction, inflammation, markers

Wiad Lek 2019, 72, 8, 1463-1465

INTRODUCTION
The endothelium is a functional barrier between vessel wall and blood stream that for a long time has been regarded as a relatively inert container for blood [1]. However, the last 20 years, researchers have found an extraordinary variety of important endothelial functions, including control of coagulation, fibrinolysis, vascular tone, growth, and immune response. It is now clear that dysfunction of the endothelium disturbs the physiological protective regulatory balance and could be associated with increased mortality in patient with different diseases including community-acquired pneumonia (CAP) [2, 3].

CAP is one of the most common causes of mortality among other infectious diseases all over the world. The mortality rate of patients with CAP depends on the severity of the disease and comorbidity [4]. Therefore, the early identification of patients at risk of serious complications is critical to the management of CAP. To improve the outcomes in the management of CAP, there has recently been a significant attention paid to evidence-based scoring systems and biological markers. They can objectively help to predict the severity, to justify hospital or ICU admission [5]. The role of inflammatory markers has been already established rather good. Researchers believe that some coagulation and thrombotic disorders during severe CAP could lead to some intravascular disorders and even be the reason of lethal end in hospitalized patients with CAP [6]. The perfect severity score and biomarker does not yet exist. There are limited data on the relationship between the severity of community-acquired pneumonia (CAP) and biomarkers of inflammation and coagulation as well [7, 8]. At present, the combination of biomarkers and clinical scores has shown promising results in predicting mortality and severe outcomes in CAP.

THE AIM
The aim was to evaluate the association between the severity of CAP (according to SMRT-CO score) and risk of thrombosis (according to Padui score) in patients with moderate and severe CAP. And furthermore, to estimate the role of parameters of systemic inflammation (C-reactive

Table I. Levels of systemic inflammation parameters in patients with moderate to severe CAP, Me [25-75%]

Parameter	Main group	Control group	p
CRP, mg/l	168.6 [102.6–277.3]	4.5 [3.4–5.5]	0.002
Fibrinogen, mcg/ml	6.8 [4.7–12.2]	2.7 [2.4–3.0]	0.000

Table II. Levels of parameters of endothelial dysfunction, hemostasis and fibrinolysis in patients with moderate to severe CAP, Me [25-75%]

Parameter	Main group	Control group	p
ET-1, ng/ml	0.64 [0.4–1.86]	0.77 [0.45–1.03]	0.778
PC, %	75.0 [64.0–95.0]	95.0 [83.0–105.0]	0.008
DD, ng/ml	1561.2 [989.4–5325.3]	267.0 [254.0–1298.0]	0.000

Table III. Levels of systemic inflammation and coagulation parameters in patients with moderate to severe CAP, Me [25-75%]

Parameter	Visit 1		Visit 2		Control group
	subgroup 1	*subgroup 2*	*subgroup 1*	*subgroup 2*	
CRP, mg/l	126,7 [68,7–192,0]*#^	197,6 [147,8–311,7]*#^	10,6 [5,0–19,2]*#^	49,0 [6,6–93,0]*#^	4,5 [3,4–5,5]
ET-1, ng/ml	0,5 [0,36–0,81]*^	1,4 [0,41–2,5]*#^	0,6 [0,4–0,7]*^	1,1 [0,7–2,4]*#^	0,77 [0,45–1,03]
PC, %	80,0 [68,0–95,0] #^	70,0 [63,0–95,0]#^	101,5 [88,5–115,0]^	106,5 [80,0–135,0]^	95,0 [83,0–105,0]

Notes: * – $p < 0.05$ between subgroups on Mann-Witney;
– $p < 0.05$ with control group on Mann-Witney;
^ – $p < 0.05$ between visits on Wilkoxon.

protein (CRP), fibrinogen), endothelial dysfunction (endothelin 1 (ET-1)), hemostasis (protein C (PC)), fibrinolysis (D-dimer (DD) on different phases of treatment.

MATERIALS AND METHODS

The main group was 75 patients with moderate to severe CAP. Depending on the severity all patients of the main group were divided into 2 subgroups: subgroup 1 – 41 patients with moderate CAP (the mean age was 48.0 [33.0–61.0] years old, men – 33 (80.0%)), subgroup 2 – 34 patients with severe CAP (the mean age was 56.0 [46.0–60.0] years old, men – 22 (65.0%). Subgroups had no significant difference according to age (p=0.178) and sex (p=0.166).

The formulation of diagnosis CAP and assessment of its severity were determined according to the international guidelines [4] and SMRT-CO scale [9].

General clinical analysis, blood coagulation test, determination of biomarkers was performed at admission before starting of antibacterial treatment (visit 1) and after clinical stability on 7–10 day after hospitalization (visit 2).

DD and CRP levels were estimated by immunoturbodimetric method [10, 11], ET-1 was estimated by immuassay test, activity of PC was established with optic method [12, 13].

The study was conducted according to Helsinki declaration with the permission of the Bioethical commission Dnipropetrovsk medical academy of the ministry of Health of Ukraine.

Received results were compared with values in control group. The control group was 16 healthy people (the mean age was 56.0 [52.0–69.0] years old, men – 13 (81.0%)).

The risk of thrombosis was estimated with the help of Padua Prediction Score for risk of lung thrombosis [14].

At died patients we observed autopsy data including electronic microscope investigations.

Statistical processing of the results in research was carried out using the methods of biometric analysis, implemented in software packages EXCEL-2003 (№ 74017-641-9475201-57075) and STATISTICA 6.0 (№ 31415926535897) [15].

RESULTS AND DISCUSSION

Analyzing the data of main group patients, we detect that during hospitalization everybody has clinical symptoms of CAP combining with mono- or bilateral lung infiltration on chest X-ray.

The severity according to the SMRT-CO scale of patients in subgroup 1 was 0.0 [0.0–1.0] scores, in subgroup 2 was 3.0 [3.0–5.0] scores (p=0.000), which proved the right distribution of patient among subgroups.

During investigation of systemic inflammatory markers on visit 1, we found that in both subgroup 1 and subgroup 2 the mean levels of CRP and fibrinogen were higher than in control group (table I). Detailed analysis showed that patients with severe CAP had significantly higher levels of CRP (197.6 [147.8–311.7] mg/l in subgroup 2 comparing with 126.7 [68.7–192.0] mg/l in subgroup 1, p=0,000), which reflects the severity of inflammation in patients with CAP. The level of fibrinogen did not differ between subgroups and was 7.3 [3.7–13.3] mcg/ml in subgroup 1 and 7.8 [5.5–12.2] mcg/ml in subgroup 2 (p=0.483).

During investigation of parameters of endothelial function (ET-1), hemostasis (PC) and fibrinolysis (DD) we found that in both subgroup 1 and subgroup 2 the mean level of DD was significantly higher and PC was significantly lower in comparing with the mean levels of these parameters in control group, but the result of ET-1 depended on the severity of the CAP. Detailed

analysis showed that patients with severe CAP had significantly higher levels of ET-1 and the lowest level of PC (table II, table III). This fact shows the highest risk of thrombosis in patients with severe CAP.

Additionally, the mean level of scores by Padua scale in patients with severe CAP was 5.0 [5.0–6.0] scores, which was significantly higher than in patients with moderate CAP, who had 1.0 [1.0–2.0] scores (p=0.000).

All patients received guided-based antibacterial treatment and mucolytic. On the visit 2 which was on 7–10 day after hospitalization most of them had signs of clinical stability: normalization of the temperature, decreasing of cough and dyspnea.

The proving of general positive effect of treatment is demonstrated by great decreasing of mean level of CRP in both patients with severe and mild CAP (table III). However, it was still differing from normal value in control group, which shows that stabilization of this marker is rather long process.

Regarding ET-1, the mean level of which was increased only in severe patients on visit 1, also significantly decreased on visit 2, but nevertheless it was still differing from the corresponding parameter in control group. This fact demonstrates that endothelial dysfunction which is one of the pathogenetical mechanisms of severe pneumonia presents its role during rather prolonged time (more than 7–10 days' period).

Opposite situation was with nature anticoagulant PC. The mean level of this parameter not only significantly increased till the visit 2 but also normalized and didn't differ from the mean level of PC in healthy persons on the 7–10 day.

CONCLUSIONS

1. In-patient cases of CAP are characterized by systemic inflammation, which comes out in intensive increasing of CRP and hypercoagulation which comes out in decreasing of PC, increasing of DD and fibrinogen.
2. Patients with severe CAP have the high risk of thrombosis which can be associated with endothelial dysfunction and manifested in maximal scores by Padua scale, the highest levels of ET-1 and the lowest level of PC.
3. Normalization of PC is coming after 7–10 days of antibacterial treatment of CAP, vice versa ET-1, which reflects prolong endothelial dysfunction in patients with severe CAP.
4. Definition of such parameters as ET-1 and PC can be useful for establishment of different coagulant disorders in patient with mild and moderate CAP, and their dynamic changes could be the initial point of prescribing or cancelling of anticoagulant treatment.

REFERENCES

1. Deanfield J.E., Halcox J. P., Rabelink T. J. Endothelial function and dysfunction testing and clinical relevance. Circulation. 2007; 115:1285–1295.
2. D.D Ribeiro, W. M. Lijfering, A. van H. Vlieg et al. Pneumonia and risk of venous thrombosis: results from the MEGA study. Thromb Haemost. –2012; 10:1179–82.
3. N. V. Bedilo, N. A. Vorob'eva, N. V. Ismaïlova et al. Evaluation of haemostasis and endothelial dysfunction characteristics in patient with community-acquired pneumonia. Anesteziol Reanimatol. –2014; 1:33-8.
4. Niederman M. S., Luna C. M. Community-acquired pneumonia guidelines: a global perspective. Semin Respir Crit Care Med. 2012; 33(3):298-310.
5. Torres A. W.E. Peetermans, G. Viegi et al. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax. 2013; 68: 1057–65.
6. Hedlund J., Hansson L. O. Procalcitonin and C-reactive protein levels in community-acquired pneumonia: correlation with etiology and prognosis. Infection. 2000; 28:68–73.
7. Fichtlscherer S., Rosenberger G., Walter D. H. et al. Elevated C–reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. Circulation. 2000; 102: 1000–1006.
8. Prasad A., Zhu J., Halcox J. P. J. et al. Relation between infections and endothelial dysfunction. Circulation 2000; 102 (Suppl II): 245.
9. Charles P. GP. et al. SMART-COP: A tool for predicting the need for intensive respiratory or vasopressor support in community-acquired pneumonia. Clinical Infectious Diseases. 2008, 47 (3):375–84.
10. Gosselin R. C., Owings J. T., Utter G.H. A new method for measuring D-dimer using immunoturbidimetry: a study of 255 patients with suspected pulmonary embolism and deep vein thrombosis. Blood Coagul Fibrinolysis. 2000; 11(8):715–21.
11. Dupuy A. M., Badiou S., Descomps B. et al. Immunoturbidimetric determination of C-reactive protein (CRP) and high-sensitivity CRP on heparin plasma. Comparison with serum determination. Clin Chem Lab Med. 2003; 41(7):948–9.
12. Abdel-Sayed S., Nussberger J.,. Aubert J.-Fr. et al. Measurement of Plasma Endothelin-1 in Experimental Hypertension and in Healthy Subjects. AJH. 2003; 16:515–521.
13. Gardiner C., Cohen H., Jenkins A. et al. Detection of acquired resistance to activated protein C associated with antiphospholipid antibodies using a novel clotting assay. Blood Coagul Fibrinolysis. 2006; 17(6):477–83.
14. Barbar S., Noventa F., Rossetto V. et al. A risk assessment model for the identification of hospitalized medical patients at risk for venous thromboembolism: the Padua Prediction Score. J Thromb Haemost. 2010; 8(11): 2450–7.
15. Hollander M., Wolfe D. A., Chicken E. Nonparametric statistical methods. John Wiley & Sons. 2014: 848 p.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mariia A. Krykhtina
Department of internal medicine №1,
State Institution "Dnipropetrovsk Medical Academy of Ministry of Health of Ukraine"
Batumska 13 street, Dnipro, Ukraine
tel: +380676327677
e-mail: mariakryhtina@gmail.com

Received: 19.02.2019
Accepted: 08.06.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

OSTRE USZKODZENIE NEREK U PACJENTÓW W WIEKU PODESZŁYM

ACUTE KIDNEY INJURY IN ELDERLY PATIENTS

Grażyna Kobus[1], Jolanta Małyszko[2], Hanna Bachórzewska-Gajewska [1]
[1]ZAKŁAD MEDYCYNY KLINICZNEJ , BIAŁYSTOK, POLAND
[2]II KLINIKA NEFROLOGII Z ODDZIAŁEM LECZENIA NADCIŚNIENIA TĘTNICZEGO I PODODDZIAŁEM DIALIZOTERAPII, WARSZAWA, POLSKA

STRESZCZENIE

Wstęp: W wieku podeszłym dochodzi do upośledzenia funkcji nerek. Choroby nerek nakładają się na zmiany anatomiczne i czynnościowe wywołane związanymi z wiekiem procesami inwolucyjnymi. Śmiertelność wśród pacjentów z ostrym uszkodzeniem nerek wynosi około 50%, pomimo postępu w leczeniu i diagnostyce AKI (*Acute Kidney injury*).

Cel pracy: Ocena częstości wystąpienia ostrego uszkodzenia nerek u pacjentów w wieku podeszłym oraz analiza przyczyn wystąpienia ostrego uszkodzenia nerek w zależności od wieku.

Materiał i metody: Analizą retrospektywną objęto dokumentację medyczną chorych hospitalizowanych w Klinice Nefrologii w okresie 6 miesięcy. W tym okresie w klinice było hospitalizowanych 452 pacjentów. Do badania włączono grupę 77 chorych z ostrym uszkodzeniem nerek jako powodem hospitalizacji.

Wyniki: Postać przednerkowa była najczęstszą przyczyną AKI w obu grupach wiekowych. W obu grupach wiekowych najczęstszą przyczyną było odwodnienie; w grupie chorych do 65. rż. odwodnienie stanowiło 29,17%; w grupie osób powyżej 65. rż. – 43.39%. Leczenie nerkozastępcze u pacjentów z AKI zastosowano u 14,29% chorych. W grupie pacjentów do 65. r.ż. hemodializę miało 16,67%, a powyżej 65 r.ż. – 13,21% chorych. Średnie stężenie kreatyniny w grupie pacjentów młodszych przy przyjęciu wynosiło 5,16±3,71 mg/dl, w grupie pacjentów starszych 3,14±1,63 mg/dl. Wielkość filtracji kłębuszkowej GFR w grupie pacjentów młodszych przy przyjęciu wynosiła 21,14±19,54 ml/min, w grupie pacjentów starszych 23,34±13,33 ml/min.

Wnioski: Główną przyczyną ostrego uszkodzenia nerek, niezależnie od grupy wiekowej, było odwodnienie. Z uwagi na duży odsetek AKI u osób starszych, ta grupa wymaga podjęcia szerszych działań prewencyjnych, nie tylko w szpitalu ale również w domu.

SŁOWA KLUCZOWE: wiek podeszły, ostre uszkodzenie nerek, terapia nerkozastępcza

ABSTRACT

Introduction: In the elderly, impairment of kidney function occurs. Renal diseases overlap with anatomic and functional changes related to age-related involutionary processes. Mortality among patients with acute renal injury is approximately 50%, despite advances in treatment and diagnosis of AKI.

The aim: To assess the incidence of acute kidney injury in elderly patients and to analyze the causes of acute renal failure depending on age.

Materials and methods: A retrospective analysis included medical documentation of patients hospitalized in the Nephrology Clinic during the 6-month period. During this period 452 patients were hospitalized in the clinic. A group of 77 patients with acute renal failure as a reason for hospitalization was included in the study.

Results: The prerenal form was the most common cause of AKI in both age groups. In both age groups, the most common cause was dehydration; in the group of patients up to 65 years of age, dehydration was 29.17%; in the group of people over 65 years - 43.39%. Renal replacement therapy in patients with AKI was used in 14.29% of patients. In the group of patients up to 65 years of age hemodialysis was 16.67% and above 65 years of age. -13.21% of patients. The average creatinine level in the group of younger patients at admission was 5.16 ± 3.71 mg / dl, in the group of older patients 3.14 ± 1.63 mg / dl. The size of glomerular filtration GFR in the group of younger patients at admission was 21.14 ± 19.54 ml / min, in the group of older patients 23.34 ± 13.33 ml / min.

Conclusions: The main cause of acute kidney injury regardless of the age group was dehydration. Due to the high percentage of AKI in the elderly, this group requires more preventive action, not only in the hospital but also at home.

KEY WORDS: old age, acute kidney injury, renal replacement therapy

Wiad Lek 2019, 72, 8, 1466-1472

WSTĘP

Obecnie możemy zaobserwować zjawisko coraz szybszego starzenia się społeczeństwa. W 2000 roku 69 mln ludzi na świecie osiągnęło wiek powyżej 80 lat i ta grupa charakteryzowała się najszybszym przyrostem liczebności. Szacuje się że do 2050 roku populacja osób w tym przedziale wieku wzrośnie prawie 6-krotnie i w większości krajów europejskich będzie stanowić 10% populacji [1]. Starzenie się organizmu człowieka to nieuchronny, złożony proces fizjologiczny, który prowadzi do wielu zmian na poziomie komórek, tkanek, jak i narządów doprowadzając do osłabienia ich funkcji. Naturalnym zjawiskiem w procesie starzenia się organizmu jest stopniowe zwiększanie się liczby zmian o charakterze

regresyjnym oraz zmniejszanie liczby procesów odnowy i regeneracji. Przykładem fizjologicznego starzenia się są nerki. Wraz z wiekiem dochodzi do upośledzenie wielu funkcji nerek. Choroby nerek nakładają się na zmiany anatomiczne i czynnościowe wywołane związanymi z wiekiem procesami inwolucyjnymi [2, 3]. Przeciętnie tempo ubytku GFR ocenia się na około 0,75 ml/min/rok po 35.–40. roku życia, przy czym po 55.–60. roku życia może ono ulec przyśpieszeniu nawet do powyżej 1 ml/min/rok [4]. W procesie starzenia się nerek dochodzi także do zmian naczyniowych [5]. Wiele innych zaburzeń czynnościowych ze strony nerek może prowadzić do istotnego zmniejszenia zdolności adaptacyjnych ustroju, zwłaszcza do zmian wolemii i natremii. Pacjenci geriatryczni są szczególnie narażeni na rozwój ostrej niewydolności nerek (AKI – *Acute Kidney injury*). Liczne badania wykazały, że wiek jest niezależnym czynnikiem ryzyka AKI. W pracy Nash i wsp. częstość AKI u pacjentów hospitalizowanych w wieku 15–39 lat wynosiła 3,7%, w wieku 40–59 lat – 5,6%, w wieku 60–70 lat – 8,6%, a u chorych >80. roku życia – 10,6% [6]. Wynika to z faktu, że osoby w wieku podeszłym są o wiele częściej narażone na odwodnienie [7], są częściej poddawane zabiegom kardiochirurgicznym (w tym zwłaszcza kardiochirurgicznym z krążeniem pozaustrojowym i zabiegom na aorcie z czasowym zamknięciem tętnic nerkowych), zabiegom z użyciem kontrastu, czy stosowanie innych leków nefrotoksycznych [8, 9].

Podatność na odwodnienie, zaburzenia procesów oszczędzania sodu, częste leczenie diuretykami, niesteroidowymi lekami przeciwzapalnymi, inhibitorami enzymu konwertującego lub lekami blokującymi receptor AT1 dla angiotensyny II, niestabilny układ krążenia, obecność subklinicznego zwężenia tętnicy nerkowej, upośledzony odpływ moczu, współistniejąca wcześniej przewlekła nefropatia to tylko niektóre czynniki wybitnie usposabiające do wystąpienia ostrego uszkodzenia nerek [10].

Według *Kidney Disease: Improving Global Outcoms* (KDIGO) kryterium rozpoznania ostrego uszkodzenia nerek jest gwałtowne upośledzenie funkcji definiowane jako: wzrost stężenia kreatyniny o ≥0,3 mg /dl (25 µmol/l) w ciągu 48 h lub wzrost stężenia kreatyniny o ≥50% w ciągu 7 dni lub zmniejszenie diurezy <0,5 ml/kg/h przez 6 h [11]. AKI charakteryzuje się szerokim zakresem zaburzeń – od niewielkich i szybko przemijających podwyższonych stężeń wskaźników biologicznych ostrego uszkodzenia nerek do bardzo ciężkiej ostrej niewydolności nerek, zaburzeń metabolicznych i klinicznych z koniecznością leczenia nerkozastępczego [12, 13] .Ostre uszkodzenie nerek w różnym stadium dotyczy aż 5% wszystkich pacjentów hospitalizowanych, a 10% chorych z tej grupy wymaga leczenia nerkozastępczego. Pacjenci, u których udało się przywrócić prawidłową pracę nerek stanowią około 17% chorych. Śmiertelność wśród chorych, u których zdiagnozowano AKI wynosi 30% [14]. Spowodowane jest to tym, że większość pacjentów leczonych z powodu AKI stanowią osoby starsze, z już upośledzoną funkcją nerek, szeregiem chorób współistniejących mających wpływ na rokowanie.

CEL PRACY

Ocena częstości wystąpienia ostrego uszkodzenia nerek u pacjentów w wieku podeszłym oraz analiza przyczyn wystąpienia ostrego uszkodzenia nerek w zależności od wieku

MATERIAŁY I METODY

Analizą retrospektywną objęto dokumentację medyczną chorych hospitalizowanych w Klinice Nefrologii w okresie od stycznia do października 2017 r. W tym okresie w klinice było hospitalizowanych 452 pacjentów. Do badania włączono grupę 77 chorych z ostrym uszkodzeniem nerek jako przyczyną hospitalizacji. Rozpoznanie AKI ustalano na podstawie ogólnie przyjętych kryteriów. Analizie poddane zostały takie parametry jak: płeć, wiek, wyniki badań laboratoryjnych (stężenie kreatyniny, GFR, morfologia krwi, CRP) choroby współistniejące, leki przyjmowane, metody leczenia). Na przeprowadzenie badań uzyskano zgodę Komisji Bioetycznej UMB numer: R-I-002/195/2017

ANALIZA STATYSTYCZNA

Analiza statystyczna wykonana została przy użyciu programu Statistica 7.0, w którym obliczony został współczynnik korelacji rang Spearmana. Za pomocą również programu Statistica wyznaczone zostały następujące parametry liczbowe: średnia arytmetyczna, odchylenie standardowe, liczebność badanej grupy

WYNIKI

Z grupy hospitalizowanych chorych (n=452), 17% (77) stanowili chorzy z ostrym uszkodzeniem nerek i tę grupę poddano analizie. Pacjenci byli w wieku od 46 do 91 lat. Średnia wieku kobiet wynosiła 73,78±10,58 roku, mężczyzn 70,88±15,11 roku. Odsetek pacjentów ≤65 r.ż wynosił 31,17%, powyżej 65. r.ż – 68,83%. Kobiety w całej grupie stanowiły 43%, mężczyźni 57%. Wszyscy pacjenci zostali przyjęci do oddziału w trybie nagłym. W grupie wiekowej do ≤65 rż -37.5% pacjentów było przyjętych z SOR-u, 33.33% z innego oddziału, ok.29% z domu. W grupie wiekowej powyżej 65 rż, z SOR-u było przyjętych 45.26% pacjentów, z domu 32.08% , z innego oddziału 22.64%.

Niezależnie od wieku chorego czas pobytu w klinice był podobny i wynosił średnio 11±8,52 dnia. Mężczyźni przebywali w szpitalu dłużej niż kobiety (12,36±8,22 *vs.* 9,48±4,62 dnia).

Cukrzyca występowała u 39,13% pacjentów do ≤65. r.ż i u co trzeciego pacjenta spośród chorych po > 65rż. W obu grupach przeważała cukrzyca insulinoniezależna.

RODZAJ AKI

Postać przednerkowa była najczęstszą przyczyną AKI w obu grupach wiekowych; do ≤65. rż stanowiła 65,2%, w grupie > 65. rż 60,38%. Postać nerkowa – 17,39% w populacji ≤65 rż i 5,66% w grupie > 65 rż. Nieokreślona po-

Tabela I. Przyczyny ostrego uszkodzenia nerek z uwzględnieniem wieku.

PRZYCZYNA AKI	≤65 rż	>65 rż
Odwodnienie	29,17%	43,39%
Nieokreślona	20,83%	18,87%
Choroba nowotworowa	8,33%	5,66%
Zespól sercowo-naczyniowy	0	3,77%
Przewlekły zespół zapalenia nerek	4,17%	1,89%
Miażdżycowo-niedokrwienna	4,17%	0
Ostre cewkowo-miąższowe zapalenie nerek	4,17%	0
Zaostrzenie przewlekłej choroby nerek	4,17%	0
Niewydolność serca zastoinowa	0	1,89%
Zatrucie lekami	8,33%	5,66%
Przerost gruczołu krokowego	0	5,66%
Zakażenie układu moczowego	0	3,77%
Zatrucie alkoholem	12,5%	0
Posocznica	0	1,89%
Złożona etiologia	0	3,77%

Tabela II. Farmakoterapia pacjentów w zależności od wieku.

LEKI PRZYJMOWANE	≤65	>65
Beta blokery	50%	58,49%
Leki hipotensyjne	84,91%	58,49%
Niesteroidowe leki przeciwzapalne	43,85%	54,72%
ACEI	13,21%	39,62%
Leki p/zakrzepowe	43,85%	64,15%
Inne leki	91,67%	100%
Diuretyki pętlowe	50%	62,26%
Aminy katecholowe	8,33%	26,42%
Mannitol	0%	1,89%
Nawodnienie	87,50%	80,77%
Antybiotyki	62,50%	61,15%

stać stanowiła około 17% w obu przedziałach wiekowych, a zanerkowa – 13,21%.

PRZYCZYNA OSTREGO USZKODZENIA NEREK

Niezależnie od wieku najczęstszą przyczyną AKI było odwodnienie. Wśród chorych do 6,5 rż. odwodnienie stanowiło 29,17%; w grupie osób powyżej 65 rż. – 43,39%. (Tab.I)

LECZENIE NERKOZASTĘPCZE

Leczenie nerkozastępcze u pacjentów z ostrym uszkodzeniem nerek zastosowano u 14,29% przypadków, częściej zabiegów hemodializ wymagali pacjenci do 65 rż (16,67%) niż pacjenci powyżej 65 rż - 13,21% .

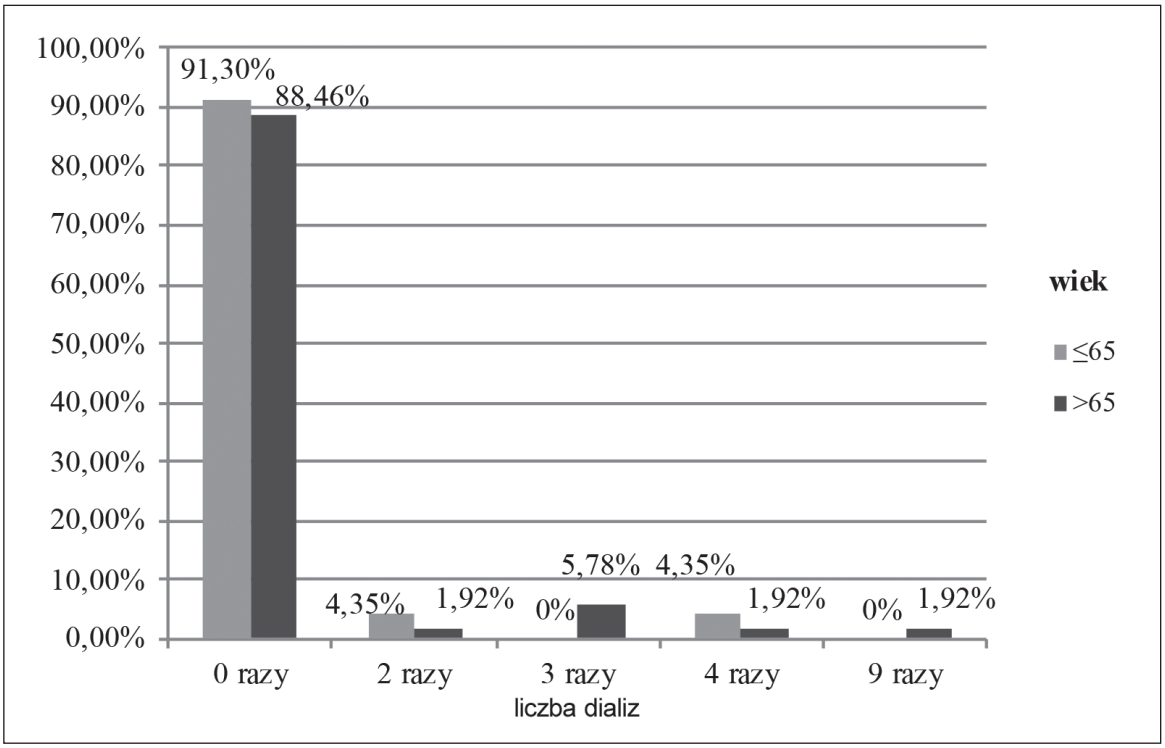
Rycina 1 przedstawia ilość zabiegów hemodializ wykonywanych u pacjentów w zależności od wieku (Ryc.1).

LOSY PACJENTA PO ZAKOŃCZENIU LECZENIA

Większość (79,17%) pacjentów do 65. rż i 71,70% pacjentów >65. rż po zakończonym leczeniu AKI była wypisana do domu. Co piąty pacjent został przeniesiony na inny oddział lub do innego szpitala/ośrodka/domu opieki społecznej. Zgon nastąpił u 9,43% pacjentów powyżej 65. rż, z towarzyszącą cukrzycą i nadciśnieniem tętniczym. Zgonów w grupie pacjentów młodszych nie było.

FARMAKOTERAPIA

Chorzy z AKI przed hospitalizacją i w trakcie niej przyjmowali leki z powodu chorób współistniejących. Antybiotykoterapię stosowano u około 60% chorych w obu grupach wiekowych. Wśród leków przyjmowanych przed hospitalizacją lub zleconych w trakcie w grupie wiekowej ≤65 około 59% przyjmowało leki hipotensyjne. W populacji >65 r.ż. najwięcej, bo aż



Ryc. 1. Ilość wykonanych zabiegów hemodializ u chorych z AKI w zależności od wieku.

(64,15%) osób przyjmowało leki przeciwzakrzepowe, beta blokery i leki hipotensyjne (58,49%). W trakcie hospitalizacji nawodnienie otrzymało 87,50% pacjentów w grupie do 65. rż. i około 81% powyżej 65. rż. (Tab. II).

WYBRANE PARAMETRY BIOCHEMICZNE PACJENTÓW

W tabeli III przedstawiono wyniki wybranych parametrów biochemicznych obu badanych grup. W badaniu uwzględniono pierwszy i ostatni pomiar wybranych parametrów biochemicznych. W grupie chorych powyżej 65 roku życia stwierdzono istotnie statystycznie wyższe stężenie GFR przy wypisie ($p < 0,05$), kwasu moczowego oraz niższe wartości CK ($p < 0,05$) (Tab. III).

PROFIL NEFROLOGICZNY

W grupie pacjentów do 65 r.ż średnie stężenie kreatyniny przy przyjęciu wynosiło 5,16±3,71 mg/dl, w grupie pacjentów >65 r.ż. – 3,14±1,63 mg/dl.

Średnie stężenie mocznika w grupie pacjentów młodszych przy przyjęciu wynosiło 136,99±74,15 mg/dl., w grupie pacjentów starszych 137,55±51,50 mg/dl.

Wielkość filtracji kłębuszkowej GFR w grupie pacjentów młodszych przy przyjęciu – wynosiła 21,14±19,54 ml/min, w grupie pacjentów starszych 23,34±13,33 ml/min.

BADANIE OGÓLNE MOCZU

W populacji ≤65 r.ż. średni ciężar moczu wynosił 1,001±0,005 g/l, obecność erytrocytów stwierdzono u 70.83%, białko w 75% przypadków. W grupie >65 r.ż. średni ciężar moczu był 1,002±0,005 g/l, obecność białka (46,30%), obecność erytrocytów w moczu (54,71%).

DYSKUSJA

Ostre uszkodzenie nerek stanowi bardzo poważny problem we współczesnej medycynie, pojawia się coraz częściej i dotyczy pacjentów w każdej grupie wiekowej. Patogeneza AKI jest bardzo złożona, chorobę tę może spowodować wiele czynników, a wiek pacjenta warunkuje jej przebieg. Chorzy powyżej 75. rż. znajdują się w grupie zwiększonego ryzyka zgonu w ciągu 3 lat [15]. Jak wynika z badań Akposso i wsp. procentowy udział pacjentów powyżej 80. rż wśród osób leczonych z powodu AKI w ciągu 25 lat zwiększył się 10-krotnie i obecnie sięga 40% [16].

W poniższej pracy ocenialiśmy częstość występowania AKI u osób w wieku podeszłym. W naszych badaniach odsetek osób po 65 rż wynosi ok.68%, i częściej dotyczył mężczyzn. Również w badaniach innych badaczy przeważali mężczyźni [17]. Czas hospitalizacji pacjentów bez względu na wiek i płeć był porównywalny i wynosił 11± 8,52 dnia.

Osoby w wieku podeszłym w większym stopniu są narażone na wystąpienie ostrego uszkodzenia nerek ze względu na wielochorobowość. W przeprowadzonej analizie uzyskaliśmy wyniki które, wskazują, że pacjenci najczęściej mieli nadciśnienie tętnicze, chorobę wieńcową, migotanie przedsionków. Z dotychczasowych prac nie wynika jednoznacznie, że nadciśnienie tętnicze może wpływać na pojawienie się AKI. Jednak związek nadciśnienia z przewlekłą

Tabela III. Parametry biochemiczne u pacjentów w zależności od wieku.

Parametry biochemiczne	Pacjenci < 65 rż.	Pacjenci >65 rż.
Glikemia przy przyjęciu [mg/dl]	178,80±137,76	126,83±88,11
Glikemia przy wypisie [mg/dl]	102.00±54,65	84,11±60,75
HBG pierwszy pomiar [g/dl]	12,02±2,95	11,36±2,60
HBG drugi pomiar [g/dl]	9,95±0,97	10,70±1,78
PLT pierwszy pomiar [x10´3/µl]	230,38±134,21	231,22±87,78
PLT drugi pomiar [x10´3/µl]	224,63±96,63	221,66±98,46
WBC pierwszy pomiar [x10´3/µl]	10,39±4,43	12,69±6,47
WBC drugi pomiar [x10'3/µl]	8,24±3,18	9,53±5,14
HCT pierwszy pomiar [%]	35,44±8,67	33,89±7,45
HCT drugi pomiar [%]	29,07±3,42	32,02±5,16
Czas protrombinowy pierwszy pomiar [sek.]	15,64±4,70	21,32±19,01
Czas protrombinowy drugi pomiar [sek.]	15,15±4,92	19,58±9,04
INR pierwszy pomiar	1,28±0,52	2,05±2,67
INR drugi pomiar	1,25±0,55	1,58±0,84
Fibrynogen [mg/dl]	502,62±271,39	505,97±224,09
Stężenie sodu pierwszy pomiar [mmol/L]	135,88±4,91	136,51±5,80
Stężenie sodu drugi pomiar [mmol/L]	137,24±3,75	139,12±3,98
Stężenie potasu pierwszy pomiar [mmol/L]	5,06±0,80	4,86±1,18
Stężenie potasu drugi pomiar [mmol/L]	4,37±0,41	4,31±0,62
Kreatynina pierwszy pomiar [mg/dl]	5,16±3,71	3,14±1,63
Kreatynina drugi pomiar [mg/dl]	2,23±1,34	1,55±0,81
GFR pierwszy pomiar [ml/min]	21,14±19,54	23,34±12,33
GFR drugi pomiar [ml/min]	44,24±28,84	52,80±26,32*
Stężenie wapnia pierwszy pomiar [mmol/L]	2,26±0,55	2,24±0,48
Stężenie wapnia drugi pomiar [mmol/L]	2,17±0,37	2,19±0,21
AST pierwszy pomiar [IU/l]	114,38±211,40	98,40±324,11
AST drugi pomiar [IU/l]	54,00±58,75	35,00±16,27
ALT pierwszy pomiar [IU/l]	90,05±181,32	113,10±428,82
ALT drugi pomiar [IU/l]	105,11±140,89	54,31±93,09
CK [IU/l]	876,08±1026,53	420,17±926,92*
CK-kinaza keratynowa drugi pomiar [IU/l]	517,25±544,96	157,08±123,79
CK-MB aktywność pierwszy pomiar [IU/l]	79,45±50,62	38,41±23,72
CK-MB aktywność drugi pomiar [IU/l]	69,50±77,21	35,40±12,13
CRP pierwszy pomiar [mg/L]	83,70±83,66	89,60±96,30
CRP drugi pomiar [mg/L]	43,86±69,03	40,51±48,54
Stężenie mocznikapierwszy pomiar [mg/dL]	136,99±74,15	137,55±51,50
Stężenie mocznika drugi pomiar [mg/dL]	62,42±33,69	98,44±50,53*
Kwas moczowy pierwszy pomiar [mg/dL]	9,35±5,09	8,34±2,21
Kwas moczowy drugi pomiar [mg/dL]	6,23±1,61	7,24±1,61*

*p<0,05

chorobą nerek nie jest bez znaczenia przy obserwowanym wzroście ciśnienia w przebiegu ostrego uszkodzenia nerek. Jest to związane z nasilonym działaniem układu renina-angiotensyna-aldosteron, który aktywuje się pod wpływem niedokrwienia i hipoperfuzji miąższu nerek w przebiegu ostrego uszkodzenia nerek [18]. Samo działanie układu nie ma wpływu na pojawienie się AKI, lecz diuretyki pętlowe, które są podawane w pierwszej kolejności podczas leczenia,

czyli korzystne dla pacjenta, mogą również zmniejszać przepływ krwi przez nerki, co może prowadzić do powstania przednerkowego ostrego uszkodzenia nerek [19, 20]. Każdy pacjent z grupy badanej miał wiele innych chorób współistniejących. Współchorobowość i związana z tym polipragmazja, która niewłaściwie stosowana zwiększa ryzyko działań niepożądanych, może wpływać na czynność nerek i prowadzić do ostrego ich uszkodzenia. Polipragmazja jest niebezpieczna dla każdego, jednak szczególnie dla osób w starszym wieku, ponieważ to właśnie seniorzy są głównymi konsumentami leków. Jedną z częstych chorób u osób starszych jest cukrzyca. Obecnie uważa się, że częstość występowania cukrzycy w populacji osób po 65. roku życia wynosi ponad 40%. W naszych badaniach na cukrzycę chorował co 3 pacjent.

Odwodnienie jest częstą przyczyną prowadzącą do ostrego uszkodzenia nerek. Potwierdziło się to również w naszych badaniach. Aż 60% pacjentów niezależnie od wieku miało kliniczne cechy odwodnienia przy przyjęciu do oddziału. Badania T. Rusinowicz i wsp. wskazują odwodnienie jako główna przyczynę wystąpienia AKI u pacjentów w podobnym przedziale wiekowym [21]. W analizie przeprowadzonej u wielu pacjentów nie można było określić przyczyny AKI, mogło być to spowodowane tym, że na pojawienie się ostrego uszkodzenia nerek wpływ miało wiele czynników i żaden nie był na tyle wyraźny aby móc stwierdzić, że on jest przyczyną. W badanej grupie pacjentów najczęstszą przyczyną ostrego uszkodzenia nerek było przednerkowe uszkodzenie nerek, do którego prowadzi między innymi właśnie odwodnienie. Jak zauważyli Selcuk i wsp., wśród pacjentów w starszym wieku szczególnie często ARF rozwija się w wyniku więcej niż jednej przyczyny, zazwyczaj działającej na różnych poziomach [22].

Rozwój wiedzy medycznej powoduje, że powstają nowe metody i techniki leczenia, które są dostępne również dla tych pacjentów. Procedury inwazyjne, które charakteryzują się dużą skutecznością u młodszych pacjentów, u osób w podeszłym wieku powodują znacznie więcej powikłań, które czasami przeważają nad korzyściami. Wybór optymalnej terapii w odniesieniu do osoby w podeszłym wieku jest obarczony również szczególnymi trudnościami natury etycznej. Wybór metody leczenia AKI zależy przede wszystkim od możliwości i doświadczenia danego ośrodka oraz aparatury, jaką posiada. W ośrodkach dysponujących wieloma metodami opiera się głównie na indywidualizacji leczenia i personalizacji postępowania w określonej sytuacji klinicznej. Pomagają nam w tym standardy i wytyczne postępowania, które są punktem wyjściowym do opracowania właściwej terapii. Dializoterapia jest metodą inwazyjną i stosunkową drogą, a osoby w wieku powyżej 80 lat ze względu na krótszy spodziewany czas przeżycia mogą być gorszymi kandydatami do jej zastosowania [23]. W naszej klinice leczenie nerkozastępcze metodą hemodializy zastosowano u około 13% pacjentów z AKI powyżej 65. roku życia i u około 16% pacjentów poniżej 65. rż .

Z analizy piśmiennictwa wynika, że śmiertelność w ostrym uszkodzeniu nerek u osób w wieku podeszłym nie jest wcale większa niż u młodszych pacjentów [24]. Paradoks ten próbuje się tłumaczyć faktem, że u młodszej osoby do rozwoju AKI konieczny jest szczególnie intensywny czynnik wywołujący (infekcja, uraz, czynnik toksyczny), który *per se* wiąże się z wysoką śmiertelnością, podczas gdy u osób starszych do powstania ARF często dochodzi w wyniku stosunkowo niewielkiego zaostrzenia przewlekłej patologii. W naszych badaniach odsetek zgonów pacjentów z AKI wynosił 9,34% i były to zgony w grupie wiekowej >65 rż. W badaniu Snarskiej i wsp. w grupie osób, które zmarły na OIOM-e obserwowano wysoki odsetek ostrego uszkodzenia nerek [25]. Po zakończonym leczeniu większość pacjentów została wypisana do domu, ewentualnie została przeniesiona na inny oddział lub do innej placówki opiekuńczej.

Ostre uszkodzenie nerek u osób w wieku podeszłym stanowi złożony problem kliniczny. Wśród czynników ryzyka AKI znajduje się większość czynników ryzyka chorób układu sercowo-naczyniowego. Dotyczy to przede wszystkim przewlekłej choroby nerek i zaawansowanego wieku, ale także cukrzycy i nadciśnienia tętniczego Utrzymanie prawidłowej wolemii i stabilizacja stanu hemodynamicznego chorego w ciężkim stanie jest najważniejszym, a w większości przypadków – jedynym sposobem zapobiegania ostremu uszkodzeniu i niewydolności nerek. W przypadku rozwoju choroby, takie postępowanie zmniejsza rozmiary uszkodzenia nerek i przyśpiesza proces zdrowienia.

WNIOSKI

1. Wśród hospitalizowanych pacjentów w Klinice Nefrologii dość wysoki odsetek stanowią chorzy z ostrą ostrym uszkodzeniem nerek.
2. Rokowanie w AKI jest szczególnie niekorzystne w grupie starszych pacjentów.
3. Najczęstszą przyczyną ostrego uszkodzenia nerek, niezależnie od wieku, jest odwodnienie, co wskazuje na niedostateczne postrzeganie problemu właściwego nawadniania pacjentów w warunkach szpitalnych, jak i pacjentów pozostających pod opieką ambulatoryjną.
4. Większą uwagę należy zwrócić, nie tylko w placówkach medycznych, ale i poza nimi, na właściwe działania prewencyjne zapobiegające ostremu uszkodzeniu nerek.

PIŚMIENNICTWO

1. United Nations Population Division. World Population Prospects. The 2002 Revision at: http://www.un.org/esa/population/publications/
2. Hoang K, Tan JC, Derby G et al. Determinants of glomerular hypofiltration in aging humans. Kidney International 2003;64:1417-24.
3. Anderson SI.Prediction, progression, and outcomes of chronic kidney disease in older adult. J Am Soc Nephrol 2009;20:1199-1209.
4. Fliser D. Ren sanus in corpore sano: the myth of inexorable decline of renal function with senescence. Nephrol Dial Transplant 2005;20:482-5.
5. Wieczorkowaska -Tobis K. Difference in estimated GFR with two different formulas in elderly individuals. Inter Urol Nephrol 2010;38:381-385.
6. Nash K, Hafeez A, Hou S. Hospital-acquired renal insufficiency. Am J Kidney Dis 2002; 39 (5): 930-936.
7. Duława J. Ostre uszkodzenie i ostra niewydolność nerek – problem interdyscyplinarny. Postepy Nauk Med 2009;10:771-775.

8. Ribichini F, Graziani M, Gambaro G et al. Early creatinine shifts predict contrast-induced nephropathy and persistent renal damage after angiography. Am J Med 2010; 123:755-63.
9. Wilhelm-Leen E, Montez-Rath ME, Chertow G. Estimating the risk of radiocontrast-associated nephropathy. J Am Soc Nephrol 2017;28:653–9.
10. Stompór T. Choroby nerek u osób w wieku podeszłym. Przew Lek 2006; 10: 78-87.
11. Group KDIGOKAKIW: KDIGO clinical practice guideline for acute kidney injury. Kidney Int 2012; (Suppl 2):1-138
12. Lines S, Lewington A. Acute Kidney Injury. Clin Med 2008;9(3):273-277.
13. Goldberg R., Dennen P. Long-term outcomes of acute kidney injury. Adv Chron Kidney Dis 2008;15:297-307.
14. Kokot M, Buława J. AKI – ostre uszkodzenie nerek – współczesne spojrzenie na zagadnienie ostrej niezapalnej niewydolności nerek. Nefrol Dializoter Pol 2009;13(3):164-170.
15. Hsu CY, Ordoñez JD, Chertow GM et al. The risk of acute renal failure in patients with chronic kidney disease. Kidney Int 2008;74:101-107.
16. Akposso K, Hertig A, Couprie R. Acute renal failure in patients over 80 years old: 25-years' experience. Intensive Care Med. 2000;26:400-406.
17. Czempik P, Cieśla D, Knapik P, Krzych ŁJ. Czynniki ryzyka ostrego uszkodzeni nerek wymagającego leczenia nerkozastępczego na podstawie danych rejestrowych. Anest Inten Ter 2016;48(3):193-198.
18. Posadas MA, Yang V, Ho B. Acute renal failure and severe hypertension from a page kidney post-transplant biopsy. Scient World J 2010;10:1539-1542.
19. Tykarski A, Narkiewicz K, Gaciong Z. Guidelines for the Management of Hypertension. Arterial Hypertens 2015;2:53-83.
20. Sołtysiak M, Widecka K. Postępowanie w nadciśnieniu tętniczym w ostrym uszkodzeniu nerek. Arterial Hypertens 2015;19(4):203-206.
21. Rusinowicz T, Telegna A, Olędzka-Oręziak M, Życińska K, Wardyn K.A.Czynniki ryzyka ostrej niewydolności nerek u osób w wieku podeszłym. Fam Medi Care Rev 2012;14(3):424-427.
22. Selcuk NY, Odabas AR, Cetinkaya R, Tonbul HZ, San A. Frequency and outcome of patients with acute renal failure have more causes than one in etiology. Renal Failure 2000;22:459-464.
23. Oadri M, Atray NK, Vachharajani TJ. Is dialysis always justified? Ethics and economics. Hemodial Int 2004;8:93.
24. Pascual J, Liano F, Gamez C. et al. Causes and prognosis of acute renal failure in the very old. J Am Geriatr. Soc. 1998;46:721-725.
25. Snarska KK, Sejnota K, Bachórzewska-Gajewska H, Małyszko J. Ostre uszkodzenie nerek wśród pacjentów z ostrą niewydolnością oddechową. Przegl Lek 2014;71(8):423-429.

Konflikt interesów:
Autorzy nie zgłaszają konfliktu interesów

AUTOR KORESPONDUJĄCY
Grażyna Kobus
Zakład Medycyny Klinicznej
Uniwersytet Medyczny w Białymstoku
ul. Szpitalna 37, 15-295 Białystok
tel. 600 310 314
e-mail: g.kobus@wp.pl

Nadesłano: 30.05.2019
Zaakceptowano: 08.07.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

CHANGES OF NITRIC OXIDE SYNTHASE AND HYDROGEN SULFIDE IN BLOOD LYMPHOCYTES IN THE ACUTE PERIOD OF POLYTRAUMA

Nataliya Matolinets, Helen Sklyarova, Eugene Sklyarov, Andrii Netliukh
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

Introduction: Polytrauma patients have high risk of shock, septic complications and death during few years of follow-up. In recent years a lot of attention is paid to gaseous transmitters, among which are nitrogen oxide (NO) and hydrogen sulfide (H2S). It is known that the rise of NO and its metabolites levels occurs during the acute period of polytrauma. Nitric oxide and hydrogen sulfide are produced in different cell types, among which are lymphocytes.

The aim: To investigate the levels of NO, NOS, iNOS, eNOS, H2S in lymphocytes lysate in patients at the moment of hospitalization and 24 hours after trauma.

Materials and methods: We investigated the levels of NO, NO-synthase, inducible NO-synthase, endothelial NO-synthase, H2S in lymphocytes lysate in patients at the moment of hospitalization and 24 hours after trauma.

Results: The study included 20 patients with polytrauma who were treated in the intensive care unit (ICU) of the Lviv Emergency Hospital. Tissue injury was associated with an increased production of NO, NOS, iNOS, eNOS during the acute period of polytrauma. At the same time, the level of H2S decreased by the end of the first day of traumatic injury.

Conclusions: In acute period of polytrauma, significant increasing of iNOS and eNOS occurs with percentage prevalence of iNOS over eNOS on the background of H2S decreasing.

KEY WORDS: polytrauma, blood lymphocytes, NO, NOS, iNOS, eNOS, H_2S

Wiad Lek 2019, 72, 8, 1473-1476

INTRODUCTION

Critically ill trauma patients with the presence of two or more body regions injured patients have increased incidence of shock and multiple organ failure which greatly increase mortality [1,2,3]. It is established that tissue damage leads to systemic inflammatory syndrome and endothelial dysfunction with implication in pathologic process various organs and systems [4,5]. The leading role in this mechanism is played by lymphocytes, followed by natural killer cells (NK-cells), macrophages, endothelial cells, neutrophils and monocytes [6,7,8]. Subsequently, the activation of the coagulation cascade and the fibrinolytic system, activation of the stress response system with synthesis of catecholamines and cortisol together with secretion of histamine, kinines and arachidonic acid derivatives (prostaglandins, leukotrienes and thromboxane) occurs [4,9].

At the same time tissue damage is associated with a changed production of gaseous transmitters. Nitric oxide (NO), which is produced in large amounts during trauma-related tissue damage, is connected with increased expression of the inducible isoform of NO-synthase. NO is considered to be crucial signaling and effector molecule in a number of biological processes which play serious role in traumatic tissue injury [10,11]. The development of multiple organ dysfunction syndrome (MODS) during the first days after polytrauma is affiliated with the systemic inflammatory response syndrome and a proinflammatory state which is T-helper-1-Lymphocyte (Th-1) mediated [4,12].

Hydrogen sulfide is the "third" gaseous transmitters that has anti-inflammatory, anti-apoptotic, and proangiogenic effect and regulates endothelial NO-synthase modulating activity [8,13]. H2S plays a key role in tissue protection against injury as it promotes repair while simultaneously downregulating the inflammatory response [14,15].

Basic researches regarding a role of nitric oxide and H2S during acute period of polytrauma are based on measurement of these components in blood plasma. However still little is known about levels of these gaseous transmitters in blood lymphocytes.

THE AIM

To investigate the levels of NO, NOS, iNOS, eNOS, H2S in lymphocytes lysate in patients at the moment of hospitalization and 24 hours after trauma.

MATERIALS AND METHODS

The investigation included 20 patients with polytrauma who were treated in the intensive care unit (ICU) of the Lviv Emergency Hospital. In average it took about a half an hour from the time of trauma till the admission to our ICU. The collected data were analyzed in terms of age, sex

Table I. Comparable levels of NO, NOS, iNOS, eNOS and H2S in lymphocytes lysate in patients in different trauma periods

	Acute period	After 24-hours	P-value
NO, µmol/l	0,38±0,01	0,47±0,02	p<0,01
NO-synthase, nmol/min×ml	0,311±0,016	0,489±0,021	p<0,01
iNOS, nmol/min×ml	0,043±0,004	0,111±0,008	p<0,01
eNOS , nmol/min×ml	0,268±0,015	0,371±0,016	p<0,01
H_2S, µmol/l	80,75±1,87	70,57±1,98	p<0,01

and causes of polytrauma. The mean age of all patients was 38,4±5,2 years. Patients with comorbidities were excluded from the investigation. Etiologies of trauma included: motor vehicle related trauma – 78 %, injuries sustained by falls – 5 %, home accident – 17 %. In the prehospital period patients were analgosedated, immobilized and received infusion therapy during transportation. All patients with polytrauma were hospitalized to the shock-ward of the ICU where a complex of clinical, laboratory and instrumental studies were conducted to assess injury nature and severity.

The severity of patient injury was determined using the Injury Severity Score (ISS) while the APACHE II-score was used to prognoses ICU-mortality. In accordance to the ISS, all patients were included in the group of a mild course (ISS within 16-25).

All subjects were evaluated before and after shock therapy. The patients underwent standard physical, laboratory and instrumental examination according to the ATLS guidelines.

Blood tests were performed on admission and 24 hours after admission. Initial blood tests were taken to assess in blood lymphocytes after lysis procedure the levels of NO, NOS, iNOS, eNOS and H_2S. The level of H_2S was measured by reaction with N,N-dymetil-para-fenildyamin catalyzed by $FeCl_3$ [16]. The determination of isoenzyme NO-synthase activity was carried out using the method of Ravaeva, M. Y., the results were reported in units of nmol/min×ml [17].

Our monitoring and assessment studies included measurement of noninvasive central hemodynamic parameters, arterial blood gas analysis, ultrasound examination of abdomen and chest, conventional x-ray of the chest, pelvis, skull and spine, whole-body computed tomography, thoraco- and laparocentesis (if indicated). Diagnostic and therapeutic measures were performed simultaneously. Each patient underwent the correction of acid-base disorders, fluid disturbances, received respiratory support (if required), antibacterial therapy, analgosedation, correction of hemostatic disorders as well as symptomatic therapy. Urgent and lifesaving surgical procedures were performed as indicated.

Continuous characteristics were represented using means and standard deviations. Independent Student t-test was used to compare discrepancies between the different groups. Analysis of data was performed and expressed as mean ± SD.

RESULTS AND DISCUSSION

During the analysis it was revealed that among the investigated patients the majority were in the moderate severity group (ISS and APACHE II). Intensive care therapy and surgical treatment during first 24 hours lead to improvement of hemodynamic parameters. On the second day an increase of systolic blood pressure and a decrease of heart rate were noted. Within the first 24 hours, the diuresis increased from 800 to 1300 ml. Standardization of hemodynamic parameters and 24-hours diuresis evidenced about patients' improvement from traumatic shock. Systemic inflammation developed after traumatic shock and manifested by the increase of systemic inflammation indicators (leukocytosis, neutrophilia), hyperglycemia, hyperfibrinogenemia, and subfebrile body temperature. Blood biochemical parameters during hospitalization were in the range of normal levels.

There were statistically significant differences in the levels of NO, NO-synthase, iNOS and eNOS, H2S in lymphocytes lysate at the time of admission and 24 hours after polytrauma.

Tissue injury is associated with an increased expression of inducible NO-synthase that leads to NO overproduction and cytotoxic effects which cause additional damage in polytrauma [11,18]. The level of Nitric Oxide in lymphocytes lysate increased after 24-hours of intensive therapy from 0,38±0,01 to 0,47±0,02 µmol/l.

Excessive NO production which is catalyzed by iNOS plays an important role in the development of septic shock [19]. The levels of NO-synthase increased in 1,57 times from 0,311±0,016 to 0,489±0,021 nmol/min×ml. The level of eNOS increased in 1,36 times from 0,268±0,015 to 0,371±0,016 nmol/min×ml. The biggest increase, by 2,63 times, occurred in the level of iNOS, which increased from 0,043±0,004 to 0,111±0,008 nmol/min×ml. Further a positive correlation between NOS and iNOS (r=0.45) and between NOS and eNOS (r=0,97) which remained high during the first day after polytrauma was discovered. Against this background H2S levels decreased from 80,75±1,87 to 70,57±1,98 µmol/l.

The results of the study showed the ambiguity of changes in the NO-synthase and H2S indices in lymphocytes lysate in patients during the acute period of polytrauma. In general, there is a significant increase in the level of NO-synthase, iNOS, and eNOS with a simultaneous H2S reduction by the end of the first day after the polytrauma

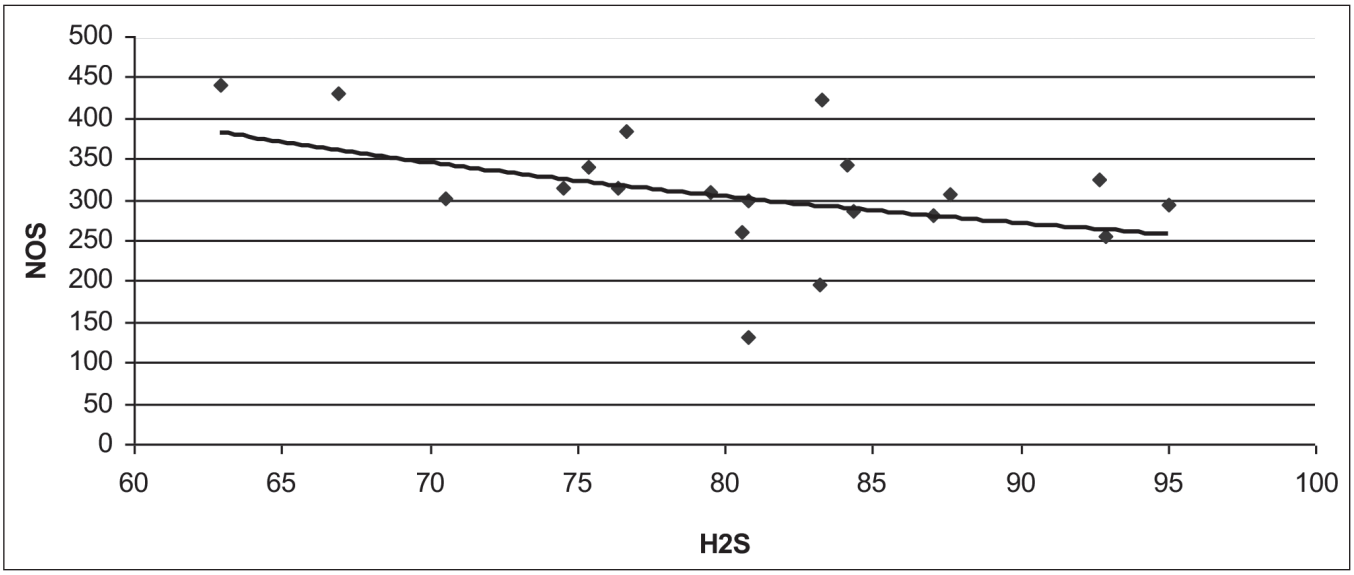


Figure 1. Correlation between levels of H2S and NO-Synthase

on the background of lifesaving stabilization of patients' hemodynamic parameters.

The decrease of H2S level occurs due to the exhaustion of H2S production in response to polytrauma and therefore leads to the attenuation of its defense role. However, attention is drawn to the change in the percentage of iNOS to eNOS, namely, the iNOS increase in the proportion from 14.16±1.12% at the start of treatment to 21.86±1.04% 24 hours after the injury. At the same time the level of eNOS, which in physiological condition plays a stabilizing role in the protection of the endothelium, increases.

Endothelial NO synthase is involved in vasodilation, inhibition of platelet aggregation and adhesion, inhibition of leukocyte adhesion and vascular inflammation, control of vascular smooth muscle proliferation [19]. The role of hydrogen sulfide in the pathogenesis of tissue damage needs further investigation. It is known that H2S, the third endogenous gaseous signaling molecule, plays an important role in vasodilation and endothelial NO-synthase modulation. Our data indicates a negative correlation between NO synthase and H2S (r-0.45).

CONCLUSIONS

During the first day of acute period of polytrauma there is increasing of inducible and endothelial NO-synthase in lymphocytes lysate.

On the background of increasing iNOS and eNOS there is a prevalence of iNOS over eNOS.

During the first day of polytrauma there is significant decrease of H2S that may indicate on disbalance between pro-inflammatory effects of iNOS and anti-inflammatory effects of H2S.

REFERENCES

1. MacKenzie EJ. Epidemiology of injuries: current trends and future challenges. Epidemiol Rev. 2000;22:112–119.
2. Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma. Injury. 2007;38:1336–1345.
3. Lausevic Z, Lausevic M, Trbojevic-Stankovic J, Krstic S, Stojimirovic B. Predicting multiple organ failure in patients with severe trauma. Can J Surg. 2008;51:97–102.
4. Trancă SD, Petrişor CL, Hagău N. Biomarkers in polytrauma induced systemic inflammatory response syndrome and sepsis - a narrative review. Romanian journal of anaesthesia and intensive care. 2014;21(2):118-122.
5. Roy J, Galano J-M, Durand T, et al. Physiological role of reactive oxygen species as promoters of natural defenses. The FASEB Journal. 2017;31(9).3729-3745.
6. Menges T, Engel J, Welters I, et al. Changes in blood lymphocyte populations after multiple trauma: association with posttraumatic complications. Crit Care Med. 1999;27(4):733-40.
7. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. Crit Care. 2010;14:1–18.
8. Namas RA, Mi Q, Namas R, et al. Insights into the Role of Chemokines, Damage-Associated Molecular Patterns, and Lymphocyte-Derived Mediators from Computational Models of Trauma-Induced Inflammation. Antioxid Redox Signal. 2015;23(17):1370-87.
9. Papurica M, Rogobete AF, Sandesc D, et al. Advances in Biomarkers in Critical Ill Polytrauma Patients. Clin Lab. 2016;62(6):977-986.
10. Hampl V, Bibova J, Banasova A, et al. Pulmonary vascular iNOS induction participates in the onset of chronic hypoxic pulmonary hypertension. Am J Physiolo Lung Cell Mol Physiol. 2006;290(1):L11-L20
11. Beitl E, Banasova A, VIcek M, Mikova D, Hampl V. Nitric oxide as an indicator for severity of injury in polytrauma. Bratisl Lek Listy. 2016;117(4):217-20.
12. Serve R, Sturm R, Schimunek L, et al. Comparative Analysis of the Regulatory T Cells Dynamics in Peripheral Blood in Human and Porcine Polytrauma. Front Immunol. 2018;9:435.
13. Bibli SI, Szabo C, Chatzianastasiou A, Luck B, Zukunft S, Fleming I, Papapetropoulos A. Hydrogen Sulfide Preserves Endothelial Nitric Oxide Synthase Function by Inhibiting Proline-Rich Kinase 2: Implications for Cardiomyocyte Survival and Cardioprotection. Mol Pharmacol. 2017;92(6):718-730.

14. Li-Long. The Role of Hydrogen Sulfide on Cardiovascular Homeostasis: An Overview with Update on Immunomodulation/. Front Pharmacol. 2017;8:686.
15. Rivers-Auty J, Bhatia M. Hydrogen sulphide, systemic inflammatory response syndrome and compensatory anti-inflammatory response syndrome following sepsis. OA Inflammation. 2013;1(1):2.
16. Dombkowski RA, Russell MJ, Olson KR. Hydrogen sulfide as an endogenous regulator of vascular smooth muscle tone in trout. Am J Physiol Regul Integr Comp Physiol. 2004;286(4):678-85.
17. Ravayeva MY, Chuyan EN. Changes of the Nitric Oxide Synthesis Activity under influence of low-intensity millimeter radiation. Tavric National University Journal. 2011;4:201-210.
18. Matolinets N, Sklyarova H, Sklyarov A. Nitric oxide and hydrogen sulfide – markers of acute period of polytrauma. Wiadomości Lekarskie 2018;71(8):1489-1492.
19. Förstermann U, Li H. Therapeutic effect of enhancing endothelial nitric oxide synthase (eNOS) expression and preventing eNOS uncoupling. Br J Pharmacol. 2011;164(2):213-223.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Nataliya Matolinets
Department of anesthesiology and intensive care FPGE
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
tel. +380677741348
e-mail: nmatolinets@gmail.com

Received: 10.02.2019
Accepted: 03.06.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

CORRECTION OF ADIPOCYTE SECRETION DISORDERS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE WITH OVERWEIGHT AND OBESITY

Kateryna V. Pivtorak[1], Natalia A. Shevchuk[2], Natalia A. Pivtorak[1], Iryna V. Fedzhaga[1]
[1]NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE
[2]REGIONAL CLINICAL ENDOCRINOLOGY CENTER, VINNYTSIA, UKRAINE

ABSTRACT

Introduction: The attention of scientists from many countries is focused on hormonal substances - adipokines at the present time. Lowering the level of the hormone adiponectin plays a central role in the development of obesity and cardiovascular disease in humans.

The aim of the work is to determine the effect of complex therapy of thiotriazolin and L-lysine escinate on adipocyte secretion indices in patients with non-alcoholic fatty liver disease (NAFLD) with overweight and obesity.

Materials and methods: 135 patients with overweight and obesity were examined, 46 of which were overweight (BMI-25-29.9 kg / m2), 34 were obesity grade I (BMI-30-34.9 kg / m2), 20 - Obesity II degree (BMI-35-39.9 kg / m2). 35 patients had normal body mass (BMI 18-24.9 kg / m2). We also examined 20 practically healthy persons. The age of the examinees varied, the median age was 55 years (intercourt scale Q1-Q3 from 40 to 61 years). The verification of the diagnosis of NAFLD was conducted in accordance with the recommendations of the Unified Clinical Protocol.

Results: The additional use of thiotriazolin and L-lysine escinate significantly influenced the adiponectin concentration level. Compared with the period before treatment, the adiponectin level increased in patients with overweight and obesity in 1,6 times ($p < 0.05$). Compared to baseline, the adiponectin content in patients with NAFLD increased by 24.6-27.6% ($p < 0.05$). Also, the level of leptin decreases significantly in patients with overweight and obesity ($p < 0.05$).

Conclusions: Integrated therapy with thiotriazolin and L-lysine escinate is an effective way to normalize the level of adipokines in patients with NAFLD with overweight and obesity.

KEY WORDS: nonalcoholic fatty liver disease, treatment, adipokines, insulin resistance

Wiad Lek 2019, 72, 8, 1477-1480

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver disease and it is closely related to metabolic syndrome. NAFLD includes a wide range of liver changes: liver steatosis, liver fibrosis, liver cirrhosis, hepatocellular carcinoma. Obesity is one of the components of metabolic syndrome, which is formed against the background of insulin resistance syndrome (IR). The endothelium dysfunction has one of the leading places in the modern concept of the pathogenesis of NAFLD [1]. It is believed that reducing the fat-derived, adiponectin hormone plays a central role in the development of IR, type 2 diabetes, obesity and cardiovascular disease in humans [2, 3].

The attention of many scientists focused on the hormone-like substances – adipokines at present time. The important role of the mechanisms of oxidative stress of adipokines in promoting the development of NAFLD is universally accepted. For leptin it is proinflammatory and profibrotic, and for adiponectin it is anti-inflammatory and insulin-sensitizing [4].

Fundamental research has shown that adiponectin increases insulin sensitivity [5], has anti-atherogenic and anti-inflammatory properties [6]. The results of the studies allowed to clarify some mechanisms that combine the development of obesity, IP and fibrogenesis processes [7].

Taking into account the body mass index (BMI), the ratio of leptin / adiponectin correlates with the presence or absence of metabolic syndrome in patients and is used as a biochemical marker for the risk of developing an IR [8]. It is believed that leptin is a link between adipocytes and β-cells of the pancreas and stimulates insulin secretion while reducing sensitivity to it.

Adipocytes of visceral adipose tissue, possessing high sensitivity to the lipolytic action of catecholamines and low sensitivity to the anti-lipolytic action of insulin, secrete free fatty acids directly into the portal vein. The high concentrations of the ones, on the one hand, become a substrate for the formation of atherogenic lipoproteins, on the other hand, prevent the binding of insulin to hepatocytes, which leads to hyperinsulinemia and potentiates IR.

However, the results obtained from leptin studies with NAFLD are more controversial and heterogeneous compared to those obtained with the use of adiponectin [9]. Insulin resistance plays a fundamental role in the pathogenesis of NAFLD. This served as a prerequisite for the study

of the relationship of IR, compensatory hyperinsulinemia and NAFLD.

Most therapeutic approaches are based on glycemic correction and reduction of IR, using drugs included in the treatment regimens of type 2 diabetes. In order to reduce the risk of CVD, patients in this group appoint agents that affect the some components of the metabolic syndrome [10].

Adiponectin can be considered as a biomarker of liver diseases such as NAFLD, NASH and liver tumors which are associated with hypoadiponectinemia. Increased adiponectin is a marked improvement in many diseases of the liver that demonstrates the outstanding role of adiponectin in protection against liver syndromes. Based on the positive effects of adiponectin, future efforts should aim at increasing adiponectin and its hepatic receptors through pharmacological strategies [11].

We have proposed and tested on the model of NAFLD [12] the applying of a new endothelioprotector of direct action - angiolin, which combines in its structure fragments of the molecules of thiotriazolin and L-lysine escinate and has high anti-ischemic, cardioprotective, neuroprotective, antioxidant and anti-inflammatory properties.

THE AIM

The purpose of the study is to determine the effect of complex therapy of thiotriazolin and L-lysine escinate on adipocyte secretion in patients with overweight and obesity.

MATERIALS AND METHODS

This work is a fragment of research work of the Department of Clinical Pharmacy and Clinical Pharmacology at National Pirogov Memorial Medical University "Efficiency and safety of chemotherapeutic agents and metabolic correctors in conditions of comorbid pathology",

The study was conducted in accordance with the requirements of the Helsinki Declaration. The permission of the bioethics committee of the National Pirogov Memorial Medical University was obtained for the study.

135 patients with overweight and obesity were examined, 46 of them were overweight (BMI-25-29.9 kg/m2), 34 - obesity I degree (BMI-30-34.9 kg/m2), 20 - Obesity of the II degree (BMI 35-39.9 kg/m2). 35 patients had normal body mass (BMI 18-24.9 kg/m2). We also examined 20 practically healthy persons. The age of the examinees varied, the median age was 55 years (intercourt scale Q1-Q3 from 40 to 61 years). An informed consent was obtained for the use of materials in scientific research from each examined patient.

All patients were examined according to a protocol for a comprehensive assessment of suspected NAFLD patients.

Elastometry was carried out according to the method - FibroScan (Echosens, France) to assess the presence and severity of liver fibrosis.

All patients were divided into three groups. Patients in the first group received baseline therapy (n = 50), according to the MOH protocol and the joint recommendations of the European Association for the Study of Liver (EASL), the European Association for the Study of Diabetes (EASD), the European Association for the Study of Obesity (EASO). Patients in the second group (n = 60) prescribed baseline therapy and thiotriazoline 100 mg for 2 tablets 3 times a day for 60 days. Patients in the third group (n = 25) were prescribed 5 ml of 0.1% L-lysine escinate in 100 ml of 0.9% sodium chloride solution in a day, except for the treatment of patients in the second group.

The control group consisted of 20 practically healthy persons without pathology of the hepatobiliary system.

Blood collection for biochemical studies was carried out in the morning by venepuncture of the elbow vein. The activity of enzyme markers of cytolysis was determined according to generally accepted methods: alanine aminotransferase (ALT), aspartate aminotransferase (AST), markers of cholestasis - alkaline phosphatase (LF), level of general bilirubin and its fractions, lipid metabolism indices: total cholesterol, low density lipoprotein cholesterol (LDL cholesterol)), triglycerides, high density lipoprotein cholesterol (HDL) in serum after centrifugation

Glucose level by glucose oxidase method (automatic glucose analyzer Bisens line, Germany), insulin by chemiluminescent method (Access2, USA), was evaluated on an empty stomach and 2 hours after receiving for 5 minutes 75 g of glucose dissolved in 250-300 ml of water. The presence of IR was assessed by the level of the HOMA-IR index (Homeostasis Model Assessment Insulin Resistance), which was calculated by the formula:

$$HOMA - IR = (insulin\ concentration\ (mcU/\ ml)\ X\ glucose\ onset\ (mmol\ /\ l))\ /\ 22.5$$

To determine the presence and degree of severity of IP, the method of determining the coefficient of IP for F.Caro, which was calculated by the formula:

$$\text{Coefficient}\ Caro = (glucose\ (mmol\ /\ l))\ /\ (insulin\ (mcU/\ ml))$$

The criteria for the presence of IP were considered to be HOMA higher than 2.77, the Caro index was less than 0.33.

The levels of adipokines (leptin and adiponectin) were determined on the Human Fax Leptin Quantification ELISA Kit (R & D, USA), Human Adiponectin ELISA (BioVendor, Czech Republic) on the immune enzyme analyzer Stat Fax 300 (USA). The ratio between the content of adiponectin and leptin was represented as the decimal logarithm (log A/L).

The statistical analysis of the obtained results was carried out using Statsoft software STATISTICA 8 using parametric and non-parametric methods for evaluating the obtained results. The data is presented as median (Me) and interquartile scope (Q1-Q3). Normality of distribution was checked using the Kolmogorov-Smirnov test. In connection with the asymmetric distribution, the Man-Whitney nonparametric rank U-criterion was used. The statistical significance of the difference between the comparative values was considered probable at $p < 0.05$.

RESULTS AND DISCUSSION

The results of the study (Figure 1) showed that the adiponectin concentrations before treatment in patients

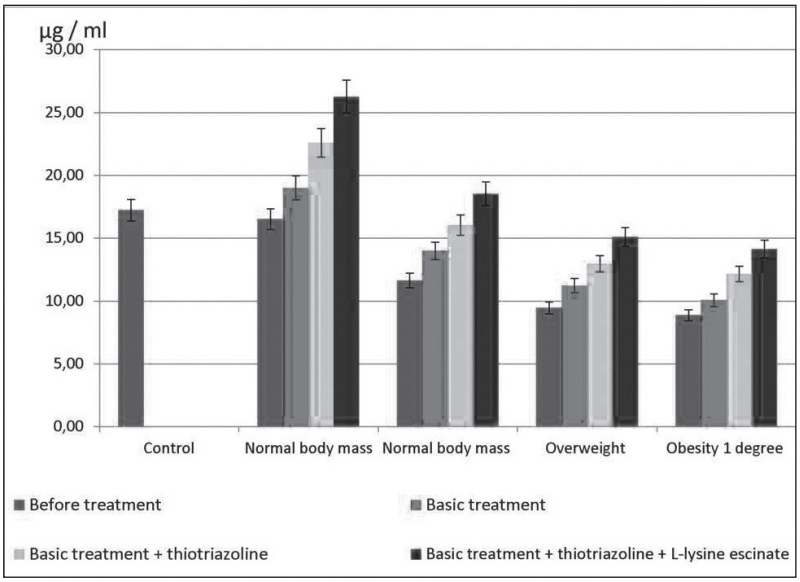


Fig. 1. The level of adiponectin in patients with overweight and obesity before and after treatment.

Table I. Indicators of leptin (ng / ml) in patients with overweight and obesity before and after treatment, Me (Q1-Q3)

	Control	NAFLD			
	Normal body mass	**Normal body mass**	**Overweight**	**Obesity 1 degree**	**Obesity 2 degree**
Before treatment	4,26 (3,83-5,25)	5,76 (5,13-6,20)	8,55 (7,50-10,00)	12,50 (11,58-13,00)	16,10 (15,40-16,90)
Basic treatment (n = 50)		5,05 (4,83-5,60)	7,60 (6,85-8,68)	10,90* (10,68-11,50)	14,80* (14,13-14,95)
Basic treatment + thiotriazolin (n = 60)		4,70 (4,30-5,40)	7,25 (5,70-8,00)	9,90*# (9,43-10,10)	10,10*# (9,90-10,20)
Basic treatment + thiotriazolin + L-lysine escinate (n = 25)		4,50* (4,30 -4,80)	5,50*# (5,20-6,63)	5,45*#Δ (5,20-5,78)	7,55*#Δ (6,63-8,03)

Note. * - statistically significant differences (p <0,05) according to the Mana-Whitney criterion in comparison with the indicators before treatment.
Are statistically significant differences (p <0,05) according to the Mana-Whitney criterion in comparison with the indicators after the basic treatment.
Δ - statistically significant differences (p <0,05) according to the Mana-Whitney criterion compared to those after baseline treatment with additional administration of thiotriazolin.

with overweight and obesity were statistically significantly lower than those of practically healthy individuals. Basic treatment in patients with NAFLD showed statistically significant differences in the increase in adiponectin concentrations in obesity classes I and II. In the group with additional administration of thiotriazolin, adiponectin concentrations were statistically significantly higher after treatment, not only in obese groups I and II, but also in patients with excess and normal body weight (p <0.05).

The additional use of thiotriazolin and l-lysine escinate significantly influenced the adiponectin concentration level. The level of adiponectin increased in patients with overweight and obesity in patients with overweight and obesity of 1.6 times (p <0.05) compared with the period before treatment. Adiponectin levels in NAFLD patients increased by 24.6-27.6% (p <0.05) compared with baseline therapy.

Adiponectin also attracts the attention of specialists in cardiovascular disease, due to the fact that many studies have shown that hypoadiponectinemia is found in arterial hypertension, obesity, coronary heart disease, heart failure, metabolic syndrome. Reduced expression of adiponectin is considered a new important risk factor for atherosclerosis [13].

We observed that the concentration of leptin in serum in patients with NAFLD with body weight and obesity was statistically significantly higher (2.05-3.78 times) compared with those of practically healthy individuals. The revealed level of leptin showed that baseline treatment slightly reduced its level (Table I). The use of thiotriazoline significantly decreases the level of leptin for patients with normal body mass by 18.4%, with overweight by 15.2%, for patients with obesity I degree - by 20.8%, for patients with obesity II degree - by 37.3%

The additional use of thiotriazoline and L-lysine escinate significantly reduces the level of leptin in patients with normal weight by 21.9%, for patients with overweight by 35.7%, for obese patients with grade I - by 56.4%, for patients with Obesity of the II degree - by 53.1% ($p < 0.05$).

The HOMA-IR index has changed in the course of treatment. In the application of basic treatment for patients with NAFLD, the index of HOMA-IR decreased slightly ($p > 0.05$). In the group with additional administration of thiotriazoline, the HOMA-IR index was statistically significantly lower in patients with normal body weight by 29.4%, with an overweight of 22.9%, with obesity I degree - by 39.2%, with obesity II degree - by 54,7% ($p < 0,05$) after treatment. The additional use of thiotriazolin and L-lysine escinate significantly influenced the index of HOMA-IR. Compared with baseline therapy, the index of HOMA-IR decreased in patients with NAFLD with normal body weight by 37.2%, with overweight - by 39.2%, with obesity 1 degree - by 36.1%, with obesity II degree - by 33.0% ($p < 0.05$).

In the study of the relationship of adipokins with the level of the HOMA-IR index, the presence of an inverse correlation interconnection of average strength ($r = -0,66$, $p = 0,0000$) with adiponectin concentration and strong direct correlation relationship ($r = 0, 80$, $p = 0.0000$) with the concentration of leptin.

Strategies that are currently used to raise adiponectin levels for treatment and prevention of IR are discussed in modern scientific literature [14].

CONCLUSIONS

Complex therapy with the use of thiotriazolin and L-lysine escinate is an effective way to normalize the level of adipokines and to reduce insulin resistance in patients with overweight and obesity.

REFERENCES

1. Khukhlina OS, Antoniv AA, Mandryk OYe, Smandych VS, Matushchak MR. The role of endothelial dysfunction in the progression mechanisms of non-alcoholic stepage hepatitis in patients with obesity and chronic kidney disease. Wiad Lek. 2019;72(4):523-526.
2. Ghoshal K, Bhattacharyya M. Adiponectin: Probe of the molecular paradigm associating diabetes and obesity. World J Diabetes. 2015 February 15; 6(1): 151-166. Doi: 10.4239 / wjd.v6.i1.151
3. Achari A, Jain S. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. Int. J. Mol. Sci. 2017 June 21; 18 (6). PII: E1321. doi:10,3390 / ijms18061321.
4. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. Q J Med. 2010 February;.103(2): 71-83. doi: 10.1093 / qjmed / hcp158
5. Silva TE, Colombo G, Schiavon L L. Adiponectin: A multitasking player in the field of liver diseases. Diabetes & metabolism. 2014 April;40(2):95-107. doi: 10,1016 / j.diabet.2013.11.004
6. Adolph TE, Grander C, Grabherr F, Tilg H. Adipokines and non-alcoholic fatty liver disease: multiple interactions. Int J Mol Sci. 2017 July 29;18(8). PII:E1649. doi:10,3390 / ijms18081649
7. Labruna G, Pasanisi F, Nardelli C, et al. High leptin/adiponectin ratio and serum triglycerides are associated with an "at–risk" phenotype in young severely obese patients. Obesity. 2011 July;19(7):1492-1496. doi: 10,1038 / oby.2010.309
8. Zhuo Q, Wang Z, Fu P, et al. Comparison of adiponectin, leptin and leptin to adiponectin ratio as diagnostic marker for metabolic syndrome in older adults of Chinese major cities. Diabetes Res.Clin.Pract. 2009 April;84(1):27-33. doi: https://doi.org/10.1016/j.diabres.2008.12.019
9. Adolph T, Grander C, Grabherr F, Tilg H. Adipokines and non-alcoholic fatty liver disease: multiple interactions. Int. J. Mol. Sci. 2017 August;18(8):1649. doi: 10.3390 / ijms18081649
10. Maurantonio M, Ballestri S, Odoardi M R, Lonardo A, Loria P. Treatment of atherogenic liver based on the pathogenesis of nonalcoholic fatty liver disease: a novel approach to reduce cardiovascular risk? Arch Med Res. 2011 July;42(5),337-353. doi: 10,1016 / j.arcmed.2011.08.004
11. Gamberi T, Magherini F, Modesti A, Fiaschi T. Adiponectin signaling pathways in liver diseases. Biomedicines. 2018 May;6(2):Article 52. doi: 10,3390 / biomedicines6020052
12. Pivtorak KV, Mazur IA, Voloshin MA.Correction of metabolic disorders caused by non-alcoholic fatty liver disease. Deutscher wissenschaftsherold. 2017;(4):74-7. DOI:10.19221/2017424. http://dwherold.de/onewebmedia/2017/4-2017/Pivtorak%2074-77.pdf.
13. Szmitko PE, Teoh H, Stewart DJ, Verma S. Adiponectin and cardiovascular disease: state of the art?. Am.J.Physiol.Heart.Circ.Physiol. 2007 April; 292(4):H1655-63. doi: 10,1152 / ajpheart.01072.2006
14. Hossain MM, Mukheem A, Kamarul T. The prevention and treatment of hypoadiponectinemia-associated human diseases by up-regulation of plasma adiponectin. Life Sci. 2015 August;135(15):55-67. doi.org/10.1016/j.lfs.2015.03.010

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Kateryna V. Pivtorak
National Pirogov Memorial Medical University
Vinnytsia, Ukraine
tel: +380632883016
e-mail: ek3727@gmail.com

Received: 01.03.2019
Accepted: 21.06.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

HISTOTOPOGRAPHY OF THE OVIDUCTS IN FETUS

Nataliia Kuzniak[1], Tetiana Protsak[1], Oleg Marchuk[1], Larysa Fedoniuk[2], Alex Kamyshnyi[3], Nataliia Penteleichuk[1], Denys Stoliar[1], Roman Dmytrenko[1]

[1]HIGHER STATE EDUCATIONAL ESTABLISHMENT OF UKRAINE "BUKOVINIAN STATE MEDICAL UNIVERSITY", CHERNIVTSI, UKRAINE
[2]STATE HIGHER EDUCATIONAL INSTITUTION "I. HORBACHEVSKY STATE MEDICAL UNIVERSITY MINISTRY OF PUBLIC HEALTH OF UKRAINE", TERNOPIL, UKRAINE
[3]ZAPORIZHZHIA STATE MEDICAL UNIVERSITY, ZAPORIZHZHIA, UKRAINE

ABSTRACT

Introduction: Nowadays, during formation and development of medicine, the main sector of health care is the protection of maternity and childhood. According to the Ministry of Health of Ukraine, inflammatory diseases of the reproductive system make up 60-65% of all gynecological diseases, among them inflammation of the fallopian tubes and ovaries is 79,6%, which occurs predominantly at young age.

The aim is to study the microscopic structure of the oviducts wall in different anatomical parts during the embryonic period of human ontogenesis.

Materials and methods: Were studied uterine tubes of the 4-10 months' embryos. We used a light microscopy method and morphometry to describe the peculiarities of oviducts' infundibulum, isthmus and ampullary part in fetus from 81 mm to 375 mm PCL.

Results: The histotopography of the oviducts tunics and its parts (infundibulum, ampullary part, isthmus and uterine part) in the fetal period of ontogenesis is described in the article. The thickness of the oviduct wall in the dynamics of the fetal period increases in the direction from the infundibulum to the uterine part of the tube. The main part of the thickness of the oviduct wall is presented by muscles. The thickness of the circumferential muscle layer predominates over the thickness of the longitudinal in 2.5-2.8 times. Mucosa is thicker the isthmus, thinnest in the ampullary part of the uterine tube.

Conclusions: The thickness of the infants oviduct wall in the dynamics increases in the direction from its infundibulum to the uterine part. The main part of the thickness of the oviduct wall is the tunica muscularis. The thickness of the circular muscle layer predominates the thickness of the longitudinal 2.5-2.8 times. Mucosa is thicker in the isthmus, the thinnest in the ampullary part. It is expedient to study the histotopography of the oviduct in the neonatal period of human ontogenesis.

KEY WORDS: uterine tubes, fetus, human

Wiad Lek 2019, 72, 8, 1481-1485

INTRODUCTION

Nowadays, during formation and development of medicine, the main sector of health care is the protection of maternity and childhood, which includes: increasing the birth rate, improving the quality of reproductive health, matching the standard of living, in particular in the embryonal and early neonatal periods [1, 2, 3]. The level of perinatal mortality in some regions of Ukraine ranges from 18.66% to 24.22%. Congenital malformations occupy the third place among the causes of perinatal mortality. In addition, in Ukraine, there is a tendency towards a decline in fertility, which is due to an increase in morbidity, which, in turn, is due in part to the untimely diagnosis of perinatal disorders of human development. According to statistics, in Ukraine, annually, 3600-3700 children with congenital anomalies are found, among which a significant proportion belongs to the defects of female reproductive organs [4, 5, 6]. According to the Ministry of Health of Ukraine, inflammatory diseases of the reproductive system make up 60-65% of all gynecological diseases, among them inflammation of the fallopian tubes and ovaries is 79,6%, which occurs predominantly at young age [7, 8].

The study of OD morphogenesis acquires a practical value, which is due to the introduction of the newest methods of prenatal diagnosis [9, 10].

THE AIM

To study the microscopic structure of the OD wall in different anatomical parts during the embryonic period of human ontogenesis.

MATERIALS AND METHODS

Were studied uterine tubes of the 19 embryos 4-10 months. The histotopographic study was conducted mainly on the right OD. In the 7- and 10-month-old embryos (2 cases), were studied parts of the left OD. The specimens were fixed in 5-6% solution of neutral formalin for 2 weeks. After fixation the OD were washed in running water for 1-2 days. Dehydration of specimens was carried out by carrying them through a battery of spirits of increasing strength (from 30 (to put degrees) to absolute, an exposition - 1-3 days), after which specimens were filled with paraffin. The series of histological sections from paraffin blocks were performed in one of three planes.

The sections of OD were stained with hematoxylin and eosin. Morphometry was performed using a microscope MBS-10 and a microscope MBI-10 with a micro line.

RESULTS AND DISCUSSION

In fetus 81,0 mm PCL (4 months), the lumen of OD is lined by single-layer columnar epithelium. The thickness of the mucosa along the length of the tube is almost the same (40-50 microns). The lamina propria of the mucous membrane is not well developed. The lamina muscularis is represented by a thick layer of mesenchyme in the thickness of 200-210 μm. There are a large number of the circular and longitudinally oriented muscle fibers. From the outside of the OD there is a mesothelium, which, without clear boundaries, passes into flattened cells of adjacent organs and structures. The lumen of the OD in the isthmus is slit-shaped, in ampullary part it has irregular shape.

In fetus 186,0-270,0 mm PCL (6-7 months) the lumen of the infundibulum is similar to the lumen of the ampullary part of the OD. The epithelium consists of columnar cells; cilia are not visible. Cell nuclei are located at different levels: in the center or near the basal pole (secretory cells). The epithelium is well separated from lamina propria of the mucosa. Basal membrane is revealed. The stroma of each fimbria is formed by loose connective tissue. Lamina propria of the mucosa contains thin collagen fibers and small cells with dark basophilic nuclei. The thickness of the mucous membrane of the infundibulum is 38.0 μm, the diameter of the lumen is 482.0 ± 6.0 μm. The tunica muscularis of the infundibulum is represented by two layers of smooth muscle cells (internal - circular, outer - longitudinal). The thickness of the longitudinal muscle layer is 10.0 ± 1.0 μm, circular - 8.0 ± 1.0 μm. In addition to circular and longitudinal fibers, oblique strands of myocytes are detected. Between myocytes in the layers of loose connective tissue, are located arterioles, which are surrounded by circularly oriented smooth muscle cells and adventitial cells, as well as thin-walled venules, surrounded by collagen fibers and single adventitial cells. Most of the vessels are concentrated in the outer layer of the tunica muscularis. Adventitia is represented by a loose connecting tissue, in which there are collected in bundles thin collagen fibers. Between the fibers there are small cells - fibroblasts and adventitial cells. The adventitia is covered with flat mesothelial cells.

The lumen of the ampullary part of the OD (1,5 ± 0,005 mm) is covered by numerous high, considerably branched folds of the mucosa - fimbriae. The epithelium of the mucosa in the ampullary part is columnar, the nuclei are mostly oval shaped, they are located in the central part of the cells. Among the epithelial cells are detected secretory cells with larger nuclei. In the stroma of each fimbria, formed by a loose connecting tissue, thin collagen fibers are determined, as well as small cells with dark basophilic nuclei. In lamina propria of the mucosa are located arterioles, venules and lymphatic capillaries. The thickness of the mucosa between the folds is 96.0 ± 5.0 μm. The lamina muscularis of the ampullary part of the OD, as well as in the infundibulum, is represented by two layers of smooth muscle cells - internal (circular) and external (longitudinal). The thickness of the longitudinal muscle layer reaches 122.0 ± 10.0 μm, circular - 246.0 ± 10.0 μm. In addition to the circular and longitudinal fibers, thin bundles of oblique-directed myocytes are detected. In the layers of loose connective tissue are present arterioles, venules and lymphatic capillaries. It should be noted that the blood vessels in microcirculatory bed of ampullary part of the OD are in less number than the blood vessels in the OD infundibulum (Fig. 1).

The lumen of the isthmus (624.0 ± 20.0 μm) is narrowed, slit-shaped, with irregular boundaries. The mucosa forms from 2 to 4 low protrusions, lined by a cuboidal epithelium, cells of which have oval nuclei. The epithelium is separated from the lamina propria of the mucosa by a basement membrane. In the connective tissue of lamina propria are present thin collagen fibers, a large number of cells, which are stem and differentiated fibroblasts. In the lamina propria also are determined arterioles, venules and lymphatic capillaries. The thickness of the mucous membrane between the folds reaches 148.0 ± 5.0 μm. The tunica muscularis of the isthmus predominates the thickness of the mucosa. The thickness of the circular layer of the tunica muscularis is 398.0 ± 20.0 μm, and the longitudinal - 156.0 ± 5.0 μm. In this layer the blood vessels of the microcirculatory bed are located.

The lumen of the uterine tube (358.0 ± 10.0 μm) is considerably narrowed. The mucosa forms single, epithelial-lined folds. The surface of folds is partly covered by a simple columnar epithelium, which is lying on the basement membrane. Among the columnar cells, there era cells with light and dark nuclei. The lamina propria of the mucosa is represented by a large number of small spindle-shaped flattened cells with dark elongated nuclei. Some cells have a large round-shaped nucleus, in which there are from 1 to 3 nucleoli (differentiated fibroblasts).

The localization of cells in the connective tissue is local. Their large number is observed under the basal membrane. Nearest tunica muscularis number of cells decreases and between them are lying thin collagen fibers. The thickness of the mucosa in this part of the tube is 112.0 ± 6.0 μm. Myocytes form the inner (circular) and outer (longitudinal) layers, without a clear border between them. Myocytes in the longitudinal layer are arranged in groups in the circular layer, and between the myocytes of the outer layer are circular groups of smooth muscle cells. Between the bundles of the muscle cells of tunica muscularis is present connective tissue where thin wavy collagen fibers and small cells with round-shaped dark nuclei are determined.

Throughout the area of muscles, lymphatic capillaries appear as a short and elongated tubules. Some of them are anastomosed with each other, in some areas there are sinusoidal enlargements. Lymphatic capillaries are covered by thin endothelial layer, some of them contain valves. Lymphatic capillaries are accompanied by bundles of collagen fibers, fibroblasts and myocytes.

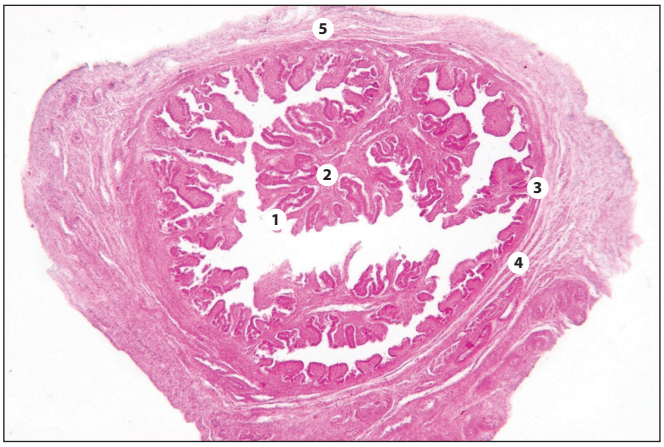


Fig. 1. Ampullary part of the oviduct of the fetus 280,0 mm PCL. Specimen. Stained with Haematoxylin and eosin x 40: 1 – epithelium; 2 – lamina propria of the mucosa; 3 – circular layer of the tunica muscularis; 4 – longitudinal layer of the tunica muscularis; 5 – adventitia.

Fig. 2. Infundibulum the oviduct of the fetus 340,0 mm PCL. Stained with Haematoxylin and eosin x 40: 1 – folds; 2 – mesentery of the uterine tube.

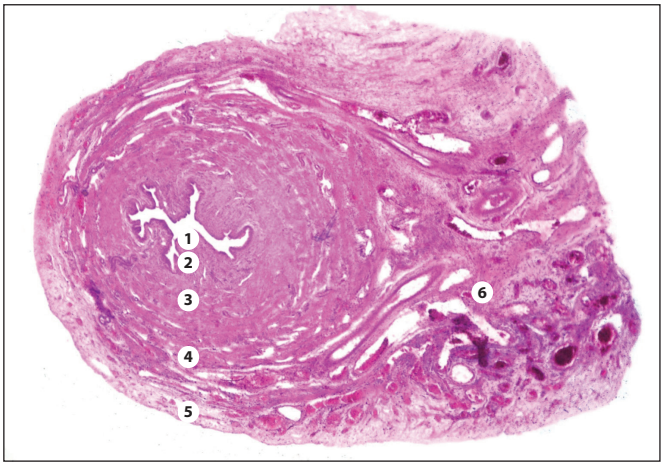


Fig. 3. The isthmus of the oviduct of the fetus 370,0 mm PCL. Stained with Haematoxylin and eosin x 40: 1 – mucosa; 2 – lamina propria of the mucosa; 3 – circular muscular layer; 4 – longitudinal muscular layer; 5 – adventitia; 6 – mesentery of the uterine tube.

The tunica muscularis contains small arteries, the wall of which is represented by three layers of cells: endothelial cells, muscle cells and adventitial cells. Near the arteries are located venules, which lined by a layer of the endothelium. The adventitia consists of connective tissue that contains thin bundles of collagen fibers, between which diffuse fibroblasts are located. Between the bundles of fibers there are the vessels of the microcirculatory bed. Adventitia is covered by flat mesothelial cells. The thickness of the longitudinal muscle layer is 110.0±5.0 μm, circular - 296.0 ± 20.0 μm.

In fetus of 271,0-375,0 mm PCL (8-10 months), the infundibulum has a well-defined lumen (988,0 ± 20,0 μm) filled with significantly branched folds of the mucous membrane. Folds are different in size: from small to very tall and narrow. Folds are so narrow that lamina propria, which forms the stroma of the folds, is visualized as a thin strip. The epithelium of the mucosa is cuboidal, cells have oval-shaped nuclei, which are located in different parts of the cells. The epithelium is separated from lamina propria of the mucosa by a basement membrane. The thickness of the mucosa of the infundibulum reaches 62.0 ± 4.0 μm. The tunica muscularis is thin, represented by smooth muscle cells, which form circular and longitudinal layers. Between smooth myocytes are detected layers of connective tissue with fine collagen fibers and small cells. Outside OD is covered with adventitia (Fig. 2). The thickness of the tunica muscularis muscle in the infundibulum is 38.0 ± 6.0 μm.

The lumen of the ampullary part of the OD is wide (1,8 ± 0,05 mm) and is closed by folders, which are different in length and width. Epithelium consists of a single layer of columnar cells which are of two types: ciliated and non-ciliated. The epithelial folds are supported by a highly vascular connective tissue core, where is located large number of arterioles, venules and lymphatic capillaries. The thickness of the mucosa is 110.0 ± 10.0 μm. The tunica muscularis is formed by two layers of smooth muscle cells. There is a predominance of the thickness of the circular layer (346.0 ± 20.0 μm) over the longitudinal (148.0 ± 10.0 μm). It should be noted that between these layers are circular oriented bundles of smooth myocytes.

The lumen of the OD isthmus (872.0 ± 20.0 μm) is much smaller than that of the infundibulum and ampullary part of the uterine tube. The mucosa (thickness 164.0 ± 20.0 μm) forms from 3 to 6 small, more often wide and unbranched folds. The epithelium is cuboidal, epithelial cells have densely located basophilic nuclei, which are at different levels. In the connective tissue of lamina propria are present thin collagen fibers, which are assembled into the bundles.

The tunica muscularis of the isthmus (Fig. 3) predominates in thickness (638.0 ± 20.0 μm) all another layers of OD. It is formed by the circular and longitudinal layers (478.0 ± 20.0 μm thick and 164.0 ± 10.0 μm respectively). The peculiarity of the tunica muscularis is the absence of a layer of connective tissue between the layers. Most vessels of the microcirculatory bed are determined between the mucosa and tunica muscularis. Uterine tube is covered with an adventitia.

The lumen of the uterine part of the OD (572,0 ± 10,0 microns) is well visible. The number of epithelial folds is 8-10. The height of the folds is different, they are branched on the apex, with a narrow or wide base. The thickness of the mucosa between the folds reaches 126.0 ± 8.0 μm. Folds of the mucosa are covered with a high columnar epithelium? Cells of which have light, round or oval nuclei. Basically they are located on two levels: closer to the apical pole and in the middle part of the cells, rarely - in the basal pole of the cells. Under the epithelium is located lamina propria of the mucosa, which forms the stroma of the folds. The epithelium in the most part is clearly separated from lamina propria. In some places there is no clear boundary between the epithelium and the components of the underlying connective tissue.

The connective tissue is represented by a large number of cells (fibroblasts and fibrocytes) with round and oval nuclei. The space between the cells is filled with thin fibers of the connective tissue. Visually the cellular component of the loose connective tissue predominates over fibers. Tunica muscularis with a thickness of 598.0 ± 10.0 μm, formed by the spindle-shaped muscle cells, in the center of which is present elongated basophilic stained nucleus. Myocytes form circular and longitudinal layers without a clear boundary. Their thickness is 460.0 ± 20.0 μm and 142.0 ± 8.0 μm, respectively. In one part of the OD section, the longitudinal layer is very thin (88,0 ± 2,0 μm), in the other part it is sufficiently wide (138,0 ± 2,0 μm). Between myocytes are located components of the connective tissue - collagen fibers. Adventitia is represented by a small number of cells of the connective tissue (fibrocytes and fibroblasts) and thin fibers, which are collected in small wave-like bundles and placed in different directions. Cells and fibers are present in an amorphous component of the intercellular substance, which is determined between the components of the connective tissue.

According to the research by Golubovsky I. A. [11, 12], morphologists study the various structures of female sexl organs, however, the main focus is on the study of their anatomical characteristics in adults. Microsurgical anatomy of the uterus is actively investigated in adults. Instead, the macroscopic anatomy of female sex organs is described only in a few articles, only indirectly in newborns. Pronyaev D. V. [6, 13] investigated the sequence of morphological uterine transformations in human fetal ontogenesis and topographic anatomical features of uterine tubes in early embryos, Klyuchko S. S. [9] performed OD studies in early postnatal ontogenesis, and we are studying histotopography of OD in human fetuses.

According to the research by Klyuchko S. S. [9] OD of the newborns, as well as in the embryos of the last month of pregnancy, have compound structure and characteristic morphofunctional peculiarities that are present in an adult organism. After birth, the thickness of uterine tubes increases near its uterine part, mainly due to the circular layer of the tunica muscularis. According to our results the thickness of the OD wall in the dynamics of the fetal period increases in the direction from its infundibulum to the uterine part, the main part of the thickness of the wall is the tunica muscularis, the thickness of the circular muscle layer prevails over the thickness of the longitudinal muscle layer 2.5-2.8 times.

Silina T.M. [14] found that OD mucosa in the early reproductive age in 53.5% of cases is covered by a simple cuboidal epithelium, in which are located a small number of ciliated and secretory cells. In the submucosa there are isolated lymphocytes. The adventitia is represented by mesothelium. Kolesnikov L. L. [15] distinguishes in uterine tubes the outer layer (spiral), the intermediate (circular) and the internal (longitudinal) layers.

CONCLUSIONS

1. The thickness of the infants OD wall in the dynamics increases in the direction from its infundibulum to the uterine part. The main part of the thickness of the OD wall is the tunica muscularis.
2. The thickness of the circular muscle layer predominates the thickness of the longitudinal 2.5-2.8 times. Mucosa is thicker in the isthmus, the thinnest in the ampullary part.
3. It is expedient to study the histotopography of the OD in the neonatal period of human ontogenesis.

REFERENCES

1. Markovskiy V.D., Kupriyanova L.S. Histologichni osoblivosti zhinochih statevih organiv plodiv z oznakami zatrimki vnutrishnoutrobnogo rozvitku. Visnik Ukrayinskoyi medichnoyi stomatologichnoyi akademiyi. 2015; 3 (51): 212-216.
2. Priymak S.G. Prenatalna diagnostika vrodzhenih vad rozvitku ploda v I trimestri vagitnosti. Materiali 94-yi pidsumkovoyi naukovoyi konferentsiyi profesorsko-vikladatskogo personalu Bukovinskogo derzhavnogo medichnogo universitetu 18, 20, 25 lyutogo 2013 roku. Chernivtsi, 2013; 142.
3. Kashperuk-Karpyuk I.S., Pronyaev D.V. Sintopichni osoblivosti vnutrishnih zhinochih statevih organiv ta sechovogo mihura plodiv. Visnik problem biologiyi i meditsini. 2014;3(109):265-268.
4. Kernesyuk M.N. Izmeneniya struktury mishechnoy obolochki mochetochnika, kak prichina posleoperatsionnoy diskinezii. Morfologiya. 2010;4:91-94.
5. Jaime Saavedra Saavedra. The Effectiviness of Hydrosalpinx Treatments in Patients that Need Assisted Reproductive Techniques. Medicine & health. 2012;Chapter IV:122–125.
6. Pronyaev D.V. Topografoanatomichni osoblivosti matkovih trub rannih plodiv. Klinichna ta eksperimentalna patologiya. 2015;(54):125-127.
7. CDC. Sexually Transmitted Disease Treatment Guidelines. Pelvic Inflammatory Disease. MMWR. 2010;59:12.
8. Bodnaryuk O.I., Andriets O.A., Lazar V.V. Chastota naybilsh znachuschih klinichnih proyaviv u divchat hvorih na salpingooforit. Medichniy forum. 2017;10(10):20-22.
9. Klyuchko S.S., Evtushenko V.M., Sokolovskiy D.N. Morfologicheskie osobennosti stenki matochnyih trub novorozhdennyih. Visnik problem biologiyi ta meditsini. 2016;2(129):143-145.
10. Urhausen C., Beineke A., Piechotta M. [et. al.] Apoptosis in the uterotubal junction and oviductal isthmus during the estrous cycle of the bitch. Anat. Rec (Hoboken). 2011;2:342-348.
11. Golubovskiy I.A. morfologichne obgruntuvannya novogo sposobu vidnovlennya prohidnosti istmichnogo viddilu matkovih trub. Visnik morfologiyi. 2009;1:72-75.

12. Golubovskiy I.A., Kostyuk G.Ya., Korol A.P. Morfologichni osoblivosti matkovih trub pri neprohidnosti yak obgruntuvannya novogo sposobu hirurgichnoyi korektsiyi neplidnosti trubnogo pohodzhennya. Galitskiy likarskiy visnik. 2010; 2:49-51.
13. Pronyaev D.V. Dvoroga matka. Klinichna ta eksperimentalna patologiya. 2014;2(48):218-221.
14. Slilna T.M. Znachennya angiogenezu v regulyatsiyi zhinochoyi reproduktivnoyi sistemi. Galitskiy likarskiy visnik. 2003;2:181-182.
15. Kolesnikov L.L. Sfinkterologiya: klassifikatsiya sokratitelnyih struktur cheloveka. Morfologiya. 2008;2:65.

The work is a fragment of inter-department al scientific research work of he Department of Human Anatomy named after M.G. Turkevich and The Department of Anatomy, Topographic Anatomy and Operative Surgery of the Higher State Education al Institution of Ukraine "Bukovinian State Medical University" "Patterns of perinatal anatomy and embryo-typography. Determination of gender-age characteristics of the structure and topographic-anatomical interrelation so for gans and structuresin human ontogenesis" (state registration number 0110U003078) and "Gender-age patterns of anatomy and topography-anatomical interrelation so for gans and structuresin human ontogenesis. Peculiarities of age and gender embryo-typography" (state registration number 0105U002927).

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Larysa Y. Fedoniuk
Head of the Medical Biology Department of the
I. Horbachevsky State Medical University
Valova street 9, 46000, Ternopil, Ukraine
tel: +380673999143
e-mail: Fedonyuk22Larisa@gmail.com

Received: 01.02.2019
Accepted: 17.06.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

STRUCTURAL AND FUNCTIONAL STATE OF THE SEMINAL GLANDS IN THE DYNAMICS OF ACUTE INFECTIOUS INFLAMMATION

Olga I. Zalyubovska[1], Tetiana I. Tiupka[1], Victor V. Zlenko[1], Yulia N. Avidzba[1], Mycola I. Lytvynenko[1], Alina O. Minaieva[2]
[1] KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE
[2] V. N. KARAZIN KHARKIV NATIONAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Introduction: Negative demographic trends are often associated with high levels of infertility, including male. In the modern literature there are data on morphofunctional changes in various organs and tissues during inflammation of various origins, obtained in experiments on animals. At the same time, there are practically no studies on changes in the seminal glands in inflammation of different etiologies.

The aim of this study is to identify the morphofunctional features of the seminal glands in the dynamics of acute infectious (staphylococcal) inflammation in rats.

Materials and methods: Experimental studies were performed on 40 nonlinear male rats weighing 180-200 g. Microscopic and histochemical studies were performed on the 7th, 14th, and 28th day.

Results: On the 7th day of staphylococcal inflammation, morphofunctional changes in the seminal glands were detected in rats in the form of a moderate rearrangement of the spermatogenic epithelium, which was manifested by a decrease in Sertoli cells and the number of type A light spermatogonia along with an increase in the number of type A dark spermatogonia and type B spermatogonia. The described changes were accompanied by a decrease in the metabolism of nucleoproteins in epithelial cells. On the 14th day, the morphological changes were characterized by a sharp decrease in Sertoli cells, the absence of type A light spermatogonia and an increase in the number of type A dark spermatogonia and type B spermatogonia. After 28 days, there is an increase in the number of tubules with the presence of type A light and dark spermatogonia, as well as single Sertoli cells, which indicates the restoration of the morphofunctional state of the seminal glands.

Conclusions: More pronounced compensatory-adaptive processes in the seminal glands occur within a period of 28 days from the start of modeling of staphylococcal inflammation. The latter is confirmed by the appearance of various shapes and sizes of tubules with restored spermatogenic epithelium of various stages of development. The presence of type A light and dark spermatogonia indicates the reserve capacity of the seminal glands.

KEY WORDS: acute infectious inflammation, seminal glands, microscopic study, histochemical reactions

Wiad Lek 2019, 72, 8, 1486-1490

INTRODUCTION

Preserving the reproductive health of the nation, as a powerful lever for stopping depopulation processes and improving the demographic situation in Ukraine, is a priority task in the field of healthcare and social development of medical science [1]. The main cause of negative demographic trends remains a high level of infertility, both female and male. According to many studies, male infertility is often associated with the presence of an inflammatory process, which is the main link in the pathogenesis of most human diseases [2, 3]. From his result depends on the recovery from the disease. In adverse environmental and social conditions, inflammatory processes in people often occur atypically with a tendency to chronicity and generalization [4, 5]. Therefore, there is a need to study various forms of inflammation, conditions affecting its course, the reasons for the transition of acute inflammation to chronic.

In the modern literature there are data on morphofunctional changes in various organs and tissues during inflammation of various origins, obtained in experiments on animals [6]. At the same time, there are practically no studies on these changes in the seminal glands in inflammation caused by various phlogogens.

Earlier, we studied the morphofunctional changes in the spermatic glands during acute aseptic inflammation [7].

THE AIM

The aim of this study is to identify the morphofunctional features of the seed glands in the dynamics of acute infectious (staphylococcal) inflammation in rats.

MATERIALS AND METHODS

Experimental studies were performed on 40 nonlinear male rats weighing 180-200 g, bred in the vivarium of the Kharkiv National Medical University following the recommendations for conducting medical and biological research using animals according to the general ethical principles of animal experiments adopted by the First National Congress of Ukraine on Bioethics (Kyiv, 2001), "European Convention for the Protection of Vertebrate Animals used

Table I. Organometric indicators of the seminal glands of rats of the control and experimental groups, (M±m)

Experiment conditions	Seminal glands			
	Weight, kg×10 $^{-3}$		Sizes, m×10 $^{-3}$	
	right	left	right	left
Control group (intact animals), n=10	6,3±0,22	6,8±0,24	2,2x1,2	2,2x1,2
Staphylococcal inflammation (7th day), n=10	8,7±0,36	8,1±0,28	4,1x1,3	3,8 x 1,3
Staphylococcal inflammation (14th day), n=10	9,7±0,34*	9,9±0,36*	4,3x1,5	4,8x1,5
Staphylococcal inflammation (28th day), n=10	5,9±0,19*	6,1±0,21*	2,7x1,2	2,3x1,0

Note. * Differences in comparison with the control group are significant ($p < 0.05$)

for Research and Other Scientific Purposes" (Strasbourg, 1986), Law of Ukraine №3447-IV dated 21.02.2006 "On the protection of animals from cruel treatment".

Animals were kept on a normal diet in standard vivarium conditions. Slaughter of animals was performed under hexenal anesthesia (50 mg/kg body weight intragastrically) by bloodletting.

Acute infectious inflammation was reproduced by intramuscular injection of 2 billion microbial bodies of *Starchusus aureus* (strain ATCC-25923), in 1 ml of isotonic sodium chloride solution [7].

Macroscopic and microscopic studies were performed in dynamics on the 7th, 14th and 28th days. Such macroscopic characteristics were evaluated: the size of the seminal glands, the condition of the membranes of the organ, the presence of hemorrhages in the areas of necrosis on the incision.

Tissue samples of seminal glands fixed in 10% neutral formalin were subjected to histological study, which were subjected to standard wiring through alcohols of increasing concentration, Nikiforov liquid (96% alcohol and diethyl ether in a 1: 1 ratio), chloroform, and then paraffin was poured. Serial sections with a thickness of 4-5×10^{-6} m were made from the blocks prepared in this way. In all cases, traditional histological methods of hematoxylin staining with eosin, as well as pikrofuksin by Van Gieson were used [8]. Micropreparations were studied on an "Olympus BX-41" microscope with subsequent processing using the program "Olympus DP-soft version 3.1" and Microsoft Excel, with which morphometric studies were performed.

To determine the level of protein-synthetic activity, the content of the ribonucleoprotein (RNP) in the cytoplasm and the deoxyribonucleoprotein (DNP) in the cell nuclei were determined by measuring the optical density in sections, processed by histochemical methods for the determination of the DNP according to Felgen-Rossenbek (the control is hydrolysis with HCI), the RNP was determined by the Brachet reaction (the control with crystal ribonuclease), and the detection of glycogen using the Periodic Acid Schif (PAS) reaction (the control is amylase) [9]. The control group was the seminal glands of intact rats.

The obtained results were processed statistically using Student's t-factor for independent samples [10].

RESULTS AND DISCUSSION

For macroscopic examination: ovoid gonads, translucent membranes, thin, whitish-gray tissue at the cut, brain-like consistency. The mass and size of the seminal glands of the control and experimental groups in the dynamics are presented in Table I.

Microscopic study of the seminal glands in the study group showed that the gonad tubules are arranged randomly, closely adjacent to each other, of ovoid, often rounded shape. All tubules have clearances. The basement membrane, on which the spermatogenic epithelium cells are located, is moderately thickened. In some cases, there are fibers of the delicate connective tissue, painted by Van Gieson in red. The tubular epithelium is represented by gonocytes, Sertoli cells, as well as type A and B spermatogonia. Part of the type A spermatogonia looks somewhat pale with a bright core, moderately saturated chromatin with a similar reaction to DNP (Table II) in conventional units of optical density. In the cytoplasm of cells there is a rather pronounced pyroninophilia, which is confirmed by an intensive histochemical reaction (Table II). The other part of the spermatogonia is represented by dark type A cells with a moderately condensed nucleus, corresponding to the intensity of the reaction to DNP and with moderate cytoplasm pyroninophilia, mainly concentrated around the nucleus (Table II). Along with this, there are type B spermatogonia, where the nucleus is saturated with chromatin, slightly larger than in type A spermatogonia and more rounded. Their cytoplasm also has moderate pyroninophilia. All described spermatogenic epithelium cells in the cytoplasm contain polymorphic PAS-positive granules diffusely located throughout the cytoplasm, with a moderate degree of intensity of histochemical reaction intensity (Table II). In the adluminal zone there are single primary sex cells or rounded gonocytes, with a large euchromatin with a nucleus in the center of the cells. The cytoplasm of many gonocytes is enlightened, vacuolation,

often with focal lysis phenomena. In some monocytes, the nuclei are shifted to the cell membrane.

Spermatozoa are between the cells of the spermatogenic epithelium and in the lumen of the tubules.

There are few rounded Leydig cells in the intertubular stroma. The cytoplasm of Leydig cells is eosinophilic, with weak pyroninophilia around the nucleus, contains PAS-positive granules, which in some places merge with each other and have a rather high intensity of the reaction to glycogen (Table II). The nuclei are small, rounded, saturated with chromatin with a moderate intensity of the reaction to DNP and a similar intensity of the reaction to RNP. Polygonal Leydig cells are smaller than rounded.

The cells of the spermatogenic epithelium are actively involved in spermatogenesis, and the light type A spermatogonia are cells that are updated (the so-called stem cells), and the type A dark cells are reserve cells that are not involved in spermatogenesis. But their presence is necessary, since they can perform the function of stem cells with the induction of spermatogenesis in emergency situations with damage to the epithelium. Sertoli cells provide a complete metabolism of sex cells and the formation of spermatozoa, as well as participate in the formation of the intratubular fluid. The interstitial Leydig cells are polygonal in shape; they perform an endocrine function and are involved in the synthesis of testosterone. Glycogen in their cytoplasm is a plastic material for energy supply of the cell [11].

In the study of the seminal glands of experimental animals, depending on the duration of their stay in the conditions of modeling staphylococcal inflammation, we identified morphological and morphometric changes that characterize the function of the gland for a period of 7, 14 and 28 days.

THE 7TH DAY

A macroscopic study showed that the gonads were slightly enlarged, the membranes are slightly tense, the focal point hemorrhages are on the incision.

Microscopically by the end of the 7th day in the gonads there is a random arrangement of tubules, rounded and slightly elongated. All tubules have wide enlightenment. The basement membrane, on which the cells of the spermatogenic epithelium are located, is slightly thickened, sometimes there are fibers of the delicate connective tissue, which is colored by Van Gieson in red color. The tubular epithelium is represented by densely located type A dark spermatogonia. The nuclei of the latter have a more intense color on the DNP than in the control group and the corresponding intensity of the histochemical reaction (Table II). The nuclei of the latter have a more intense color on the DNP than in the control group and the corresponding intensity of the histochemical reaction (Table II). At the same time, the intensity of the reaction to RNP is reduced compared with the control group (Table II). In accordance with this, the intensity of the reaction of the PAS-reaction is also reduced. Type A light spermatogonies are small, with an increase in the intensity of the histochemical reaction to DNP in the nucleus and a decrease in the cytoplasm in determining the RNP and the PAS-positive material (Table II).

Along with the above, the number of type B spermatogonia increases. The latter increase the intensity of the reaction to RNP in the cytoplasm and the optical density when determining glycogen, that is, the PAS-reaction. Spermatocytes appear in leptotene and pachytene, spermatids. Sertoli cells are few, as are type A light spermatogonies. Primary sex cells or round-shaped gonocytes, in the adluminal zone, their moderate number. They have a large euchromatin-containing nucleus located in the center of the cells. The cytoplasm of many gonocytes is enlightened, vacuolated. In some gonocytes, the nuclei are displaced to the cell membrane.

Single polygonal Leydig cells were found in the intertubular stroma. Cytoplasm of Leydig cells is eosinophilic with mild pyroninophilia and low intensity of histochemical reaction. The nuclei have a rounded shape, moderately saturated with chromatin with few nucleoli with a weakly pronounced intensity of the reaction to RNP (Table II). The oval Leydig cells are also slightly. The lumen of the vessels dramatically expanded, filled with red blood cells, and there are small perivascular hemorrhages, unevenly pronounced edema of interstitial tissue.

In some cases, the canaliculi, predominantly represented by cells of the pathogenic epithelium in the form of type B spermatogonia, sparse gonocytes, as well as indifferent sex cells, are devastated.

Spermatozoa are between the cells of the spermatogenic epithelium, and in the lumen of some tubules.

Thus, on the 7th day, staphylococcal inflammation in the seminal glands experimental animals show signs of moderate adjustment of spermatogenic epithelium, Sertoli cells manifesting decrease, as well as reducing the amount of type A light spermatogonia and increasing the number of type A dark spermatogonia and increased number of type B spermatogonia.

The described morphological changes are accompanied by a decrease in the exchange of nucleoproteins in epithelial cells and emptying of the tubular epithelium, combined with a decrease in the activity of Leydig cells and documented by a decrease in more mature cell forms and a decrease in the total number. Along with the above, a decrease in the protein-synthetic function of cells and a decrease in glycogen are noted.

THE 14TH DAY

A macroscopic study showed that the gonads were slightly enlarged, small focal point hemorrhages occur on the incision.

The dimensions of the tubules are polymorphic.

All tubules have openings with few spermatozoa. A small number of tubular epithelial cells is observed (partial emptying). The basement membrane with cells of the spermatogenic epithelium located on it is slightly thickened. The tubular epithelium is represented mainly by first-order spermatocyte in pachytenas and few type A dark spermatogonia whose nuclei contain dense chromatin with a pronounced reaction intensity. Cytoplasmic pyroninophilia is mild with an appropriate intensity of the reaction (Table II).

Table II. The intensity of histochemical reactions to DNP, RNP and glycogen in the cells of the spermatogenic epithelium of the seed glands, Leydig cells (n=10, M±m).

Cells of the spermatogenic epithelium of the seminal glands	DNP in cell nuclei, c.u. of optical density	RNP in the cytoplasm of cells, c.u. of optical density	PAS-reaction in the cytoplasm of cells, c.u. of optical density
Control group (intact animals)			
Type A light spermatogonia	0,210±0,007*	0,298 ± 0,011*	0,285±0,011
Type A dark spermatogonia	0,245±0,019*	0,275± 0,010*	0,244± 0,003*
Type B spermatogonia	0,224±0,008	0,198± 0,007	0,221±0,005
Leydig cells	0,198±0,007	0,132±0,005	0,297±0,012
Staphylococcal inflammation (the 7th day)			
Type A light spermatogonia	0,188±0,033*	0,248 ± 0,031*	0,265±0,031
Type A dark spermatogonia	0,255±0,021*	0,269± 0,033*	0,244± 0,003*
Type B spermatogonia	0,224±0,008	0,198± 0,007	0,221±0,005
Leydig cells	0,179±0,006	0,112±0,002	0,278±0,012
Staphylococcal inflammation (the 14th day)			
Type A light spermatogonia	-	-	-
Type A dark spermatogonia	0,285± 0,011*	0,235 ± 0,008*	0,223± 0,004*
Type B spermatogonia	0,237±0,008*	0,179±0,007*	0,194±0,005*
Leydig cells	0,177±0,006	0,128±0,033	0,168±0,009
Staphylococcal inflammation (the 28th day)			
Type A light spermatogonia	0,206±0,007*	0,279 ± 0,010*	0,275±0,010
Type A dark spermatogonia	0,238±0,008*	0,234± 0,008*	0,232± 0,008*
Type B spermatogonia	0,220±0,008	0,168± 0,006	0,208±0,007
Leydig cells	0,179±0,006	0,112±0,004	0,267±0,009

Note. * Differences in comparison with the control group are significant ($p < 0.05$)

In the area of the basement membrane with a sufficiently condensed nucleus, type B spermatogonia are few. It turns out a small number of small spermatids and sperm groups, single Sertoli cells. There are single gonocytes that have a large euchromatin-containing nucleus with a central location. The cytoplasm of many gonocytes is enlightened, vacuolated, with focal lysis phenomena. In some gonocytes, the nuclei are shifted to the cell membrane. The intertubular stroma is empty, Leydig cells are mostly absent, but in some gonads they are found and have vacuolated cytoplasm with a slightly stained nucleus.

Thus, in the period of 14 days of staphylococcal inflammation in the seminal glands of experimental animals, signs of pronounced rearrangement of the spermatogenic epithelium are revealed, manifested by a sharp decrease in Sertoli cells, as well as the absence of type A light spermatogonia and the appearance of a larger number of type A dark spermatogonia, an increase in the number of type B spermatogonia.

Moreover, the described morphological changes are accompanied by a decrease in the metabolism of nucleoproteins in epithelial cells and begins by emptying the tubular epithelium, accompanied by a decrease in the activity of interstitial tissue, in particular Leydig cells, and is documented by the appearance of only mature forms in single specimens.

THE 28TH DAY

After 28 days in a macroscopic study of the gonads, the latter approaching the control group, the shells are sometimes slightly thickened, sclerotized, on the cut visible small grayish-white cells of the tissue of the seminal glands. In microscopic study, the testis shell is thickened, represented by thin elastic fibers, in some places by coarse-fibrous tissue. Compared with the study on the 14th day, the number of tubules increased, they are of different size and shape (some are ovoid, others are close to rounded). There are small round tubules. The tubule wall is represented by small myoid cells and cells of the spermatogenic epithelium, which are loosely attached. In many tubules, the wall is significantly thickened due to the proliferation of spermatogenic epithelial cells. In the basal part of such tubules is a large number of type A and B spermatogonia. Among the type A spermatogonia, there are two options: light and dark. Type A light spermatogonia with a large oval-shaped bright nucleus, containing euchromatin, and small nucleoli scattered near the shell of the nucleus, their cytoplasm is small, moderately stained with eosin. Type A dark spermatogonia are represented by a small number of cells with a darker nucleus, intensely stained with hematoxylin, with few nucleoli located near the nuclear envelope.

Also on the basement membrane there are few type B spermatogonia and much more type A spermatogonia.

The nuclei of these cells contain light euchromatin and multiple nucleoli.

Histochemical changes in spermatogenic epithelium cells are close in intensity to the control group (Table II), but the average group indices are somewhat lower. Perhaps a slight decrease in optical density can be explained by the presence of polymorphic tubules both ordinary and smaller ones.

Closer to the adluminal compartment of the seminiferous tubule there are a large number of spermatocytes and spermatids, as well as spermatozoa. Spermatozoa are localized both between the described cells and in the lumen of the tubules.

Among the epithelium of the tubules, in the adluminal compartment, single supporting Sertoli cells with a bright, irregularly shaped euchromatin-containing nucleus with the nucleolus are found.

Also, there are single small ovoid tubules with a depleted spermatogenic epithelium, represented mainly by type A dark spermatogonia, a small amount of type B spermatogonia and a small number of spermatocytes. Sertoli cells are few and are located mainly on the basement membrane.

The tubules are quite tight in relation to each other. Between them there are polygonal Leydig cells, whose nuclei are small, eccentrically located. There are also small round Leydig cells.

Vascular lumens are unevenly dilated, filled with red blood cells. Small foci of excessively developed coarse-fibrous connective tissue, intensely colored by Van Gieson in red color, occur between the tubules.

CONCLUSIONS

Analyzing the morphological data obtained in the experiment, it should be noted that the most pronounced compensatory-adaptive processes in the seminal glands occur in the period of 28 days from the beginning of the modeling of staphylococcal inflammation. This was confirmed by the appearance of various shapes and sizes of tubules with restored spermatogenic epithelium of various stages of development. The presence of type A light and dark spermatogonia indicates the reserve capacity of the seminal glands.

REFERENCES

1. Gojda N.G., Moiseyenko R.O., Majstruk G.P. et al. Osnovni dosyagnennya vykonannya Derzhavnoyi programy «Reproduktyvne zdorovya naciyi» za 2005-2015 rr. In: Zdorov`e zhenshhyny. 2016; 4(110): 14-16.
2. Gorpynchenko I.I. Choloviche bezpliddya: etiologiya, patogenez, diagnostyka ta suchasni metody likuvannya. In: Zdorovie muzhchiny. 2016; 1(56): 8-16.
3. Klimenko N.A. Rol vospaleniya v patologii. In: Zagalna patologiya ta patologichna fiziologiya. 2010; 2: 20-21.
4. Klimenko N.A. Gematologicheskie mehanizmy hronizacii vospaleniya. – Kharkiv: HNMU, 2010: 88.
5. Weiss U. Inflammation. In: Nature. 2008; 454: 427.
6. Tatarko S.V. Morfofunkcionalnoe sostoyanie selezenki pri raznyh po techeniyu i etiologii vidah vospaleniya. In: Medicina sogodni i zavtra. 2014; 2-3(963-640): 50-56.
7. Avidzba Yu.N., Zalyubovska O.I., Zlenko V.V. Morfofunkcionalnye osobennosti semennyh zhelez pri karaginenovom vospalenii. In: Eksperimentalna i klinichna medicina. 2016; 4(73): 5-10.
8. Merkulov G.A. Kurs patogistologicheskoj tehniki. – Leningrad: Medgiz, 1961: 340.
9. Lilli R. Patogistologicheskaya tehnika i prakticheskaya gistohimiya – Moskow: Mir, 1969: 648.
10. Lapach S.N., Chubenko A.V., Babich P.N. Statisticheskie metody v mediko-biologicheskih issledovaniyah s ispolzovaniem Exel. – Kyiv.:MORION, 2001: 408.
11. Hess R.A., de Franca L.R. Spermatogenesis and Cycle of the Seminiferous Epithelium. In: C.Yan Cheng (Eds.), Molecular Mechanisms in Spermatogenesis. Advances in Experimental Medicine and Biology. 2009; 636: 1-15.

Author's contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interests.

CORRESPONDING AUTHOR

Mycola I. Lytvynenko
Kharkiv National Medical University, Kharkiv, Ukraine
4 Nauky Avenue, Kharkiv, Ukraine, 61022
tel: +380994012393
e-mail: kkld1@ukr.net

Received: 14.02.2019
Accepted: 11.06.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

CONDITION OF HEMODYNAMICS IN THE PULMONARY CIRCULATION OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) CONCURRENT WITH METABOLIC SYNDROME WITH HYPERTROPHY AND ATROPHY OF THE MYOCARDIUM

Viktor P. Boriak, Svitlana V. Shut', Tetiana A. Trybrat, Olena V. Filatova
UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY, POLTAVA, UKRAINE

ABSTRACT

Introduction: In recent years, COPD is observed as not an isolated, but an associated pathology, in particular, concurrent with metabolic syndrome.

The aim of the research is to identify the differences in changes of the rheopulmonography parameters (RPG) depending on the presence of hypertrophy or atrophy of the right ventricular myocardium in patients with COPD concurrent with metabolic syndrome..

Materials and methods: We studied changes in rheopulmonography (RPG) in 145 patients with chronic obstructive pulmonary disease (COPD) concurrent with metabolic syndrome.

Results: We detected precapillary hypertension of the pulmonary circulation in patients with right ventricular myocardial hypertrophy: anacrotism serration; flattened peak of the systolic wave; decreased Vcp; high placement of incisura; horizontal course of catacrotism; decreased amplitude of the systolic wave (in this case, due to a greater increase in the resistance of the blood flow in the pulmonary vessels than the decreased impact volume of the right ventricle); prolonged Q-a (in this group of patients, it depends more on hypertension of the pulmonary circulation than on the reduction of contractile function of the myocardium).

In atrophy of the right ventricular myocardium, the following changes in the RPG were revealed: decreased systolic wave at its dramatic rise; prolonged Q-a (in this case, due to the weakened heart contraction); Vmax reduction (it reflects the reduction of myocardial contractility); in hypertrophy of the myocardium, Vcp., unlike RPG, does not decrease, which is explained by the decrease in the pressure of the pulmonary circulation.

Conclusions: We believe that these changes in RPG allow differentiating hypertrophy and right ventricular myocardial atrophy along with established diagnostic criteria, and can be used as markers for the diagnosis and treatment of COPD concurrent with metabolic syndrome.

KEY WORDS: chronic obstructive pulmonary disease, metabolic syndrome, rheopulmonography, myocardial hypertrophy, myocardial atrophy

Wiad Lek 2019, 72, 8, 1491-1493

INTRODUCTION

In recent years, there has been an increase in the specific weight of morbidity and mortality of the population caused by chronic obstructive pulmonary disease (COPD). This is due to a number of causes: pollution of the air pool, smoking, bacterial flora resistance to some antibiotics [1,2]. Chronic obstructive pulmonary disease (COPD) is an example of a multifactorial illness, which involves many factors [3-6]. In a significant number of cases, COPD development is preceded by acute bronchitis and pneumonia, which tend to the prolonged course. Patients with COPD account for 2.5-3% of the total population, and mortality from this pathology constitutes 20-40% of all deaths from circulatory failure [7-8]. The incidence of chronic bronchitis in various regions of Ukraine reaches 3-11%, and in hazardous industrial and agricultural production - 15-50%, with 25-30% of patients suffering from the obstructive form of the disease.

Pulmonary heart disease (PHD) is characterized by the staged development. At the first stage, one can observe a restructuring process at the level of the external respiration apparatus with subsequent respiratory failure; at the second stage – pulmonary hypertension (PH), changes of central and peripheral hemodynamics are joined. At the third stage, an increase in the minute volume of the heart is characteristic against the background of already changed contractile function of the myocardium and at the fourth one, right ventricular heart failure is observed.

PH is the main manifestation of hemodynamic impairment of the pulmonary circle COPD. It leads to overload and (or) dilation of the right ventricle, which subsequently plays an important role in the occurrence of circulatory insufficiency by the right ventricular type. The main causes for the development of LH are increased pulmonary vascular resistance and decreased lumina of the vessels of the lungs as a result of hypoxia.

However, it should be noted that the pulmonary pathology, including chronic pulmonary heart, has not been sufficiently studied in all aspects. In addition, in recent years, scientists have focused on COPD as not an isolated

Table I. Reopulmonography parameters in patients with COPD concurrent with metabolic syndrome

Parameters	Healthy people n=16	Patients with COPD concurrent with metabolic syndrome in whom the following was detected:			
		Hypertrophy of the myocardium n=68	P	Atrophy of the myocardium n=77	P
Amplitude of the systolic wave (Ohm)	0.291±0.025	0.126±0.018	<5%	0.103±0.022	<5%
Amplitude of the diastolic wave (Ohm)	0.163±0.034	0.089±0.012	<5%	0.059±0.015	<5%
Right ventricular tension period (sec)	0.115±0.023	0.212±0.018	<5%	0.173±0.019	<5%
Time of maximum systolic filling (sec)	0.165±0.021	0.289±0.024	<5%	0.185±0.018	>5%
Time of rapid blood filling (sec)	0.082±0.240	0.109±0.017	>5%	0.119±0.014	>5%
Time of slow blood filling (sec)	0.119±0.015	0.169±0.012	<5%	0.072±0.022	>5%
The maximum speed of rapid blood filling (Vmax) (Ohm / sec)	1.76 ±0.210	0.21.410±0.120	>5%	1.080.940±0.120	<5%
The average speed of slow blood filling (Vcp.) (Ohm / sec)	0.46 ±0.060	0.17 ±0.032	<5%	0.390±0.021	>5%

Note: P indicates reliability with respect to the corresponding group

disease, but a combined pathology. In particular, the combination of COPD with metabolic syndrome, has come to the forefront of research [9,10].

Rheopulmonography is one of the research methods (both safe and economically beneficial), allowing to assess the state of hemodynamics in the pulmonary circulation.

THE AIM

The aim of the research is to identify the differences in changes of the rheopulmonography parameters (RPG) depending on the presence of hypertrophy or atrophy of the right ventricular myocardium in patients with COPD concurrent with metabolic syndrome.

MATERIALS AND METHODS

Changes of RPG were studied in 145 patients with chronic obstructive pulmonary disease (COPD) concurrent with metabolic syndrome.

We determined the nosological form of COPD and metabolic syndrome according to WHO criteria. The stage of circulatory failure was evaluated in accordance with the classification by N.D. Strazhesko and V.Kh. Vasylenko. The diagnosis of COPD and metabolic syndrome was made on the basis of anamnestic, clinical, biochemical and instrumental data, obtained during the examination of patients. The study group consisted of patients with at least one main type of obesity (central (abdominal) - waist circumference of more than 80 cm in women and more than 94 cm in men and / or increased body mass index of more than 30) and two additional criteria of metabolic syndrome (arterial hypertension AH>140 / 90mm Hg)), increased triglyceride levels>1.7 mmol / l, decreased HDL cholesterol levels<1.0 mmol / l in men; <1.2 mmol / l in women, increased LDL cholesterol>3.0 mmol / l, fasting hyperglycemia (fasting blood glucose> 6.1 mmol / l), impaired glucose tolerance - glucose in the blood plasma 2 hours after the glucose tolerance test within> 7.8 and <11.1 mmol / l). The diagnosis of hypertrophy and atrophy of the right ventricular myocardium was made on the basis of echocardiography and electrocardiogram analysis.

For rheopulmonography, rheograph P4-02 was used, with the recording device - electrocardiograph ELCAR-6 and diagnostic automated complex "Cardio +". Zonal rheopulmonography was performed according to standard methodology (Zhukovsky L.I., Frinerman E.A. [11])

The reliability of difference in the comparable average values was determined by the Student's t-criterion. At the same time, the difference was considered reliable at $t \geq 2.0$, which corresponded to the probability of an error-free prediction equal to 95% or more ($p \geq 95\%$).

Before launching the study, all patients signed an informed consent to participate in the study, in accordance with the requirements of Helsinki Declaration as of 1975 and the Order of the Ministry of Public Health of Ukraine No. 690 as of September 23, 2009, "On Approval of the Procedure for Conducting Clinical Trials of Medicines and Examination of Materials for Clinical Trials" and "Typical Provision on the Ethics Commission". The ethical, moral and legal aspects of the study have been agreed with the Bioethics Commission of Ukrainian Medical Stomatological Academy.

RESULTS AND DISCUSSION

Considering the changes in pulmonary blood flow in these groups of patients, we revealed the difference in RPG changes in patients with COPD with hypertrophy and atrophy of the right ventricle myocardium. Different indices were characteristic for these groups of patients (Table I).

Thus, while studying the changes in rheopulmonogram in patients with hypertrophy of the right ventricular myocardium, we revealed precapillary hypertension of the pulmonary circulation, which is manifested by the following symptoms:

- anacrotism serration;
- flattened peak of the systolic wave;
- decreased Vcp.
- high placement of incisura;
- horizontal course of catacrotism;

- decreased amplitude of the systolic wave (in this case, due to a greater increase in the resistance of the blood flow in the pulmonary vessels than the decreased impact volume of the right ventricle);
- prolonged Q-a (in this group of patients, it depends more on hypertension of the pulmonary circulation than on the reduction of contractile function of the myocardium).

In atrophy of the right ventricular myocardium, the following changes in the RPG were revealed:
- decreased systolic wave at its dramatic rise;
- prolonged Q-a (in this case, due to the weakened heart contraction);
- Vmax reduction (it reflects the reduction of myocardial contractility);
- in hypertrophy of the myocardium, Vcp., unlike RPG, does not decrease, which is explained by the decrease in the pressure of the pulmonary circulation.

Our data suggest that hypertrophy and atrophy of the myocardium in patients with COPD are combined with different changes in rheopulmonogram. This allows us to interpret the identified RPG changes as characteristic of hypertrophy and atrophy of the right ventricular myocardium in the groups under consideration. It is possible to study the dynamics of RPG changes in patients with COPD concurrent with metabolic syndrome in further more broad and differentiated research.

CONCLUSIONS

We believe that the detected changes in RPG allow differentiating hypertrophy and right ventricular myocardial atrophy along with established diagnostic criteria and can be used as markers for the diagnosis and treatment of COPD concurrent with metabolic syndrome.

REFERENCES

1. Feshchenko YU.I., Yashina L.A., Gorovenko N.G.. Khronicheskiye obstruktivnyye zabolevaniya legkikh [Chronic obstructive pulmonary diseases.]. K.: Morion; 2002: 3-19(In Ukrainian)
2. Treumova S.I. , Petrov YE.YE., Boryak V.P .Khronichne obstruktyvne zakhvoryuvannya lehen u poyednanni z metabolichnym syndromom [Chronic obstructive pulmonary disease in combination with metabolic syndrome] Visnyk problem biolohiyi i medytsyny. 2015; 1 (117): 30-36 (In Ukrainian)
3. Cheng W., Xing X., L. Li. Natural abundance in soils and plants in relation to N cycling in a rangeland in Inner Mongolia. Journal of Plant Ecology. 2007; 3: 201
4. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO workshop report. Last updated 2013. www.goldcopd.org/.
5. Buist A. S., McBurnie M. A., Vollmer W. M. et al. International variation in the prevalence of COPD: a population-based prevalence study. Lancet. 2007; 370: 741-750.
6. Stanford R. H., Shen Y., McLaughlin T. Cost of chronic obstructive pulmonary disease in the emergency department and hospital: an analysis of administrative data from 218 US hospitals. Treat Respir Med. 2006; 5: 343–349.
7. Mannino D. M., Buist A. S. Global burden of COPD: risk factors, prevalence, and future trends. Lancet. 2007; 370: 765–773.
8. Agusti A. G. Systemic effects of chronic obstructive pulmonary disease. Proc. Am. Thorac. Soc. 2005; 2: 367–370.
9. Boriak V.P., Trybrat T.A., Treumova S.I. et al. Vyvchennya faktoriv ryzyku khronichnoho obstruktyvnoho zakhvoryuvannya lehen' na foni metabolichnoho syndromu [study of the risk factors of chronic obstructive pulmonary disease on the background of metabolic syndrome]. Visnyk problem biolohiyi i medytsyny. 2015; 3, 1(122):15-17 (In Ukrainian)
10. Trybrat T. A., Shut' S. V., Sakevych V. D. Metabolichnyy syndrom v praktytsi simeynoho likarya. [metabolic syndrome in the practice of a family doctor]. Visnyk problem biolohiyi i medytsyny. 2015; 2, 4(121):36–38(In Ukrainian).
11. Zhukovskiy L.I., Frinerman Ye.A. Osnovy klinicheskoy reografii legkikh. [Basics of clinical rheography of the lungs.]; Tashkent, 1976 :276. (In Russian)

The present paper is a part of the research project "Features of the course and prognosis of metabolic syndrome, taking into consideration genetic, age, and gender aspects of patients, the presence of various components of metabolic syndrome and specific concomitant pathology, as well as the ways for correction of the detected disorders" state registration No. 0114U001909.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Viktor P. Boriak
Ukrainian Medical Stomatological Academy
23 Shevchenko Str., 36011, Poltava, Ukraine
tel: +380662479946
e-mail: wiktorbor3@gmail.com

Received: 23.02.2019
Accepted: 10.07.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

ASSOCIATIONS OF IRS-1 POLYMORPHISM WITH VARIOUS COMPONENTS OF THE METABOLIC SYNDROME IN HYPERTENSIVE PATIENTS

Maryna Kochuieva[1], Valentyna Psarova[2], Larysa Ruban[3], Nataliia Kyrychenko[2], Olena Alypova[4], Olha Matlai[2], Anna Shalimova[5],

[1]KHARKIV MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KHARKIV, UKRAINE
[2]SUMY STATE UNIVERSITY SUMY, UKRAINE
[3]KHARKIV STATE ACADEMY OF PHYSICAL CULTURE, KHARKIV, UKRAINE
[4]STATE INSTITUTION "ZAPORIZHZHIA MEDICAL ACADEMY OF POSTGRADUATE EDUCATION MINISTRY OF HEALTH OF UKRAINE", ZAPORIZHZHIA, UKRAINE
[5]GOVERNMENT INSTITUTION "L.T. MALAYA THERAPY NATIONAL INSTITUTE OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE", KHARKIV, UKRAINE

ABSTRACT

Introduction: The metabolic syndrome is one of the most discussed cross-disciplinary problems of modern medicine. Now there are various definitions and criteria of diagnostics of metabolic syndrome. The abdominal obesity is considered the main component of the metabolic syndrome, as a reflection of visceral obesity which degree is offered to be estimated on an indirect indicator – a waist circumference. Alongside with abdominal obesity, a number of classifications distinguish insulin resistance (IR) as a diagnostic criterion of metabolic syndrome. It is proved that IR is one of the pathophysiological mechanisms influencing the development and the course of arterial hypertension (AH), type 2 DM and obesity. There are two components in the development of IR: genetic (hereditary) and acquired. In spite of the fact that IR has the accurate genetic predisposition, exact genetic disorders of its appearance have not been identified yet, thus demonstrating its polygenic nature.

The aim: To establish possible associations of the insulin receptor substrate-1 (IRS-1) gene polymorphism with the severity of the metabolic syndrome components in patients with arterial hypertension (AH).

Materials and methods: 187 patients with AH aged 45-55 years and 30 healthy individuals. Methods: anthropometry, reactive hyperemia, color Doppler mapping, biochemical blood analysis, HOMA-insulin resistance (IR), glucose tolerance test, enzyme immunoassay, molecular genetic method.

Results: Among hypertensive patients, 103 had abdominal obesity, 43 - type 2 diabetes, 131 - increased blood triglycerides, 19 - decreased high density lipoproteins, 59 - prediabetes (33 - fasting hyperglycemia and 26 - impaired glucose tolerance), 126 had IR. At the same time, hypertensive patients had the following distribution of IRS-1 genotypes: Gly/Gly - 47.9%, Gly/Arg - 42.2% and Arg/Arg - 10.7%, whereas in healthy individuals the distribution of genotypes was significantly different: Gly/Gly - 86.8% ($p<0.01$), Gly/Arg - 9.9% ($p<0.01$) and Arg/Arg - 3.3% ($p<0.05$). Hypertensive patients with Arg/Arg and Gly/Arg genotypes had significantly higher HOMA-IR ($p<0.01$), glucose, insulin and triglycerides levels ($p<0.05$), than in Gly/Gly genotype. At the same time, body mass index, waist circumference, blood pressure, adiponectin, HDL, interleukin-6, C-reactive protein, degree of endothelium-dependent vasodilation, as well as the frequency of occurrence of impaired glucose tolerance did not significantly differ in IRS-1 genotypes.

Conclusions: in hypertensive patients, the genetic polymorphism of IRS-1 gene is associated with such components of the metabolic syndrome as hypertriglyceridemia and fasting hyperglycemia; it is not associated with proinflammatory state, endothelial dysfunction, dysglycemia, an increase in waist circumference and decrease in HDL.

KEY WORDS: metabolic syndrome, insulin receptor substrate-1 gene, arterial hypertension, genetic polymorphism

Wiad Lek 2019, 72, 8, 1494-1498

INTRODUCTION

The metabolic syndrome is one of the most discussed cross-disciplinary problems of modern medicine. Initially the term "metabolic syndrome" was offered for the persons having the increased risk of cardiovascular diseases development and type 2 diabetes mellitus (DM) [1–3]. Then this term was complemented with new criteria.

Now there are various definitions and criteria of diagnostics of metabolic syndrome (World Health Organization; European Group for the Study of Insulin Resistance; National Cholesterol Education Program-Adult Treatment Panel III; American Association of Clinical Endocrinologists; International Diabetes Federation; International Atherosclerosis Society, International Association for the Study of Obesity [4–8]. Irrespective of one or another definition, criteria of diagnostics of metabolic syndrome is existence of at least three of its components (various combination of obesity, the increased arterial blood pressure (BP), increases of the low density lipoproteins (LDL), triglycerides, decrease of the high density lipoproteins (HDL) and violations of carbohydrate metabolism).

The abdominal obesity is considered the main component of the metabolic syndrome, as a reflection of visceral obesity which degree is offered to be estimated on an indirect indicator – a waist circumference [9–14]. Alongside with abdominal obesity, a number of classifications distinguish insulin resistance (IR) as a diagnostic criterion of metabolic syndrome.

It is proved that IR is one of the pathophysiological mechanisms influencing the development and the course of arterial hypertension (AH), type 2 DM and obesity [6, 15, 16].

There are two components in the development of IR: genetic (hereditary) and acquired. In spite of the fact that IR has the accurate genetic predisposition, exact genetic disorders of its appearance have not been identified yet, thus demonstrating its polygenic nature.

At the present stage, it is known that the sensitivity of peripheral tissues to insulin is defined also by the existence of specific receptors, which mediate the stimulating influence of insulin on glucose utilization with the participation of glucose conveyors and start the whole range of cellular reactions [15, 17]. Receptor level of IR formation is one of three levels of disorders and consists of the reduction of the number of insulin receptors, abnormal shapes of receptors to insulin and substrate of an insulin receptor (IRS) of type 1 and also the disorder in affinity of insulin receptors [15–18]. At the present stage, there are known 4 main IRS among which IRS-1 and IRS-2 are leading because of their mediation the majority of biological effects of insulin.

Despite the considerable achievements in genome researches, the majority of the genetic factors contributing to the development and progressing of various components of the metabolic syndrome remain uncertain. The existence of contradictory views of a contribution of polymorphism Gly972Arg of IRS-1 gene to the formation of IR in various populations [18–26] caused carrying out this research in the Ukrainian population of patients.

THE AIM

The aim of the research: establishment of possible associations of polymorphism of IRS-1 gene with the expressiveness of various components of the metabolic syndrome in patients with AH.

MATERIALS AND METHODS

We examined 187 patients aged 45-55 years with AH stage II grade 2 (the main group) and 30 healthy individuals (the control group). Patients of the main group (92 men and 95 women) had a history of hypertension for less than 5 years, medicated with antihypertensive drugs non-systemically and/or did not reach target BP levels when prescribing drug therapy.

In this study, using standard biochemical methods on the patients, we defined venous blood glucose concentration, and insulin levels. IR was determined using the homeostasis model assessment index (HOMA-IR). In patients without previously established DM, a glucose tolerance test was conducted. For studying endothelial function, the degree of endothelium-dependent vasodilation (EDVD) in reactive hyperemia was determined in all patients. Investigations were carried out using a broadband linear transducer 5-12 MHz Doppler color mapping with three readings being taken arteries at 15-min intervals between samples on the left and right brachial arteries, according to the method of Celermajer D.S. (in the modification of the method by Ivanova O.V.) [27, 28]. Normally, the maximum vasodilation of the brachial artery should exceed 10% of the original diameter. Simultaneously, we measured the intima media thickness (IMT) of the carotid artery (CA, 2 cm proximal to the bifurcation of the common CA). Determining the pulse wave velocity (PWV) of the abdominal aortic (AA, on the left subclavian artery to the femoral artery) was performed using a phased transducer with a frequency of 2-4 MHz.

Levels of interleukin-6 (IL-6), C-reactive protein (CRP) and adiponectin in blood were determined during enzyme immunoassay. The rs1801278 polymorphism of the IRS-1 gene was assessed by the molecular genetic method. Three genotypes of the IRS-1 gene (Gly/Gly, Gly/Arg and Arg/Arg) were identified. Processing of statistical data was performed using the software package "Statistics". The values are presented as the average value of parameters (M) and standard error (m).

The study protocol was approved by the Ethics Committee. All participants were informed about the aim of the study and signed a written consent form.

RESULTS

It was established that among 187 examined persons with AH the main components of the metabolic syndrome were present at such percentage: 55.1% had abdominal obesity, 33% – fasting hyperglycemia (≥ 5.6 mmol/l), 22.9% – diagnosed earlier (established less than 2 years ago) type 2 DM, 70.1% had increased blood triglycerides (≥ 1.7 mmol/l), and 10.2% – decrease in HDL <1.03 mmol/l in men and <1.29 mmol/l in women). IR estimated by the index of HOMA is established in 67.8% of patients (table 1).

Dysglycemia (impaired glucose tolerance) as an additional criteria of the metabolic syndrome was noted in 13.9% of patients, the endothelial dysfunction (estimated on such indicators as EDVD according to test with reactive hyperemia, PWV and IMT) – in 72.7%, and the pro-inflammatory state (diagnosed according to the content of the CRP, IL-6 and adiponectin) – in 65.8% of patients (table I).

At the same time patients with AH had the following distribution of IRS-1 genotypes (table II): Gly/Gly – 47.9%, Gly/Arg – 42.2% and Arg/Arg – 10.7% whereas in control group there were reliable differences in genotypes distribution: Gly/Gly – 86.8% ($p<0.01$), Gly/Arg – 9.9% ($p<0.01$) and Arg/Arg – 3.3% ($p<0.05$).

Thus, the frequency of occurrence of various options of genotypes IRS-1 gene authentically differed at almost healthy patients and in hypertensive patients with various components of the metabolic syndrome. At the following stage, associations

Table I. Presence of the metabolic syndrome components at hypertensive patients

Components of the metabolic syndrome	Hypertensive patients with different components of the metabolic syndrome	
	n	%
Abdominal obesity	103	55.1
Triglycerides ≥ 1.7 mmol/l	131	70.1
HDL <1.03 mmol/l at men and <1.29 mmol/l at women	19	10.2
Fasting hyperlycemia ≥ 5.6 mmol/l	33	17.6
Earlier diagnosed type 2 DM	43	22.9
IR (↑HOMA- IR)	126	67.4
Impaired glucose tolerance (dysglycemia)	26	13.9
Endothelial dysfunction (↓EDVD, ↑ PWV, ↑IMT)	136	72.7
Pro-inflammatory condition (↑CRP, ↑IL-6, ↓adiponectin)	125	65.8

Table II. Distribution of IRS-1 genotypes in the main and control groups

Genotypes	Main group, n=187		Control group, n=30		Reliability of a difference between groups
	n	%	n	%	
Gly/Gly	88	47,9	26	86.8	p<0.01
Gly/Arg	79	42,2	3	9.9	p<0.01
Arg/Arg	20	10,7	1	3.3	p<0.05

of polymorphism of IRS-1 gene with anthropometrical, metabolic and hemodynamic indicators in hypertensive patients were estimated. It was established that patients of the main group with Arg/Arg and Gly/Arg genotypes had significantly higher levels of glucose ($p<0.05$), insulin ($p<0.05$), HOMA-IR ($p<0.01$), triglycerides ($p<0.05$), than patients with Gly/Gly genotype. Although BMI, waist circumference, BP levels, adiponectin, LDL, IL-6, CRP, EDVD degree and also frequency of occurrence of the impaired glucose tolerance significantly did not differ in various IRS-1 genotypes (table III).

Considering the fact that genotypes of Arg/Arg and Gly/Arg significantly differed from Gly/Gly genotype in lager expressiveness of metabolic disorders, and among themselves – only HOMA-IR, at a further stage genotypes of Arg/Arg and Gly/Arg were united in one group – with (Gly/Arg + Arg/Arg) genotypes (table IV).

DISCUSSION

The features of distribution of genotypes of IRS-1 gene established in our research at hypertensive patients in the presence of different components of the metabolic syndrome, confirm results of a number of researches according to which Gly972Arg the polymorphism of the specified gene is associated with development of IR in the European and Mexican populations [16–20].

In spite of the fact that according to some authors [17] Gly972Arg the polymorphism is associated with BMI, in this research there was not established the reliable distinctions of BMI in various IRS-1 genotypes in patients with AH.

The data obtained in our research, coincide with results of a number of researchers according to which existence of Gly/Arg and Arg/Arg genotypes was associated with higher levels of atherogenous lipoproteins [17–19].

Reliable distinctions of levels of insulin, glucose and HOMA-IR in various IRS-1 genotypes are confirmation of Gly972Arg polymorphism association with development and progressing of receptor level of IR in hypertensive patients. At the same time, the most expressed IR is characteristic of hypertensive patients with homozygous Arg/Arg genotype of IRS-1.

In spite of the fact that endothelial dysfunction is one of links between IR and cardiovascular diseases and also is considered as additional criterion of the metabolic syndrome, in our research the analysis of a structurally functional condition of vessels in various IRS-1 genotypes did not show reliable distinctions of indicators depending on IRS-1 polymorphism.

It should be noted that one more component of the metabolic syndrome – the pro-inflammatory condition that is estimated on the CRP, IL-6 and adiponectin levels, also did not show reliable distinctions in different IRS-1 genotypes.

Thus, results of our research showed that in hypertensive patients, Gly972Arg polymorphism of IRS-1 gene is associated only with separate components of the metabolic syndrome.

CONCLUSIONS

In hypertensive patients, the polymorphism of IRS-1 gene is associated with such components of metabolic syndrome as hypertriglyceridemia and fasting hyperglycemia; the

Table III. Comparative assessment of indicators in hypertensive patients depending on IRS-1 polymorphism

Indicators	Hypertensive patients, n=187		
	Genotype		
	Gly/Gly, n=88	Gly/Arg, n=79	Arg/Arg, n=20
SBP, mm Hg	168.7 ± 0.41	169.4 ± 0.43	169.9 ± 0.62
DBP, mm Hg	99.8 ± 0.24	100.5 ± 0.28	101.1 ± 0.34
BMI, kg/m^2	29.8 ± 0.39	31.1 ± 0.35	30.7 ± 0.44
Waist circumference, cm	94.5 ± 2.46	96.3 ± 3.57	97.1 ± 4.04
IMT, mm	0.85 ± 0.008	0.87 ± 0.012	0.86 ± 0.009
PWV AA, m/s	8.46 ± 0.12	8.49 ± 0.15	8.47 ± 0.12
EDVD, %	7.71 ± 0.07	7.57 ± 0.08	7.49 ± 0.14
Cholesterol, mmol/l	5.97 ± 0.05	6.12 ± 0.04	6.07 ± 0.05
Triglycerides, mmol/l	1.82 ± 0.04	2.12 ± 0.05*	2.18 ± 0.06**
LDL, mmol/l	4.49 ± 0.05	4.89 ± 0.04*	4.94 ± 0.08**
HDL, mmol/l	1.14 ± 0.01	1.08 ± 0.01	1.05 ± 0.02
Blood glucose, mmol/l	5.65 ± 0.02	6.43 ± 0.03*	6.51 ± 0.04**
Insulin, mcU/ml	16.8 ± 0.48	22.1 ± 0.52*	23.8 ± 0.43**
HOMA-IR	3.96 ± 0.11	5.21 ± 0.08*	6.05 ± 0.09**°
Adiponectin, ng/ml	7.82 ± 0.03	7.54 ± 0.02	7.65 ± 0.03
IL-6, ng/ml	139.6 ± 4.12	145.9 ± 5.31	148.7 ± 4.99
CRP, mg/dl	6.3±0.22	6.7±0.25	6.6±0.34
Distribution of patients with impaired glucose tolerance, %	46.2	38.5	15.3

Note: * – statistically significant difference between genotypes of Gly/Gly and Gly/Arg; ** – statistically significant difference between genotypes of Gly/Gly and Arg/Arg; ° – statistically significant difference between genotypes of Gly/Arg and Arg/Arg.

Table IV. Comparative assessment of indicators at Gly/Gly and Gly/Arg + Arg/Arg genotypes IRS-1 in patients with AH

Indicators	Hypertensive patients, n=187	
	Genotype	
	Gly/Gly, n=88	Gly/Arg + Arg/Arg, n=99
SBP, mm Hg	168.7 ± 0.41	169.6 ± 0.45
DBP, mm Hg	99.8 ± 0.24	100.9 ± 0.31
BMI, kg/m^2	29.8 ± 0.39	30.0 ± 0.32
Waist circumference, cm	94.5 ± 2.46	96.8 ± 3.29
IMT, mm	0.85 ± 0.008	0.87 ± 0.011
PWV AA, m/s	8.46 ± 0.12	8.48 ± 0.14
EDVD, %	7.71 ± 0.07	7.55 ± 0.09
Cholesterol, mmol/l	5.97 ± 0.05	6.09 ± 0.05
Triglycerides, mmol/l	1.82 ± 0.04	2.14 ± 0.05*
LDL, mmol/l	4.49 ± 0.05	4.91 ± 0.05*
HDL, mmol/l	1.14 ± 0.01	1.07 ± 0.02
Blood glucose, mmol/l	5.65 ± 0.02	6.46 ± 0.03*
Insulin, mcU/ml	16.8 ± 0.48	22.9 ± 0.44*
HOMA-IR	3.96 ± 0.11	5.42 ± 0.07*
Adiponectin, ng/ml	7.82 ± 0.03	7.57 ± 0.03
IL-6, ng/ml	139.6 ± 4.12	146.9 ± 6.13
CRP, mg/dl	6.3±0.22	6.7±0.27
Distribution of patients with impaired glucose tolerance, %	46.2	53.8

Note: * – statistically significant difference between genotypes of Gly/Gly and Gly/Arg +Arg/Arg.

specified polymorphism is not associated with pro-inflammatory state, endothelial dysfunction, dysglycemia, increase in waist circumference and decrease in HDL.

REFERENCES

1. Moebus S, Stang A. The metabolic syndrome – a controversial diagnostic concept. Herz. 2007;32(7):529–40.
2. Nilsson PM, Engstrom G, Hedblad B. The metabolic syndrome and incidence of cardiovascular disease in nondiabetic subjects: a population-based study comparing three different definitions. DiabetMed 2007; 24:464–472.
3. Benetos A, Thomas F, Pannier B, Bean K, Jego B, Guize L. All-cause and cardiovascular mortality using the different definitions of metabolic syndrome. Am J Cardiol 2008; 102:188–191.
4. Prasad H et al. Metabolic syndrome: definition and therapeutic implications. Postgraduate medicine. 2012;124(1):21–30.
5. Kassi E et al. Metabolic syndrome: definitions and controversies. BMC medicine. 2011;9(1):48.
6. Gallagher EJ, LeRoith D, Karnieli E. The metabolic syndrome – from insulin resistance to obesity and diabetes. Medical Clinics of North America. 2011; 95(5):855–873.
7. Nolan P.B., Carrick-Ranson G., Stinear J.W., Reading S.A., Dalleck L.C. Prevalence of metabolic syndrome and metabolic syndrome components in young adults: A pooled analysis. Preventive Medicine Reports. 2017:7; 211-215.
8. Alberti K. et al. Harmonizing the Metabolic Syndrome A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16): 1640–1645.
9. Romero-Corral A, Somers VK, Sierra-Johnson J, et al. Normal weight obesity: a risk factor for cardiometabolic dysregulation and cardiovascular mortality. Eur Heart J. 2010;31:737–46.
10. Clark AL, Fonarow GC, Horwich TB. Obesity and the Obesity Paradox in Heart Failure. Prog Cardiovasc Dis. 2014;56 (4):409–14.
11. Boban M, Persic V, Jovanovic Z, et al. Obesity dilemma in the global burden of cardiovascular diseases. Int J Clin Pract. 2014;68 (2):173–9.
12. Coutinho T, Goel K, Corrêa De Sá D, et al. Combining body mass index with measures of central obesity in the assessment of mortality in subjects with coronary disease: Role of "normal weight central obesity". J Am Coll Cardiol. 2013;61:553–60.
13. Lainscak M, Haehling S, Doehner W, et al. The obesity paradox in chronic disease: facts and numbers. Journal of Cachexia, Sarcopenia and Muscle. 2012;3 (1):1–4.
14. De Schutter A, Lavie CJ, Patel DA, et al. Obesity paradox and the heart: which indicator of obesity best describes this complex relationship? Curr Opin Clin Nutr Metab Care. 2013;16(5):517–24.

1. Aileen J.M., Edward P.F., Ronald K.J. Human Insulin Receptor Substrate-1 (IRS-1) Polymorphism G972R Causes IRS-1 to Associate with the Insulin Receptor and Inhibit Receptor Autophosphorylation. J. Biol. Chem. 2005; 280:6441–6446.
2. Rung J., Cauchi S., Albrechtsen A. et al. Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat. Genet. 2009;41(10):1110–1115.
3. Kilpeläinen T.O., Zillikens M.C., Stančákova A. et al. Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. Nat. Genet. 2011;43(8):753–760.
4. Morini E., Prudente S., Succurro E. et al. IRS1 G972R polymorphism and type 2 diabetes: a paradigm for the difficult ascertainment of the contribution to disease susceptibility of «low-frequency-low-risk» variants. Diabetologia. 2009;52(9):1852–1857.
5. Burguete-Garcia A.I., Cruz-Lopez M., Madrid-Marina V. et al. Association of Gly972Arg polymorphism of IRS-1 gene with type 2 diabetes mellitus in lean participants of a national health survey in Mexico: a candidate gene study. Metabolism. 2010;59(1):38–45.
6. Celi F.S., Negri C., Tanner K. et al. Molecular scanning for mutations in the insulin receptor substrate-1 (IRS-1) gene in Mexican Americans with type 2 diabetes mellitus. Diabetes Metab. Res. Rev. 2000;16(5):370–377.
7. Jellema A., Zeegers M.P., Feskens E.J. et al. Gly972Arg variant in the insulin receptor substrate-1 gene and association with Type 2 diabetes: a meta-analysis of 27 studies. Diabetologia. 2003;46(7):990–995.
8. Sesti G., Federici M., Hribal M.L. et al. Defects of the insulin receptor substrate (IRS) system in human metabolic disorders. FASEB J. 2001;15(12):2099–2111.
9. Taniguchi C.M., Ueki K., Kahn R. Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. J. Clin. Invest. 2005;115(3):718–727.
10. Hribal M.L., Tornei F., Pujol A. et al. Transgenic mice overexpressing human G972R IRS-1 show impaired insulin action and insulin secretion. J. Cell. Mol. Med. 2008;12(58):2096–2106.
11. Bodhini D., Radha V., Mohan V. Association Study of IRS1 Gene Polymorphisms with Type 2 Diabetes in South Indians. Diabetes technology & therapeutics. 2011;13(7):767–772.
12. Orkunogulu Suer F.E., Mergen H., Bolu E., Ozata M. Molecular scanning for mutations in the insulin receptor substrate-1 (IRS-1) gene in Turkish population with type 2 diabetes mellitus. Endocrinol. J. 2005;52(5):593–598.
13. Celermajer DS, Sorensen KE, Cooh VM, et al. Noninvasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340: 1111–1115.
14. Ivanova OV, Rogoza AN, Balahonova TV i dr. Opredelenie chuvstvitelnosti plechevoy arterii k napryazheniyu sdviga na endoteliy, kak metod otsenki sostoyaniya endoteliy zavisimoy vazodilatatsii s pomoschyu ultrazvuka vyisokogo razresheniya u bolnyih s arterialnoy gipertoniey. Kardiologiya. 1998; 3:37– 41 (Article in Russian).

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Anna Shalimova
Government Institution "L.T. Malaya Therapy National Institute of the National Academy of Medical Sciences of Ukraine"
Kharkiv, Ukraine
e-mail: anna.shalimova83@gmail.com

Received: 24.02.2019
Accepted: 01.07.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

QUERCETIN POTENTIATES ANTIRADICAL PROPERTIES OF EPIGALLOCATECHIN-3-GALLATE IN PERIODONTIUM OF RATS UNDER SYSTEMIC AND LOCAL ADMINISTRATION OF LIPOPOLISACCHARIDE OF SALMONELLA TYPHI

Alina M. Yelins'ka, Lilia I. Liashenko, Vitalii O. Kostenko
UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY, POLTAVA, UKRAINE

ABSTRACT

Introduction: There has been demonstrated that pharmaceutical effect of epigallocatechin-3-gallate (EGCG), a polyphenol, which is found in green tea *(Camellia sinensis),* is implemented through the activation of Nrf2 (Nuclear Factor Erythroid 2-Related Factor 2).The importance of Keap1 / Nrf2 / antioxidant response element (ARE) system is determined by the fact that the state of NF-κB- and AP-1-associated pathways depends on its activity. Recent studies have demonstrated the property of quercetin to suppress ubiquitin-dependent proteolysis of complex of NF-κB and its inhibitory protein IκB. All this provides preconditions to eliminate the potentiality of NF-κB-dependent expression of the number of genes of pro-oxidant and pro-inflammatory proteins. However, co-effect produced by quercetin and EGCG on the oxidative nitrosative stress markers in the periodontal tissues is still unclear.

The aim: To investigate the co-effect produced by quercetin and an inducer of the Keap1 / Nrf2 / ARE epigallocatechin-3-gallate on markers of oxidative-nitrosative stress in rats' periodontium under the systemic and local administration of *Salmonella typhi* lipopolysaccharide (LPS).

Materials and methods: The studies were conducted on 30 white rats of the Wistar line, divided into 5 groups: the 1st included intact animals, the 2nd was made up of animals after their exposure to combined systemic and local LPS administration, the 3rd and 4th groups included animals, which were given injections with water-soluble form of quercetin (corvitin) and EGCG respectively, and the 5th group involved rats, which were injected with co-administered corvitin and EGCG. The formation of superoxide anion radical ($\cdot O_2^-$) was evaluated by a test with nitro blue tetrazolium using spectrophotometry of the periodontal soft tissue homogenate. The total activity of NO-synthase and concentration of peroxynitrite in the homogenate of the soft components of periodontium were evaluated spectrophotometrically.

Results: Co-effect produced by corvitin and EGCG under systemic and local LPS administration is accompanied with reduced O_2^- production by NADPH-dependent electron transport chains (microsomal and NOS) by 20.0 % ($p<0.05$) compared with values for the animals received separate corvitin during the experiment. $\cdot O_2^-$ generation by the mitochondrial respiratory chain yielded to comparable data of the 3rd and 4th groups by 27.6 % ($p<0.01$) and 23.8 % ($p<0.05$) respectively. No differences were found between the groups exposed to combined or separate action of the above mentioned agents in the experiment when assessing O_2^- generation by leukocyte NADPH-oxidase. Combined effect of corvitin and EGCG during systemic and local LSP administration showed the decrease in NOS activity and peroxynitrite concentration in periodontal tissues by 53.3 % ($p<0.001$) and 27.0 % ($p<0.02$) compared with the findings in the 3rd group, and by 42.0 % ($p<0.01$) and 22.3 % ($p<0.01$) in the 4th group.

Conclusions: the co-administration of water-soluble form of quercetin and epigallocatechin-3-gallate under systemic and local introducing of lipopolysaccharide *Salmonella* typhi has been proven to be more effective means for preventing and correcting oxidative-nitrosative stress in the periodontal tissues than this occurs at separate administration of each of the polyphenols.

KEY WORDS: signal pathway Keap1 / Nrf2 / ARE, epigallocatechin-3-gallate, quercetin, lipopolysaccharide-induced systemic inflammatory response, oxidative-nitrosative stress, periodontium

Wiad Lek 2019, 72, 8, 1499-1503

INTRODUCTION

It has been known that impairment of the periodontium structure and functions result from a number of somatic diseases (metabolic syndrome, atherosclerosis, chronic pyelonephritis, traumatic disease, etc), whose development includes lipopolysaccharide (LPS)-induced systemic inflammatory response as a key component of pathogenesis [6]. Free-radical processes, associated with prolonged activation of certain redox-sensitive transcription factors (NF-κB, AP-1), contribute much to this process [2, 27]. This leads to the expression of genes of inflammatory cytokines, metalloproteinases, inducible nitric oxide synthase (NOS), cellular adhesion molecules, cyclooxygenase-2, etc. that can induce production of reactive oxygen and nitrogen species [16, 19, 29].

The redox-sensitive signaling pathway Keap1/Nrf2 plays a key role in the highly regulated cell systems. It activates gene expression through the interaction Nrf2 (Nuclear Factor Erythroid 2-Related Factor 2) and cis-acting enhancer sequence, known as antioxidant response element (ARE) [21, 24].

It has been reported that Nrf2 signalling is important in controlling oxidative stress in healthy periodontium

that helps to maintain integrity of periodontium despite of its constant exposure to aggressive factors as bacteria, neutrophiles, and macrophages [6].

Recently there has been demonstrated that pharmaceutical effect of epigallocatechin-3-gallate (EGCG), a polyphenol, which is found in green tea *(Camellia sinensis)* [12], is implemented through the activation of Nrf2 due to proteolysis of an inhibitory protein Keap1 [10, 22].

We have demonstrated than the introduction of EGCG in the lipopolysaccharide-inducible systemic inflammatory response is an effective mean to correct oxidative and nitrosative stress in the tissues of periodontium of the rats: it reduces the generation of superoxide anion radicals, in particular, its production by mitochondrial electron transport chain, microsomal and NOS electron transport chains, leukocyte NADPH-oxidase, as well as reduces the total activity of NOS and peroxynitrite concentration [30]. The administration of EGCG under modeled systemic inflammatory response is an effective means of preventing and correcting the disruption of connective tissue of periodontium in rats: it reduces collagenolysis and depolymerization of proteoglycans and glycoproteins [28].

The importance of Keap1 / Nrf2 / ARE system is determined by the fact that the state of NF-κB- and AP-1-associated pathways depends on its activity. In this ways Nrf2 inductors phenethyl isothiocyanate, sulforaphane and curcumin suppress the NF-κB activation [15].

Recent studies have demonstrated the property of quercetin to suppress ubiquitin-dependent proteolysis of complex of NF-κB and its inhibitory protein IκB that impairs the IκB degradation due to the proteasome activity [9]. All this provides preconditions to eliminate the potentiality of NF-κB-dependent expression of the number of genes of pro-oxidant and pro-inflammatory proteins (cytokines, matrix metalloproteinases, inducible NOS, etc.) [18].

However, co-effect produced by quercetin and EGCG on the oxidative and nitrosative stress markers in the periodontal tissues is still unclear. Solving this problem will enable to evaluate the role of this polyphenols as a potential means of pathogenetic therapy for inflammatory and dystrophic diseases of periodontal tissues.

THE AIM

The aim of the present study was to investigate the co-effect produced by quercetin and an inducer of the Keap1 / Nrf2 / ARE epigallocatechin-3-gallate on markers of oxidative and nitrosative stress in rats' periodontium under systemic and local administration of *S. typhi* LPS.

MATERIALS AND METHODS

The studies were conducted on 30 white rats of the Wistar line weighing 180-220 g, divided into 5 groups: the 1st included intact animals, the 2nd was made up of animals after the combined systemic and local LPS administration, the 3rd and 4th groups included animals, which were given injections with water-soluble form of quercetin (corvitin) and EGCG respectively, and the 5th group involved rats, which were injected with co-administered corvitin and EGCG.

For systemic administration, *S. typhi* LPS (pyrogenalum, "Medgamal", Russia) was injected intraperitoneally in a dose, which stimulated rise in temperature by 1.5 °C according to the scheme [30]: during the first week, 4 minimum pyrogenic doses (MPD) of 0.4 μg / kg of rat body weight were given 3 times a week. During the following 7 weeks of the experiment, rats were given 4 MPD / kg of body weight once a week.

For local administration, LPS *S. typhi* was performed once in as dose of 1 μg / kg, equally divided into four injections into the gum at the level of the second molars 7 days prior to the decapitation (acute gingivitis model).

Water-soluble form of quercetin (corvitin, "Borshchahivskiy CPP", Ukraine) was administered intraperitoneally in a daily dose of 10 mg / kg recalculated for quercetin [11], and EGCG (Sigma-Aldrich, Inc., USA) was administered in a dose of 21.1 mg / kg [28] 3 times a week, starting on the 30th day of the systemic LPS administration.

The research was conducted in compliance with the standards of the Convention on Bioethics of the Council of Europe 'European convention for the protection of vertebrate animals used for experimental and other scientific purposes' (Strasbourg, 18.III.1986). The animals were decapitated with ethereal anesthesia. Soft components of periodontium (gingiva and periodontal ligament) served as the objects of the study.

The formation of superoxide anion radical ($^{\cdot}O_2^-$) was evaluated by a test with nitro blue tetrazolium using spectrophotometry of the periodontal soft tissue homogenate by the following inductors: NADH was used to evaluate O_2^- production by the mitochondrial electron transport chain, NADPH was used to evaluate O_2^- production by endoplasmic reticulum and NOS, and *S. typhi* LPS was used to assess O_2^- production by NADPH oxidase of white blood cells [13].

The activity of NOS was determined by the difference in the concentration of nitrite ions before and after the incubation of homogenate into the medium containing L-arginine (NOS substrate) and NADPH. The concentration of nitrite ions was assessed by the formation of diazo-compounds in the reaction with sulfanilic acid, and then we carried out the reaction with α- naphthylethylenediamine, resulting in the production of red color derivatives [1]. The concentration of peroxynitrite in the homogenate was evaluated spectrophotometrically by wavelength of 355 nm [1].

The findings obtained were statistically processed. To verify the normality distribution, the Shapiro-Wilk test was applied. If they corresponded to the normal distribution, then the Student's t-test was used to compare independent samples. When the results ranges were not subject to normal distribution, statistical processing was performed using a nonparametric method, the Mann-Whitney test. Statistical calculations were performed using the "StatisticSoft 6.0" program.

RESULTS AND DISCUSSION

Systemic and local LPS co-administration led to significant changes in the O_2^-generation in the tissues of periodontium (Table I). Thus, the $\cdot O_2^-$ production by NADPH-dependent electron transport chains (microsomal and NOS) increased by 71.0 % (p<0.001), while the $\cdot O_2^-$ production by the mitochondrial respiratory chain increased by 85.5 % (p<0.001). Generation of $\cdot O_2^-$ by NADPH oxidase of white blood cells also elevated by 85.4 % (p<0.001).

According to the literature, the largest amount of $\cdot O_2^-$ is produced by the mitochondrial electron transport chain as a result of the one-electron reduction of oxygen at the level of the following complexes: NADH:ubiquinone oxidoreductase, ubiquinol:cytochrome c oxidoreductase and cytochrome bc1 [5].

Resent studies have demonstrated the activation of NF-κB and AP-1 enhances O_2^- generation by NADH- and NADPH-dependent electron transport chains (mitochondrial, microsomal and NOS) in the periodontal tissues homogenate [14, 27]. This process is accelerated by the formation of peroxynitrite in the tissues [3, 14].

At the same time, a powerful level of $\cdot O_2^-$ production under the conditions of LPS-inducible systemic inflammatory response is provided by NADPH-dependent electron transport chains: in the reactions of microsomal oxidation (with cytochrome P450) [7] and by NOS itself, which is capable of switching from NO to $\cdot O_2^-$ under unfavourable conditions [20]. When the bacterial LPS are introduced into the mammalian body, the $\cdot O_2^-$ generation by leukocyte NADPH oxidase naturally increases [4]. In addition to LPS, proinflammatory cytokines, whose synthesis depends on the activation of NF-κB [8, 23], are also known as effective stimulants of $\cdot O_2^-$synthesis.

Combined systemic and local LPS administration increased NOS activity and peroxynitrite concentration (Table II) in periodontal tissues in three times (p<0.001) and 85.5 % (p<0.001) respectively. This can be explained by the ability of LPS to provide NF-κB-dependent activation of inducible NOS [23].

It was found that the separate administration of both corvitin and EGCG reduced $\cdot O_2^-$ production by NADPH-dependent electron transport chains by 27.0 % (p<0.001) and by 31.0 % (p<0.001) (Table I). Under the same conditions, O_2^- production by the mitochondrial respiratory chain declined by 31.2 % (p<0.01) and 34.6 % (p<0.001) respectively. Generation of O^-_2 by leukocyte NADPH-oxidase in periodontal tissues was lessened by 34.5 % (p<0.001) and 37.9 % (p <0.001) respectively to comparable data of the 2nd group.

Under these conditions, NOS activity in periodontal tissues decreased by 32.8 % (p<0.01) and 45.9 % (p<0.001), peroxynitrite concentration declined by 35.1 % (p<0.001) and 39.0 % (p<0.001) respectively to comparable data of the 2nd group (Table II).

Co-effect produced by corvitin and EGCG under systemic and local LPS administration is accompanied with reduced O_2^-production by NADPH-dependent electron transport chains (microsomal and NOS) by 20.0 % (p<0.05) compared with values for the animals received separate corvitin during the experiment (Table I). $\cdot O_2^-$ generation by the mitochondrial respiratory chain yielded to comparable data of the 3rd and 4th groups by 27.6 % (p<0.01) and 23.8 % (p<0.05) respectively.

No differences were found between the groups exposed to combined or separate action of the above mentioned agents in the experiment when assessing O_2^- generation by leukocyte NADPH-oxidase.

Combined effect of corvitin and EGCG during systemic and local LSP administration showed the decrease in NOS activity and peroxynitrite concentration in periodontal tissues by 53.3 % (p<0.001) and 27.0 % (p<0.02) compared with the findings in the 3rd group, and by 42.0 % (p<0.01) and 22.3 % (p<0.01) in the 4th group.

The results obtained point out the synergic effect produced by water-soluble form of quercetin and EGCG on

Table I. Co-effect produced by corvitin and epigallocatechin-3-gallate on generation of superoxide anion-radical in periodontium soft tissues under local and systemic administration of S. typhi lipopolysaccharide (M+m, n=35)

Groups of the animals studied	Inductors of superoxide anion radical production, nmol / g • s		
	NADPH	NADH	LPS
Control group I (intact animals)	12.47±0.87	15.41±1.08	1.58±0.12
Control group II (combined systemic and local LPS introduction)	21.32±0.41 *	28.59±1.41 *	2.93±0.12 *
+ water-soluble form of quercetin (corvitin)	15.57±0.85 */**	19.68±1.32 */**	1.92±0.13 **
+ EGCG	14.72±0.81 **	18.69±1.31 **	1.82±0.14 **
+ corvitin + EGCG	12.45±0.83 **/***	14.24±0.90 **/***/****	1.72±0.10 **

Note (in table I-II):
* – p<0.05 compared with values in the control group I (intact rats);
** – p<0.05 compared with values in the control group II (combined systemic and local LPS administration);
*** p<0.05 compared with values in the animals received corvitin only under systemic and local LPS administration;
**** p<0.05 compared with values in the animals received EGCG only under systemic and local LPS administration

Table II. Co-effect produced by corvitin and epigallocatechin-3-gallate on nitrosative stress markers in periodontium soft tissues under local and systemic administration of S. typhi lipopolysaccharide (M+m, n=35)

Groups of the animals studied	Total activity of NO-synthase, µmol / min ·g· of protein	Concentration of peroxynitrite- ions, µmol / g, of homogenate
Control group I (intact animals)	4.20±0.22	0.83±0.04
Control group II (combined systemic and local LPS administration)	12.53±0.61 *	1.54±0.06 *
+ water-soluble form of quercetin (corvitin)	8.42±0.64 */**	1.00±0.08 **
+ EGCG	6.78±0.54 */**	0.94±0.05 **
+ corvitin + EGCG	3.93±0.48 **/***/****	0.73±0.03 **/***/****

the correction of oxidative and nitrosative stress markers in the periodontal tissues.

Resent studies have demonstrated the potential of Keap1 / Nrf2 / ARE system to control other redox-sensitive elements, including NF-κB i AP-1 [15, 25]. We have found out the activation of NF-κB is an important component in the mechanism of free radical damage to the periodontal tissues during systemic inflammatory response. The use of an inhibitor of the nuclear translocation of NF-κB ammonium pyrrolidine dithiocarbamate in these conditions limits the manifestations of oxidative and nitrosative stress in the periodontium [29].

Previously it was shown that LPS-induced NF-κB activation could be attenuated by certain Nrf2 activators, such as phenethyl isothiocyanate, sulforaphane and curcumin [15]. The administration of these compounds significantly inhibits phosphorylation in the site of IκB kinase (*IKK*) / IκB and p65 NF-κB subunit nuclear translocation, consequently alleviating NF-κB signaling [26].

Co-administration of water-soluble form of quercetin and EGCG allows to limit NFκB-dependent mechanisms of oxidative and nitrosative stress more effectively as well as to enhance antioxidant potential of periodontal tissues associated with ARE-dependent gene expression.

Non-toxicity of quercetin and EGCG is a benefit, which distinguishes them from most NF-κB activation inhibitors, whose applying is known to cause a number of adverse reactions [17].

Thus, the co-administration of water-soluble form of quercetin and epigallocatechin-3-gallate under systemic and local introducing of *S. typhi* lipopolysaccharide has been proven to be more effective means for preventing and correcting oxidative and nitrosative stress in the periodontal tissues than this occurs at separate administration of each of the polyphenols.

REFERENCES

1. Akimov O Ye., Kostenko VO. Functioning of nitric oxide cycle in gastric mucosa of rats under excessive combined intake of sodium nitrate and fluoride. Ukr Biochem J. 2016;88(6):70-5.
2. Ambili R, Janam P. A critique on nuclear factor-kappa B and signal transducer and activator of transcription 3: The key transcription factors in periodontal pathogenesis. J Indian Soc Periodontol. 2017 Sep-Oct;21(5): 350-6.
3. Bohdanov AV, Hryshko YuM, Kostenko VO. Mechanisms of nitroxide-ergic dysregulation in periodontal tissues of rats under combined excessive sodium nitrate and fluoride intake. Wiad Lek. 2016;69(3, pt 2):457-61. (In Russian).
4. Cachat J, Deffert C, Hugues S, Krause KH. Phagocyte NADPH oxidase and specific immunity. Clin Sci (Lond). 2015 May 1;128(10):635-48.
5. Dröse S, Brandt U. Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. Adv Exp Med Biol.2012; 748:145-69.
6. Hasturk H, Kantarci A, Van Dyke TE. Oral inflammatory diseases and systemic inflammation: role of the macrophage. 2012;3:118.
7. Im SC, Waskell L. The interaction of microsomal cytochrome P450 2B4 with its redox partners, cytochrome P450 reductase and cytochrome b(5). Arch Biochem Biophys. 2011;507(1):144-53.
8. Kaidashev IP. NF-kB Activation as a molecular basis of pathological process by metabolic syndrome. Fiziol Zh. 2012;58(1):93-101. (In Ukrainian).
9. Kang CH, Choi YH, Moon SK et al. Quercetin inhibits lipopolysaccharide-induced nitric oxide production in BV2 microglial cells by suppressing the NF-κB pathway and activating the Nrf2-dependent HO-1 pathway. Int Immunopharmacol. 2013 Nov; 17(3):808-13.
10. Kanlaya R, Khamchun S, Kapincharanon C, Thongboonkerd V. Protective effect of epigallocatechin-3-gallate (EGCG) via Nrf2 pathway against oxalate-induced epithelial mesenchymal transition (EMT) of renal tubular cells. Sci Rep. 2016 Jul 25;6:30233.
11. Khmil' DO, Kostenko VO. Combined effect of L-arginine and water-soluble form of quercetin on markers of oxidative-nitrosative stress in skin of rats exposed to excessive sodium nitrate. Fiziol Zh. 2017; 63(6):53-9. (In Ukrainian).
12. Kim HS, Quon MJ, Kim JA. New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biol. 2014 Jan 10;2:187-95.
13. Kostenko VO, Tsebrzhins'kii OI. Production of superoxide anion radical and nitric oxide in renal tissues sutured with different surgical suture material. Fiziol Zh. 2000;46(5):56-62. (In Ukrainian).

14. Kostenko VA, Yelins'ka AM, Ljashenko LI et al. NO- and peroxynitrite-dependent changes in superoxide anion-radical production in rats' organs under modeled metabolic syndrome. Zhurn. Grodnenskogo gos. med. univ. 2014;(2):74-7. (In Russian).
15. Li W, Khor TO, Xu C et al. Activation of Nrf2-antioxidant signaling attenuates NFkappaB-inflammatory response and elicits apoptosis. Biochem Pharmacol. 2008 Dec 1;76(11):1485-9.
16. Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. / Matrix Biol. 2002 Apr;21(3):251-62.
17. Lin Y, Bai L, Chen W, Xu S. The NF-kappaB activation pathways, emerging molecular targets for cancer prevention and therapy. Expert Opin Ther Targets. 2010 Jan;14(1):45-55.
18. Liu X, Lin R, Zhao B et al. Correlation between oxidative stress and the NF-κB signaling pathway in the pulmonary tissues of obese asthmatic mice. Mol Med Rep. 2016 Feb;13(2):1127-34.
19. Ljashenko LI, Denisenko SV, Kostenko VA. Role of transcription nuclear factor κB in mechanisms of free radical processes impairment and connective tissue disorganization in periodontium under modeled metabolic syndrome. Aktual'ni problemy suchasnoyi medytsyny: Visn Ukr med stomatol akad. 2014; 14(1):97-100. (In Ukrainian).
20. Luo S, Lei H, Qin H, Xia Y. Molecular mechanisms of endothelial NO synthase uncoupling. Curr Pharm Des. 2014;20(22):3548-53.
21. Ma Q. Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol. 2013;53:401-26.
22. Sun W, Liu X, Zhang H et al. Epigallocatechin gallate upregulates NRF2 to prevent diabetic nephropathy via disabling KEAP1. Free Radic Biol Med. 2017 Jul;108:840-857.
23. Tornatore L, Thotakura AK, Bennett J et al. The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation. Trends Cell Biol. 2012 Nov;22(11):557-66.
24. Vomhof-Dekrey EE, Picklo MJ Sr. The Nrf2-antioxidant response element pathway: a target for regulating energy metabolism. J Nutr Biochem. 2012 Oct;23(10):1201-6.
25. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. Biochem Soc Trans. 2015 Aug;43(4):621-6.
26. Xu C, Shen G, Chen C et al. Suppression of NF-kappaB and NF-kappaB-regulated gene expression by sulforaphane and PEITC through IkappaBalpha, IKK pathway in human prostate cancer PC-3 cells. Oncogene. 2005 Jun 30;24(28):4486-95.
27. Yelins'ka AM, Akimov OYe, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. Ukr Biochim J. 2019;91(1):80-5.
28. Yelins'ka AM, Shvaykovs'ka OO, Kostenko VO. Epigallocatechin-3-gallate prevents disruption of connective tissue in periodontium and salivary glands of rats during systemic inflammation. Wiad Lek. 2018;71(4):869-73.
29. Yelins'ka AM, Shvaykovs'ka OO, Kostenko VO. Influence of ammonium pyrrolidine dithiocarbamate on production of reactive oxygen and nitrogen species in tissues of periodontium and salivary glands of rats exposed to systemic administration of Salmonella typhi lipopolisaccharide. Fiziol Zh. 2018;64(5):63-9. (In Ukrainian).
30. Yelins'ka AM, Shvaykovs'ka OO, Kostenko VO. Sources of production of reactive oxygen and nitrogen species in tissues of periodontium and salivary glands of rats under modeled systemic inflammation. Probl ekol med. 2017;21(3-4):51-4.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Vitalii O. Kostenko
Department of Pathophysiology,
Ukrainian Medical Stomatological Academy,
Shevchenko St., 23, 36000 Poltava, Ukraine
tel: +380532226966
e-mail: patofiziolog@umsa.edu.ua

Received: 18.02.2019
Accepted: 05.07.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

ENVIRONMENTAL AND OCCUPATIONAL HAZARDS OF METAL NANOCOMPOUNDS PRODUCTION AND APPLICATION: HYGIENIC, CLINICAL AND TOXICOLOGICAL ASPECTS

Oleksandr Yavorovskiy, Sergii Omelchuk, Lyudmyla Sokurenko, Tetyana Zinchenko, Nina Solokha, Vasyl Aleksiichuk, Roman Brukhno
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Introduction: Nanometals are the most common nanoobjects, used nearly in all industrial branches. Considerable advances in the nanotechnological production have led to progressive use of nanomaterials in industry, though occupational safety of the nanoindustry staff is insufficiently studied.

The aim: Estimation of labor safety during production of metal nanoparticles for the purpose of defining necessary and efficient preventive measures.

Materials and methods: The personnel of the hygienic departments of the O.Bogomolets National medical university have conducted numerous physiological, hygienic, biochemical, morphological and toxicological studies. The scientists have studied and revealed hazardous workplace factors of various metal nanoparticle production technologies, particularly those of metal nanoparticles: nanosilver, titanium nitride, chromium disilicide, lead sulphide, etc.

Results and conclusions: The authors have developed method of the occupational exposure metal nanoparticles exposure air control, assessed and analyzed health of the personnel engaged in production of the nanometals. The paper contains data of the blood cells functional activity assessment, with the detected possible molecular nanoparticles toxicity mechanisms due to the altered gene expression. The authors have studied effect of nanoaerosols onto the laboratory rats respiratory organs, evaluated their blood biochemical characteristics, liver lipid content of fatty acids as well as defined morpho-functional hepatic transformations of the laboratory animals which underwent lead sulfide nanoparticles treatment.

KEY WORDS: metal nanocompounds production, hazardous workplace factors, laboratory animals

Wiad Lek 2019, 72, 8, 1504-1511

INTRODUCTION

Industrial globalization leads to significantly increased production of new compounds with unique physical, chemical and biological properties. Nanometals take up the priority position among these compounds as they are widely used in medicine, pharmacology, cosmetology, textile production and electronic industry [1]. Considerable economic and scientific development is directly related to increased number of employees affected by new factors [2, 3]. The nanostructure vast specific surface, high surface curvature, polymorphy and insolubility in water and biological media may stipulate for their high bioactivity, higher toxicity and personal human hazards. Unfortunately, high rates of nanoindustry development significantly leave behind scientific trials of nanocompounds production and use negative consequences [3 - 5].

The personnel of Hygienic departments of O.Bogomolets National medical university have recently been studying hygienic effects of nanotechnologies, accepted by leading institutions of the National academy of sciences of Ukraine [6, 7]. The obtained data are offered in the paper.

THE AIM

Aims – estimation of labor safety during production of metal nanoparticles for the purpose of defining necessary and efficient preventive measures.

MATERIALS AND METHODS

The trials on metal nanoparticles production technology were held in the International Center of Electron Beam Technologies, affiliated to E.Paton Institute of Electrical Welding as well as I.Frantsevich Institute for Problems of Materials Science.

The authors, considering basic unfavorable environmental factors, have observed timing, studied psychophysiological factors (particularly, severity of the labor conditions), occupational noise, microclimate, X-ray emission and the operating content of the aerosols containing nanoparticles.

In order to evaluate personnel's health, the authors have analyzed regular medical examination charts and conducted the following clinical and instrumental procedures : objective examination, electrocardiography, electroencephalography and echocardiography. The liver and thyroid gland were examined using the ultrasound.

The range of conducted examination procedures includes: defining the enzymatic activity of aspartate transaminase, alanine-aminotransferase, total bilirubin, cholesterol and glucose blood content. To study nanoparticle effect onto the immune system the enzyme-linked immunosorbent assay ELISA was employed.

Then the authors held trials with the male Wistar rats, weighing 230 – 240g, and white rats, weighing 170-200g. The scientists used nanoparticles of silver in the NaCl matrix,

sized 16±5 nm, and suspension of 50mg of titanium nitride ultramicroscopic powder with 1ml of saline solution.

In other trials colloid solutions of lead sulphide nanoparticles, sized 10 and 30nm, were used, which were daily introduced intraabdominally, 5 times a week (working week modeling), concentrated as 1/100 of the ionic lead median effect concentration EC50. To compare the obtained data distilled lead nitrate solution with particles sized 400nm was used.

Nanopowder toxicity was estimated by defining the median lethal dose in intragastric and intraabdominal introduction as well as by studying the nanoparticle effect on the rabbit eye mucosa and skin.

To conduct histological studies the lungs were fixed in 10% neutral formaldehyde solution. Then the material was dewatered in ascending concentration alcohols and treated with chloroform - alcohol, chloroform, chloroform-paraffin (37°C) and paraffin (57°C), the paraffin being poured into the wax. Paraffin sections, 7μm in width, were obtained using sliding microtome. Serial sections were stained with hematoxylin, eosin and Van Gison.

To evaluate nanosilver effect onto expression of SNARK proteinkinase, caseinkinase-1ε and circadian genes, total RNA was obtained from the rat liver, lungs, heart, kidneys and testicles, using the Trizol reagent (Trizol; Invitrogen, USA) according to the manufacturer's instructions. The method was described by O.G.Minchenko et al. [8].

The murine blood serum was studied for such biochemical characteristics as total protein concentration, albumin concentration, total fat concentration, cholesterol concentration, triglyceride concentration and enzymatic activity(ALT and AST), using biochemical analyzer Vitros-250. Sample preparation and biological data estimation were conducted according to the manual.

For histological examination of murine liver, preparations were fixed in 12% formaline solution with the phosphate buffer (pH=7.0-7.2) the sections were stained according to the study needs (Azure II-eosin, Van Gison, PAS reaction) to be further light optically studied. To detect acidic and neutral mucopolysaccharides samples were stained with PAS reagent.

The samples with 30 cells, stained after Einarson technique, were examined for: nucleus intersection area (N area), nucleus specific optical density (N DM), Hertwig index (IH), nucleus integrated optical density (N IntDen), as well as measured nucleic acids in the modified nucleus volume (N Coeff NA) for extrapolation of the results in the section plane: 1: N Coef NA = N IntDen × ¾×N area ×√(N area/π) [9,10].

Cell cytoplasm was studied for: cell intersection area (C area, μm^2), cell specific optical density (C DM, units), cell integrated optical density (C IntDen, μm×units), cell nucleic acids in the modified volume (using the same formula as in 1) (C Coef NA). The departure point index for estimation of the nucleic acids content was equal to 1, which is characteristic for the lymphocyte nuclei, situated in the organ stroma. In each trial the paired correlation analysis was conducted.

Morphometric studies were performed using image analyzer microscope Olympus BX51 with digital camera C-4040zoom and PC. Sizing was conducted using micrometer E.LEITZ WETZIAR [11].

The obtained data were statistically analyzed using Microsoft Excel software, mean averages compared regarding the Student's t-criterion. The difference was considered reliable at $p<0.05$.

RESULTS AND DISCUSSION

Introduction of nanotechnologies directly affects labor conditions, so we have decided to study how production affects the operating personnel's health. According to the obtained results, the operating personnel's work refers to the acceptable severity group, hazardous class, "exhausting work" of the 1st degree due to the "continuous attention concentration" parameter, which may lead to increased morbidity with subsequent disability. The next stage was defining health conditions of personnels' work. The staff work under acceptable parameters of microclimate, infrared exposure, production noise and X-ray exposure [12].

Nanoparticle production is characterized by a real possibility of their invasion into the operating area air and penetration into the personnel's bodies by inhalation. During nanosilver synthesis by electron beam technology method, the highest concentrations of aerosol which contains silver nanoparticles were determined during vacuum chamber purification (0.618±0.004 mg/m^3) and during separation of condensate from template (0.0018±0.0001 mg/m^3). Estimation of nanosilver concentration is a challenging issue as this is new man-made factor, the health hygienic evaluation of which can't be conducted using maximum concentration limit obtained by gravimetric method for silver metal (1 mg/m^3). It is necessary to consider that the silver nanoparticles operating area air concentration during purification of the vacuum chamber purification 61.8 times exceeds permissible standards accepted in the USA, Austria, Germany, Japan and Switzerland (0.01 mg/m^3) [12, 13]. The obtained results substantiate the necessity of defining and introducing new health standards for the nanoparticle maximum concentration limit according to their absolute number in the air volume unit, their size, specific surface and other physical-chemical parameters.

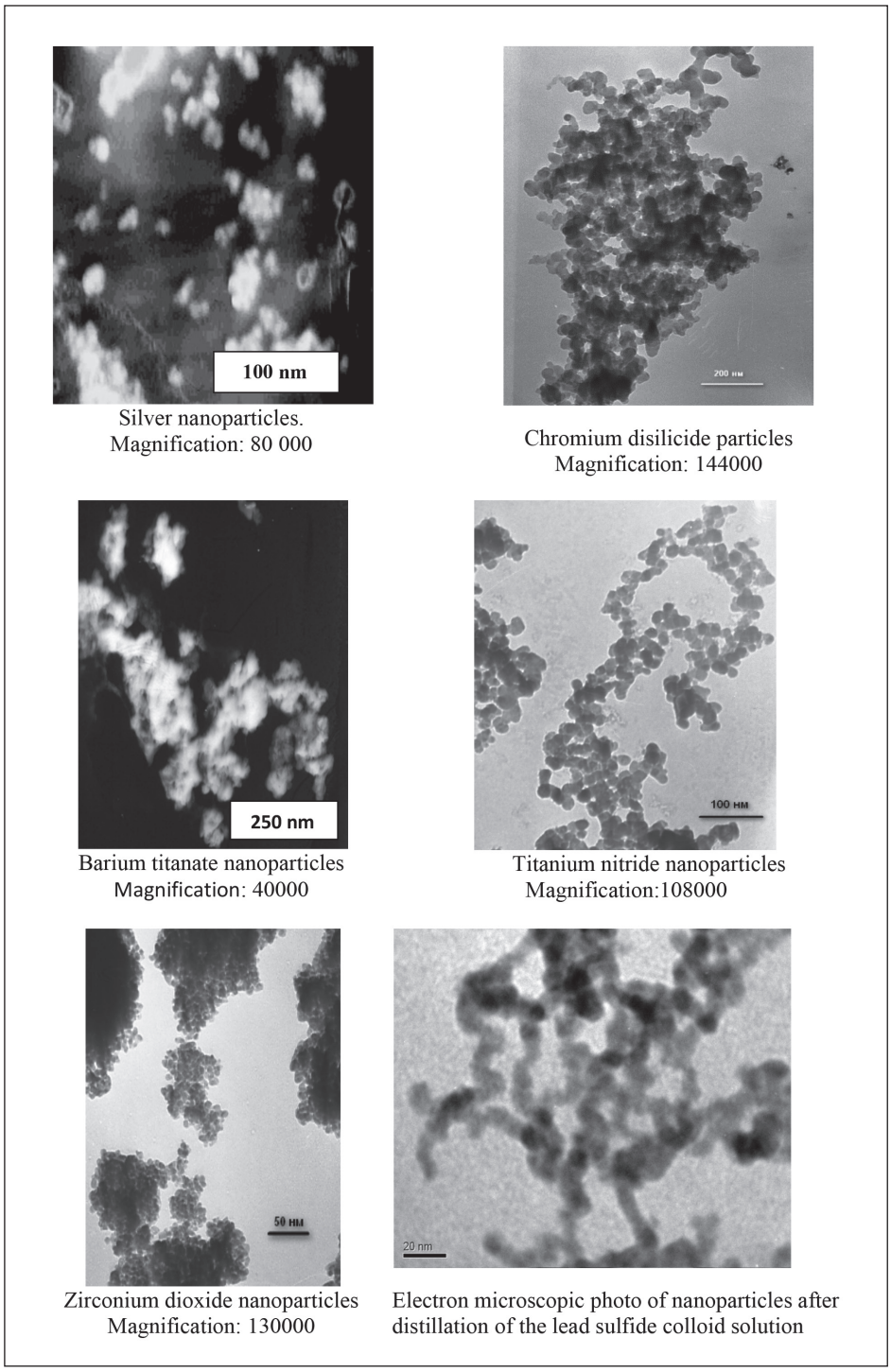
Nowadays, the scientists possess sufficient range of nanoparticle visualization methods as well as methods of estimating nanoparticles air concentration, though most of them have drawbacks. The authors have offered a new approach to the hygienic control of nanoparticle operating area air content and battle-tested the polyvinyl pyrolidone water-soluble nanofilters, which may arrest the aerosol particles, sized 1nm-10 μm, with air aspiration speed below 30 l/min. Chemical properties of polyvinyl pyrolidone water-soluble filters provide for obtaining suspension with already stable nanoparticles. This permits for using the obtained suspension to analyze the nanoparticles shape and dispersion content by electron microscopy and atom absorption spectral measurement (fig. 1). Besides defining

shape and size of nanoobjects, water-soluble filters permit to calculate their total surface area, which is required for complete toxicological assessment [14, 15].

The size and shape of metal nanocompounds have been assessed by the authors in collaboration with the O.Bogomolets National medical university department of microbiology and virusology personnel (head of the department – academician Shyrobokov V.P.). Nanopowders are presumably of round shape, with capacity for aggregation (fig.1) [12, 16].

To establish the upper toxicity parameters, we introduced to the laboratory animals (mice and rats) nanopowders of refractory metal compounds (titanium nitride and chromium disilicide) dosed as 5000, 10000 and 15000 mg/kg. None of nanopowders caused animals death upon its introduction into the stomach, only slight general depression and lethargy were noted in 24 hours after introduction. Introduction of nano CrSi2, nano TiN and nano ZrO2 in the conjunctival eye sac and on the skin of the rabbit didn't lead to visible changes on the conjunctiva or skin [16].

Clinical assessment of hepatobiliary system showed increased activity of alanine aminotransferase and aspartate



Silver nanoparticles.
Magnification: 80 000

Chromium disilicide particles
Magnification: 144000

Barium titanate nanoparticles
Magnification: 40000

Titanium nitride nanoparticles
Magnification:108000

Zirconium dioxide nanoparticles
Magnification: 130000

Electron microscopic photo of nanoparticles after distillation of the lead sulfide colloid solution

Fig.1. Electron-diffraction photographs of studied nanoparticles of metals and their compounds.

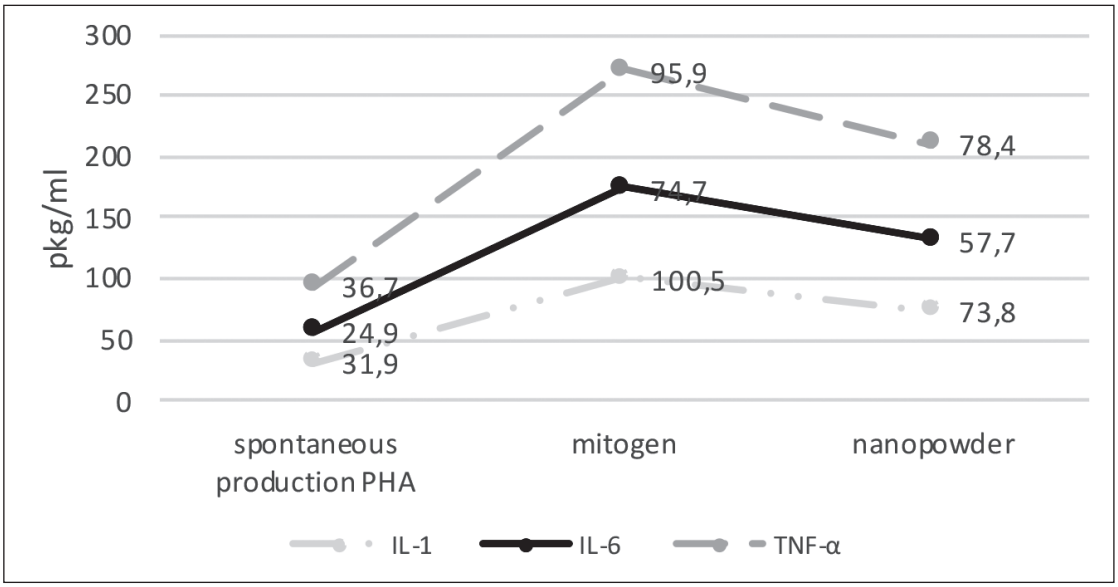


Fig. 2. Production of IL-1, IL-6 and TNF-α by mononuclear cells in vitro, by operating personnel dealing with nanocomposites.

aminotransferase, combined with hypercholesterolemia and hyperglycemia. Continuous action of nonoxide refractory metal compound nanopowders causes systemic autoimmune response with cytokines. Such processes make up a pathogenetic factor of onset and development of structural vascular changes as well as toxic digestive conditions [16] (fig. 2, 4).

The parallel trial detected that after 30 introductions of lead sulphide nanoparticles, sized 10 and 30 nm, to the animals, murine blood alanine tranferase activity reliably increased ($p \leq 0.05$) 2.5 times, after 60 introductions – almost threefold, compared to the control animals.

Murine blood aspartate transferase activity, after 60 introductions of toxins, reliably increased, compared to the control ($p \leq 0.05$, fourfold).

The authors have also established that with 30 and 60 toxins introductions, total lipids and cholesterol concentration tended to decrease, while triglycerides and glucose increased, regardless of the studied toxicants action duration [17].

Morphological examination showed reinforcement of dystrophy in the animals' hepatocytes, according to the lead sulphide nanoparticles action duration. After 30 introductions of the substances the authors revealed granular cytoplasm and off-centered nuclei, congestion of the central vein and inconsiderable amount of erythrocytes in sinusoids (fig. 3). After 60 introductions the dystrophy was even more expressed [18].

Morphometric and densitometric studies of the hepatocyte nuclei and cytoplasm after 30 and 60 introductions of the substances revealed decompensation processes.

The *in vitro* studies showed that the nanocomposites may increase functional activity of the monocyte-macrophage line cells regarding production of IL-1, IL-6 and TNF-α, which standardly coordinate interaction of the immune cells and activate inflammation.

IL-4, inducing the B-lymphocytes proliferation, is supposed to increase receptor expression for the IgE Fc-fragment, being an antagonist of IFN-α, suppressing production of IL-1, TNF-α, IL-6, IL-8 and cytotoxic effect of T-lymphocytes. It is of extreme importance in the development of immediate allergic reactions.

In order to reveal the silver nanoparticles possible genotoxic and cancerogenic action the authors, in cooperation with O.Palladin Biochemistry institute personnel, studied gene expression. The article covers only some issues of the study of nanosilver effect onto the caseinkinase-1ε gene expression, more detailed information on the trials is represented in further publications [8, 19, 20]. Silver nanoparticles may affect caseinkinase-1ε gene expression in the murine lungs, liver, myocardium and testicles in 24 hours after introduction of silver nanoparticles. Silver nanoparticles stimulating effect onto the caseinkinase-1ε mPNA expression in these organs was also detected in 3 and 14 days after the nanoparticles introduction.

The authors have revealed that the caseinkinase-1ε gene expression intensifies in kidneys under the silver nanoparticles effect, but only in 3 and 14 days after their introduction. The obtained results evidence about possible effect of silver nanoparticles on important cell metabolic processes regulation within the level of caseinkinase-1ε gene expression, which may lead to disordered signaling cascade in cells and development of pathological conditions, as well as serve as a sensitive marker of metal nanoparticles biosafety [20].

According to the conducted hygienic trials of the production process, the authors have established that the thermosynthesis operating personnel underwent titanium nitride influence. The conducted electron miscoscopic analysis showed present titanium nitride powder conglomerates in intraalveolar septae macrophages, viewed as large or small aggregations which decreased in number throughout the experiment (fig. 6, 7) [16, 21].

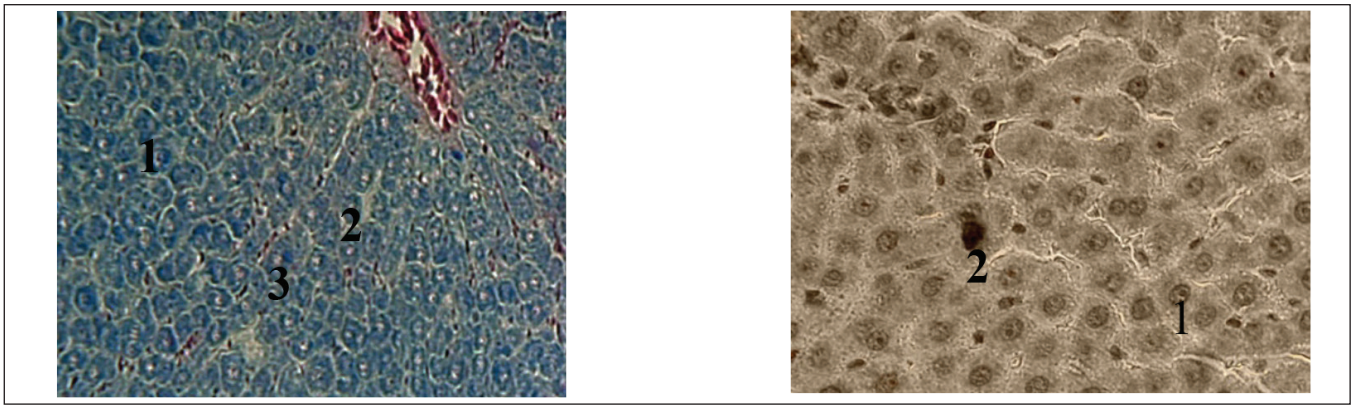


Fig. 3. Murine hepatic structure after introduction of lead sulphide nanoparticles, sized 30nm, after 30 introductions (A – staining with Azure II-eosin, magn. 200; B – staining with picro-fuchsin according to Van Gieson, magn. 400). 1 – hepatocyte, 2 – hepatocyte nucleus, 3 – blood corpuscles.

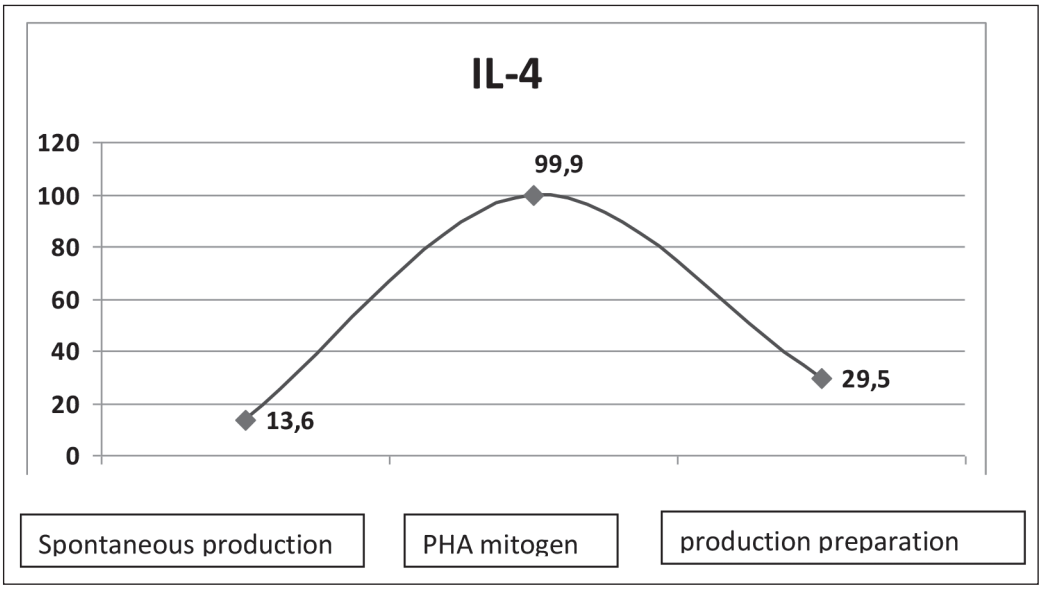


Fig. 4. Production of IL-4 by mononuclear cells in vitro by donors who contact with nanocomposites.

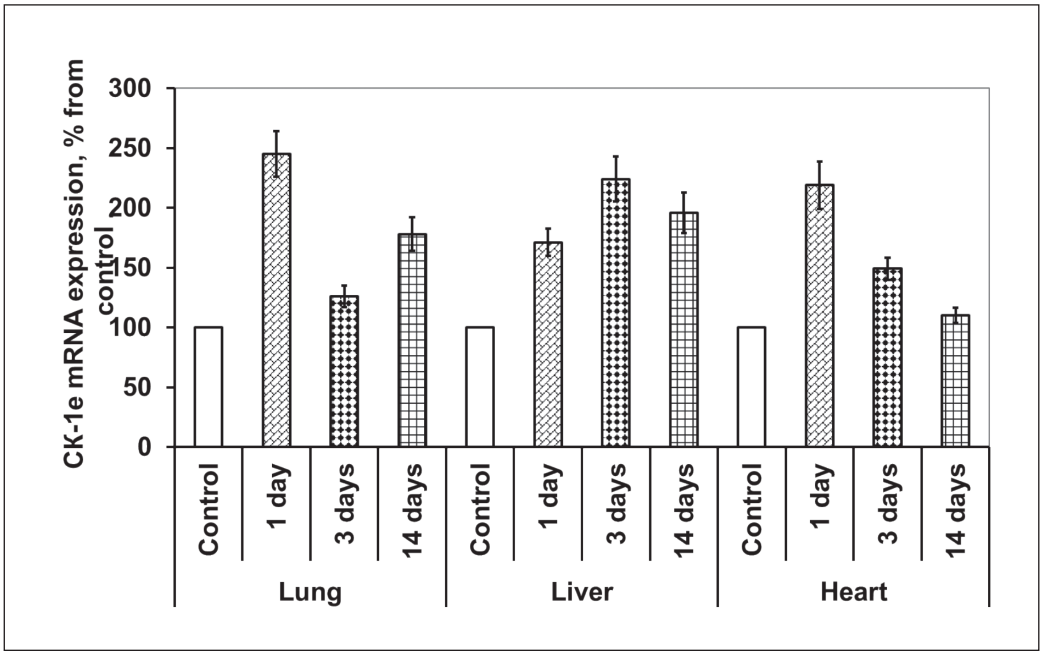


Fig. 5. Analysis of silver nanoparticles effect onto the caseinkinase-1ε mRNA expression in the lungs, liver and myocardium of rats.

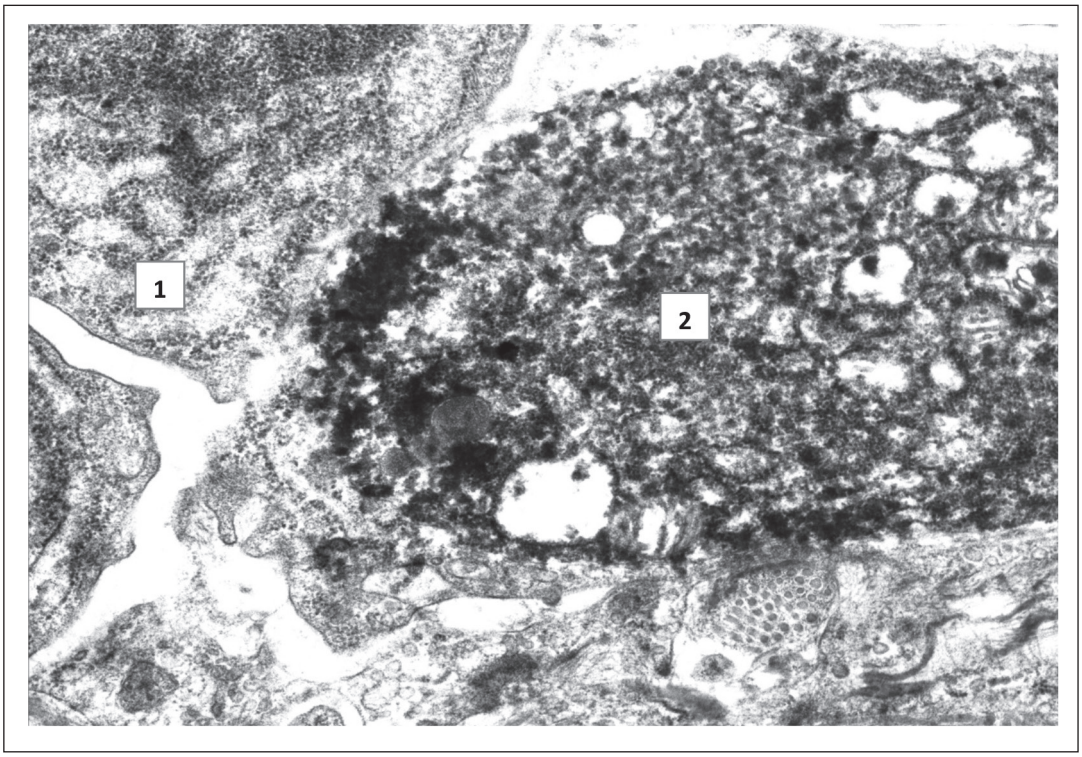


Fig. 6. Lungs in 7 days after introducing titanium nitride nanoparticles.
Cell debris (1). Macrophage necrosis (2).
Electron microscopic photo. Magnif.:A-18000,B- 18000.

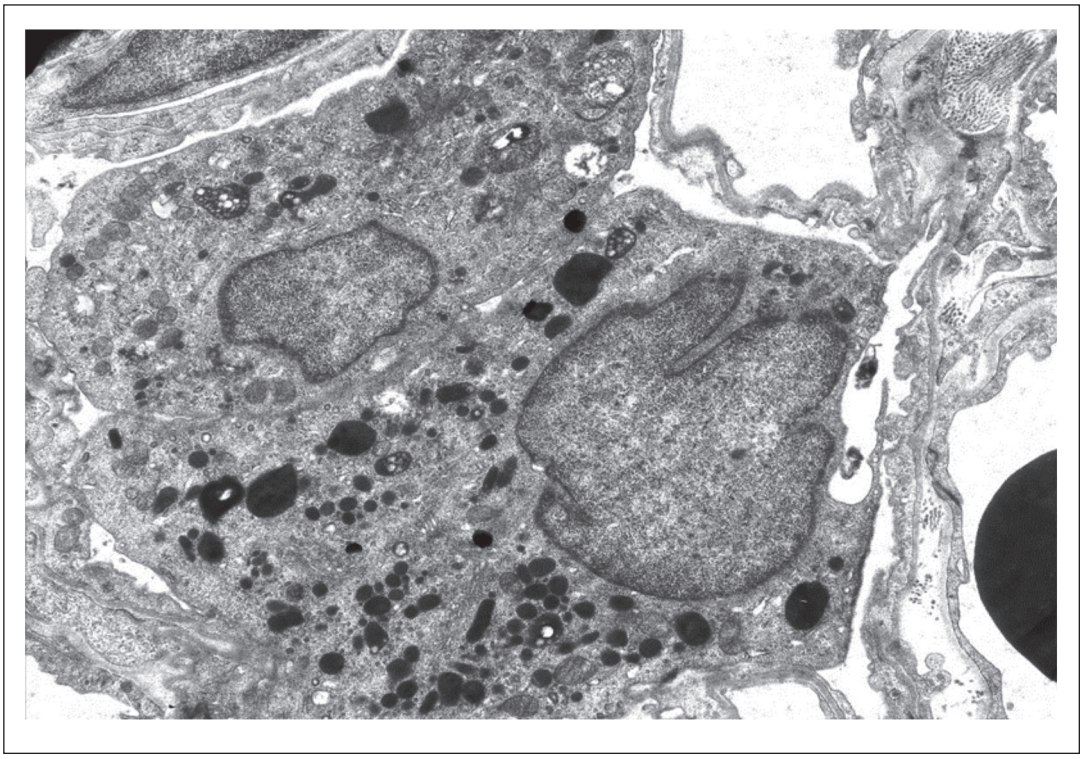


Fig. 7. Lungs in 1 year after introduction of the titanium nitride nanoparticles. Macrophage. Electron-microscopic photo. Magnif.:A-12000, B-9000.

The intraalveolar septae within the material accumulation were thickened, infiltrated with lymphocytes and plasmocytes, tissue basophils, macrophages and rarely neutrophils. Also, morphological signs of chronic bronchitis were noted: thickening, swelling and infiltration of all wall layers, dystrophic changes and exfoliation of ciliated epithelium.

The data on health and occupational morbidity of the personnel engaged in nanoparticle production are poorly

represented in medical sources or absent at all. As for the personnel affected by the barium titanate nanocrystal powder, they are most commonly affected with respiratory and haemopoetic diseases. The clinical-instrumental assessment detected changes in the bronchopulmonary system: chronic obstructive pulmonary disease and the right ventricular hypertrophy. The other common diseases are asthenic syndrome with cerebral angiospasm and cerebral functional disorder signs [22, 23].

The obtained data make up a basis for preparing the substantiated professional safety principles aimed at the nanoproduction personnel healthcare.

CONCLUSIONS

According to the conducted hygienic trials, the authors have established that the operating conditions of the nanoproduction personnel are characterized with many hazardous production factors: physical, dynamic, static and nervous overload, production noise, X-ray exposure, effect of aerosols with silver or any other metal nanoparticles and their oxygen-free compounds.

The described methods of air nanoparticles content assessment are quite informative and easy to use, they may be applied during production technological operations, which are characterized by high dust exposure.

To establish the nanoaerosols effect on the development of personnel health disorders it is recommended to supervise health dynamic patterns of the personnel.The authors have offered new sequence of periodical personnel's medical check-ups in order to improve their quality.

A single introduction of titanium nitride ultramicroscopic particles leads to structural metamorphoses in the bronchial tree and respiratory organs, the degree of expression of which depends on aerosol action.

The authors have established basic law principles of lead nanoparticles effect, which is a global pollutant, onto the body: in 30-fold and 60-fold introduction of studied substances we observed impaired liver protein synthesis, leading to dysproteinosis, disordered lipid and carbohydrate metabolism, which is related to cytotoxic effect of the studied nanoparticles. While evaluating liver morphology, pronounced dystrophic changes, dependent on exposure, were noticed.

After 30 introductions of lead sulphide the effect was observed in ALT, while after 60 introductions both ALT and AST increased their activity equally.

Continuous effect of non-oxide refractory metal compound nanopowders leads to systemic immune-inflammatory response with cytokines, which make up a pathogenetic factor of onset and progress of the vascular structural changes and hepatobiliary toxic disorders.

It has been established that molecular mechanisms of metabolic abnormalities which affect the circadian rhythms, necessary for human activity cycle, may, in their turn, provoke various pathological conditions and trigger oncogenesis. Altered expression of these genes is a genetic marker of nanoparticles hazardous effect.

The reliable nanoindustry development should be supported with a long-term studies of potential toxicological risks and their management, basing on the scientifically substantiated standardization of the nanoparticles production environment composition.

REFERENCES

1. Manzoor Ahmad Gatoo, Sufia Naseem, Mir Yasir Arfat. Physicochemical Properties of Nanomaterials: Implication in Associated Toxic Manifestations. BioMed Research International. 2014;2:1 – 8.
2. Yzmerov N.F., Kyrylov V.F. Hyhyena truda. Uchebnyk dlia vuzov s prylozhenyem na kompakt dyske. – M.: Yzdatelskaia hrupa "НЭОТАР-Medya", 2008;583 s.
3. A.Y. Potapov, V.N. Rakytskyi, A.V. Tulakyn i dr. Bezopasnost nanochastyts y nanomateryalov dlia okruzhaiushchei y proyzvotsvennoi sredy. Hyhyena y sanytaryia. 2013;3:8–14.
4. Muhammad Sajid, Muhammad Ilyas, Chanbasha Basheer. Impact of nanoparticles on human and environment: review of toxicity factors, exposures, control strategies, and future prospects. Environmental Science and Pollution Research. 2015;22 (6):4122–4143.
5. Johnston H.J., Hutchison G., Christensen F.M., Peters S., Hankin S. A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. Critical Reviews in Toxicology. 2010;40:328–346.
6. Movchan B. A., Yakovchuk K. Yu. Novyi podkhod k poluchenyiu mykroporystykh materyalov y pokrыtyi эlektronno-luchevыm ysparenyem neorhanycheskykh veshchestv. Zhurnal «Sovremennaia elektrometallurhyia». 2001;2.
7. Rahulia A.V., Vasylkyv O.O., Skorokhod V.V. Syntez y spekanye nanokrystalycheskoho poroshka tytanata baryia v neyzometrycheskykh uslovyiakh. I. Upravlenye dyspersnostiu tytanata baryia v protsesse eho synteza yz tytanyl-oksalata baryia. Poroshkovaia metallurhyia. 1997;3/4:59-65.
8. Minchenko D.O., Zinchenko T.O., Yavorovskyi O.P. i dr. Ekspresiia SNF1/AMP-aktyvuiemoi proteinkinazy (SNARK) u pechintsi, leheniakh, nyrkakh, holovnomu mozku, simianykakh ta miokardi pry dii na orhanizm shchuriv nanochastok sribla. Naukovyi visnyk Natsionalnoho medychnoho universytetu imeni O.O. Bohomoltsia. 2010;1:33 – 39.
9. Avtandylov H. H. Osnovy kolychestvennoi y patolohycheskoi anatomyy. M. : Medytsyna, 2002;240s.
10. Hrabovyi O. M., Zaretskyi M.B., Klymniuk H.I. Heterohennist neiroblastom za vmistom u yikhnikh klitynakh nukleinovykh kyslot (poperednie sposterezhennia). Klynycheskaia onkolohyia. 2012;6:12–20.
11. Sokurenko L. M. Morfolohycheskye yssledovanyia deistvyia lekarstvennыkh veshchestv v toksykolohyy. Liky Ukrainy. 2012;5 (161):62–68.
12. Yavorovskyi O.P., Shyrobokov V.P., Shevtsova V.M. Fizioloho-hihiienichna otsinka umov pratsi operatoriv pry oderzhanni nanochastok sribla za dopomohoiu elektronno-promenevoi tekhnolohii. Zhurnal Akademii medychnykh nauk Ukrainy. 2009;3:543 – 554.
13. Silver Nanoparticles. Material Safety Data Sheet. US Research Nanomaterials to OSHA and ANSI, 2010.7s.
14. Yavorovskyi O.P., Shyrobokov V.P., Veremei M.I. ta in. Metodychni pidkhody do hihiienichnoho kontroliu za vmistom nanochastynok u povitri robochoi zony pry otrymanni sylytsidiv metaliv metodom vysokoenerhetychnoi mekhanoaktyvatsii. Zhurnal Akademii medychnykh nauk Ukrainy. 2012;1:126 – 131.

15. Yavorovskyi O.P., Veremei M.I., Demetska O.V. ta in. Do pytannia hihiienichnoho kontroliu u povitri robochoi zony aerozoliv z nanorozmirnoiu dyspersnoiu fazoiu. Dovkillia i zdorovia. 2013;1:56 – 59.
16. Yavorovskyi O.P. ta in. Hihiienichna otsinka chynnykiv vyrobnychykh chynnykiv yak osnova upravlinnia ryzykamy u roboti operatoriv z oderzhannia poroshkiv nitrydu tytanu, dysytsylidu khromu i dioksydu tsyrkoniiu. Dovkillia i zdorovia. 2016;4:63 – 68.
17. Omelchuk S.T., Aleksiichuk V.D., Sokurenko L.M. Biokhimichni pokaznyky krovi ta morfofunktsionalnyi stan pechinky eksperymentalnykh shchuriv, yaki zaznaly dii nanochastok sulfidu svyntsiu u rizni terminy doslidzhennia. Likarska sprava. 2014;3-4 (1127):114–119.
18. Omelchuk S.T., Aleksijchuk V.D., Sokurenko L.M. et al. Characteristics of rat liver exposed to nanoparticles of lead compound. Georgian Med News. 2016;12 (261):94–99.
19. Minchenko D.O., Bozhko I.V., Zinchenko T.O. ta in. Zminy u rivni ekspresii tsyrkadialnykh heniv Per1, Clock i BMal1 u pechintsi, leheniakh, simianykakh, nyrkakh ta sertsi shchuriv za dii na orhanizm nanochastok sribla. Biolohichni studii/Studia Biologica. 2010;1:5 – 14.
20. Yavorovskyi O.P., Minchenko O.H., Minchenko D.O. et al. Ekspresiia kazeinkinazy-1ε ta Per2 u pechintsi, leheniakh, nyrkakh, simianykakh ta miokardi pry dii na orhanizm shchuriv nanochastok sribla. Zhurn. AMN Ukrainy. 2010;1:160-167.
21. Solokha N.V., T.Iu. Kuftareva T.Iu. Strukturne pidhrunttia patohennoi dii ultramikroskopichnykh chastok nitrydu tytanu na dykhalnu systemu. Dovkillia i zdorovia. 2016;1:18-21.
22. Yavorovskyi O.P., Tkachyshyn V.S., Shevtsova V.M. ta in. Stan zdorovia pratsivnykiv, zainiatykh vyrobnytstvom nanochastynok tytanatu bariiu. Dovkillia i zdorovia. 2013;4:57 – 61.
23. Yavorovskyi O.P., Tkachyshyn V.S. ta in Nanochastky i nanomaterialy: budova, fizyko-khimichni i toksykolohichni vlastyvosti, vplyv na orhanizm pratsivnykiv. Dovkillia i zdorovia. 2016;3:29 – 36.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Sergii Omelchuk
Saliutna str. 25A, ap.6, 04111, Kyiv, Ukraine
e-mail. sergii.omelchuk@nmu.ua

Received: 12.02.2019
Accepted: 08.07.2019

 © Wydawnictwo Aluna

ORIGINAL ARTICLE
PRACA ORYGINALNA

LABORATORY CRITERIA OF PERINATAL DAMAGE OF CENTRAL NERVOUS SYSTEM AT PREMATURE NEWBORNS

Viktoria A. Petrashenko, Andrii M. Loboda, Olexandr I. Smiyan, Sergii V. Popov, Svetlana N. Kasyan, Igor E. Zaitsev, Elena K. Redko
SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

Introduction: Fetal and neonatal hypoxia takes a special place among the damaging factors of central nervous system (CNS). All forms of oxygen deficiency are accompanied by the development of bioenergetic hypoxia, which leads to tension of metabolic processes of the organism. Metabolic effect of hypoxia includes stark reduce of mitochondrial activity due to a significant inhibition enzymes of the Krebs cycle: succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH). In newborn babies is not always possible to objectively assess the condition of the CNS defeat, because very often the severity of lesions does not correspond to clinical symptoms, especially in premature newborns. So far determination the severity of hypoxic-ischemic CNS lesions is still very actual in modern medicine. More objective method of such an assessment is determine the activity of neurospecific enolase (NSE).

The aim of the paper is to increase the efficiency of diagnosis of hypoxic CNS lesions in premature infants by determining the activity of NSE and study energy supply during the neonatal period.

Materials and methods: The concentration of NSE, SDH and LDH were determined in 15 conventionally healthy preterm infants (CHPI), which made the comparison group, and 64 premature babies with hypoxic-ischemic CNS lesions, which were divided into three groups: I group – 26 premature children with mild CNS lesions; II group – 20 premature children with severe hypoxic lesions and low birth weight; III group – 18 premature newborns with severe damage of central nervous system and extremely low birth weight. NSE activity was determined by enzyme immunoassay using reagents of the company «Fujirebio» (Sweden) on an automatic analyzer «Multiscan Plus» company «Labsystems» (Finland). Material for investigation was peripheral venous blood of newborns, which collected by vein punction at morning on an empty stomach.

Results and conclusions: Metabolic effect of hypoxia in premature infants manifested by severe inhibition of mitochondrial respiratory activity, which appears in the reduction of aerobic enzyme activity of SDH and activation serum LDH. During the neonatal period in infants with perinatal hypoxic- ischemic lesions of the CNS levels of the of NSE, SDH and LDG aren't normalized, that indicated on energy deficiency and requires the development of effective methods of correcting this condition. Perinatal hypoxia in premature neonates causes significant alteration of neuronal membranes and increase concentration in blood such neurospecific protein as NSE, whose concentration correlates with the degree of severity of CNS injury.

KEY WORDS: hypoxia, succinate dehydrogenase, lactate dehydrogenase, neurospecific enolase, premature newborns

Wiad Lek 2019, 72, 8, 1512-1516

INTRODUCTION

Fetal and neonatal hypoxia takes a special place among the damaging factors of central nervous system (CNS). This pathology usually is a result of placental insufficiency, which oversees almost all complications of pregnancy – toxicosis, intrauterine growth retardation, prematurity, infection. More important place hypoxic damage occurs in premature infants, in which it is 10-15 times more often cause death of children [1].

All forms of oxygen deficiency are accompanied by the development of bioenergetic hypoxia, which leads to tension of metabolic processes of the organism [2]. Metabolic effect of hypoxia includes stark reduce of mitochondrial activity due to a significant inhibition enzymes of the Krebs cycle: succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH).

Some authors believe that activity of oxidative enzymes of lymphocytes, particularly succinate dehydrogenase, is adequate reflection of dysmetabolical processes and energy cell metabolism [3, 4].

Abovementioned enzymes (SDH, LDH) are key in the processes of aerobic and anaerobic glycolysis, and decrease their activity – is as a marker of whole mitochondrial dysfunction [5, 6]. Therefore, objective criteria for assessing the severity of CNS lesions are indicators of cells energy metabolism.

Mechanisms of hypoxic damage of brain cells are characterized by a complex cascade of pathophysiological processes. Final result of this mechanism is the death of neurons due to necrosis and apoptosis [7].

In newborn babies is not always possible to objectively assess the condition of the CNS defeat, because very often the severity of lesions does not correspond to clinical symptoms, especially in premature newborns. So far determination the severity of hypoxic-ischemic CNS lesions is still very actual in modern medicine. More objective method of such an assessment is determine the activity of neurospecific enolase (NSE) [7].

THE AIM

The aim of the paper is to increase the efficiency of diagnosis of hypoxic CNS lesions in premature infants by determining the activity of NSE and study energy supply during the neonatal period.

The objects of research are premature infants with hypoxic CNS lesions of different severity.

MATERIALS AND METHODS

The concentration of NSE, SDH and LDH were determined in 15 conventionally healthy preterm infants (CHPI), which made the comparison group, and 64 premature babies with hypoxic-ischemic CNS lesions, which were divided into three groups:

I group – 26 premature children with mild CNS lesions;

II group – 20 premature children with severe hypoxic lesions and low birth weight;

III group – 18 premature newborns with severe damage of central nervous system and extremely low birth weight.

Severity of hypoxia was determined by considering pregnancy and childbirth, state after birth (Apgar score, signs of dysfunction of the central nervous system, respiratory, cardiovascular and urinary systems in the first three days of life), laboratory (concentration of urea and creatinine in blood, urine analysis, blood pH) and instrumental (ultrasound) data.

NSE activity was determined by enzyme immunoassay using reagents of the company «Fujirebio» (Sweden) on an automatic analyzer «Multiscan Plus» company «Labsystems» (Finland). Material for investigation was peripheral venous blood of newborns, which collected by vein punction at morning on an empty stomach.

The energy supply of the newborns was evaluated after activity of succinate dehydrogenase (SDH) in blood lymphocytes and lactate dehydrogenase (LDH) in serum of blood.

SDH activity in the lymphocytes of peripheral blood determined by quantitative cytochemical method using reagents of the company «SIGMA-ALDRICH» (Suisse).

Anaerobic metabolism in newborns was studied through measuring LDH activity in plasma by kinetic method on semi-automatic photometer PV 1251(Byelorussia) using reagent kits of the company "Diacon" (Russia) according to a standardized method that optimized by German Society for Clinical Chemistry – test pyruvate – lactate.

The content of enzymes in the blood was studied in the early neonatal (1-7th days of life) and late neonatal (20-30th days of life) periods.

Statistical analysis of results carried out with a program Microsoft Excel. We used statistics variation methods which are suitable for medical and biological research. For all parameters measured the average (M), the average error (m). Using the Student's criterion (t) determined reliability index (R).

RESULTS AND DISCUSSION

The average gestational age and body weight of babies in the different groups were respectively: 33,77 ± 0,26 weeks and 2024 ± 32,1 g in the first group, 33,16 ± 0,58 weeks and 2015,48 ± 34,2 g – in the second, and 28,88 ± 0,72 weeks and 1126,48 ± 24,3 g – in the third group. The comparison group included children which were born at term 35,26 ± 0,51 weeks, body weight was 2194,8 ± 81,11 g.

Determining the level of NSE in serum of premature infants found that at the end of the early neonatal period in brain cells of children with hypoxic-ischemic CNS lesions showed destructive changes of neuronal membranes. About this evidenced significant increase the level of enzyme. So, if perinatal CNS lesions of mild degree occur, NSE content in the blood of children of I group increased by 45% relative to the comparison group ($p < 0,05$). Thus, even mild hypoxia caused a significant alteration of neuronal membranes and damage brain tissue. In the second group of infants with low birth weight on the base of severe hypoxia there was further increase activity of this enzyme in the blood, which manifested by increased serum concentrations of NSE in 2,2 times relative to the children of I group ($p < 0,001$). It should also be noted that its activity in case of severe hypoxia was almost 3,3 times higher relative to the comparison group ($p < 0,001$).

Maximum concentration of enolase reached in premature infants with very low birth weight and severe hypoxic-ischemic injury of the CNS. Its contents in serum of premature neonates of III group was 4 times greater than in comparison group ($p < 0,001$), increased 2,9 ($p < 0,001$), and 1,3 ($p < 0,05$) times relative to infants of I and II groups, respectively (Figure 1).

Thus, hypoxic injury of the nervous tissue causes increased permeability of cell membranes and leave into the blood such neurospecific protein as NSE. The high rates of NSE in serum of premature infants on a base of hypoxic injury describe breach of the functional condition of cell membranes of neurons and correspond to the severity of brain damage due to hypoxia [7]. Therefore, to assess the severity of hypoxia is necessary determine the level of NSE in serum in the early neonatal period in premature infants.

During the neonatal period in the serum of all groups of children one can see a significantly lower enolase concentration, indicating a gradual recovery of neurons. It should be noted that at the end of the 30th day of life the level of this enzyme in premature infants with hypoxic-ischemic CNS lesions remained significantly higher than in comparison group. In the children of I, II and III group it was 1,4, 2,8 and 3,5 times greater than in the comparison group of kids.

So, in premature infants in the case of perinatal hypoxic-ischemic CNS lesions in the absence of any clinical symptoms at the end of neonatal period there is no stabilization of cell membranes of neurons, as indicated by high rates of neurospecific enolase in serum. These data suggest the possibility of remote effects due to central nervous system injury and the need to control the level of NSE during the neonatal period [8,9].

Furthermore, perinatal hypoxia may cause tension all metabolic processes in the organism. Changes of energy

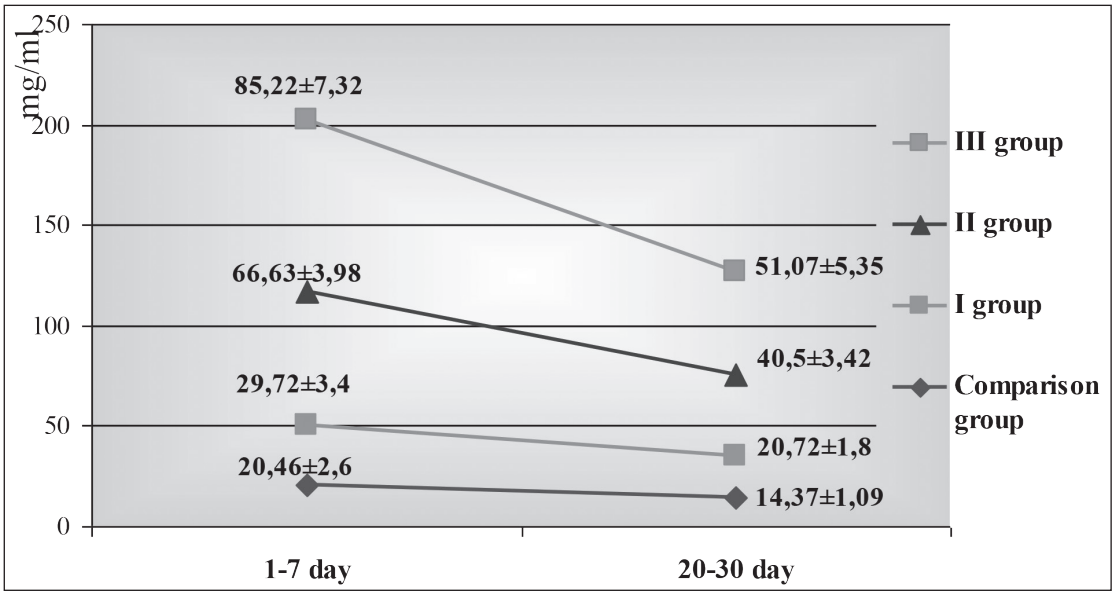


Fig. 1. Dynamics of NSE in premature newborns with hypoxic-ischemic CNS lesions in neonatal period, mg/ml.

metabolism can manifest by violation of almost all organs and systems, especially the central nervous system, heart and kidneys. Most accessible cells, which indicates early metabolic shifts in the body, are peripheral blood lymphocytes. Enzyme activity of lymphocytes is a "enzymatic mirror" of metabolic processes in different tissues. An oxidative enzyme succinate dehydrogenase is a marker of energetic processes of Krebs cycle. This enzyme is a part of the complex II of mitochondrial respiratory chain. It is strongly associated with the inner membrane of mitochondria, and the low activity of SDH evidences about inhibition of Krebs cycle functions. Enzymatic status of lymphocytes provides an opportunity to assess the degree of hypoxic influence on the newborn organism [10, 11].

In the premature newborn metabolic effect of hypoxia occurs in the early neonatal period through severe inhibition of mitochondrial respiratory activity, as testified by reduce activity of the main aerobic enzyme – SDH.

In infants with mild perinatal CNS injury in the early neonatal period the total number of granule of formazan into cells decreased on 33% relative to the comparison group ($p < 0{,}001$). In same time for infants in second and third groups were quantity 1,7 and 2,0 times lower respectively than in comparison group ($p < 0{,}001$) (Table I).

Along with a decreased of the total number of formazan granules, were decreased number of cells containing these granules, and the average number of granules per cell.

Reduced activity of SDH indicates the initial stages of the process of decompensation, which is accompanied by disturbance of energy metabolism and leads to the formation of tissue hypoxia. At severe CNS injury further decrease activity of this enzyme shows the progression of decompensation and the formation of severe energetical disorders.

During the neonatal period in peripheral blood lymphocytes in premature newborns with hypoxic- ischemic CNS lesions observed only a trend towards recovery SDH activity. Even at the end of the 30th day, the total number of formazan granules in infants with severe hypoxia was 1,5 times less relative to the comparison group. Significantly low remained also the number of cells containing the enzyme.

These data indicate the ineffectiveness of aerobic glycolysis even at the end of the first month of life, so you can talk about development in premature infants energetical deficiency due to hypoxic and ischemic CNS lesions and requires the development of effective methods of correction.

Thus, the results of cytochemical studies of lymphocytes found that in premature infants with perinatal hypoxic-ischemic CNS lesions occurred pronounced changes in metabolic adaptation [12,13].

The brain, as the main target organ in case of hypoxia, is very sensitive to hypoxia. Energy supply of the brain caused most of all by aerobic mechanisms. Hypoxia causes an energy stress that activates compensatory anaerobic way of glucose utilization, for which is high specific increase activity of anaerobic enzymes, especially LDH.

Research LDH levels in serum of premature infants with perinatal hypoxic-ischemic CNS lesions found that in case of oxygen deficiency in children of all groups was significant increase level of this enzyme, and thus activation of anaerobic glycolysis. In the early neonatal period in children with mild hypoxic lesions enzyme concentration increased 2,5 times relative to the comparison group ($p < 0{,}001$). This shows the maximum tension of compensatory adaptive mechanisms aimed to the effective utilisation of energy substrates to prevent the energy deficiency.

In case of severe hypoxia observed certain exhaustion of compensatory mechanisms of anaerobic glycolysis activation, but even among newborns of second and third groups with severe hypoxic injury LDH level in serum was 1,7 times greater ($p < 0{,}001$ and $p < 0{,}01$, respectively) than in neonatal comparison group (Figure 2).

During the neonatal period, the gradual recovery of aerobic glucose utilization way was found. Indicator of this process was some reduction of serum LDH in the newborns with perinatal hypoxic lesions of the CNS. Significant

Table I. Morphocytochemical indicators of SDH activity in peripheral blood lymphocytes.

		Total number of granules	Total number of cells with granules	Number of granules per cell
Comparison group	1-7 day	424,62±14,9	43,5±0,5	9,76±0,3
	20-30 day	417,43±19,2	43,4±0,5	9,66±0,5
I group	1-7 day	319,0±17,3 p	36,4±0,7 p	8,73±0,4
	20-30 day	358,08±20,1 p	39,9±0,7 p	8,95±0,4
II group	1-7 day	249,37±10,4 p, p_1	32,5±0,9 p, p_1	7,70±0,4 p
	20-30 day	260,75±11,6 p, p_1	34,2±0,9 p, p_1	7,74±0,4 p, p_1
III group	1-7 day	207,87±18,1 p, p_1	30,6±1,9 p, p_1	6,72±0,2 p, p_1, p_2
	20-30 day	233,5±12,6 p, p_1	32,8±1,1 p, p_1	7,09±0,2 p, p_1

Notes: p – significantly difference of indexes relative to the comparison group;
p_1 – significantly difference of indexes relative to the Ist group;
p_2 – significantly difference of indexes relative to the IInd group.

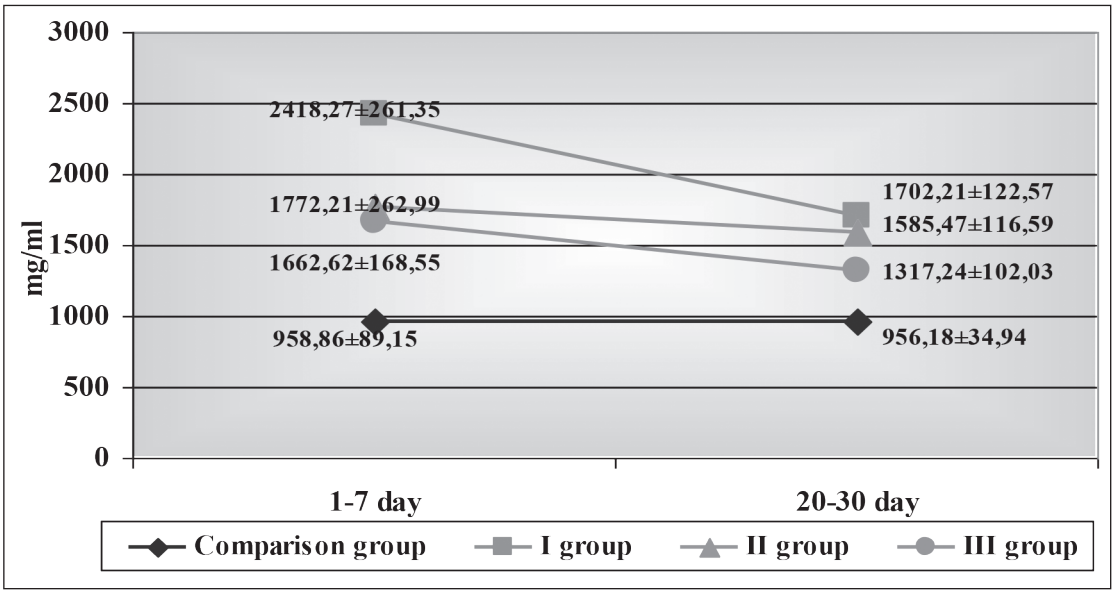


Fig. 2. Dynamics of LDH in premature babies with hypoxic-ischemic injury of the central nervous system, mg/ml.

decrease concentration of this enzyme was observed only in children with mild hypoxia. But even at the end of the first month LDH contents in the blood of children of all groups with hypoxia remained significantly higher than in comparison group, that indicated the absence of adequate recovery of energy metabolism into cells.

Thus, the process of aerobic glycolysis occurs in the mitochondria of brain cells with enzymes of Krebs cycle, the main of which is SDH, only in case of normal diffusion oxygen from intercellular space into neurons [14,15].

Hypoxia dramatically reduces the activity of aerobic glycolysis, which is manifested by low activity of such enzyme of Krebs cycle as SDH. Metabolism in brain cells become anaerobic with the activation of the corresponding enzyme (LDH) [16, 17]. Functional brain activity is suppressed, which is manifested clinically by progressive impairment of consciousness. Hypoxia blocks include pyruvic acid into Krebs cycle, that's why piruvate isn't oxidized and converted to lactic acid. Increasing the concentration of the latter causes acidosis, which is a factor of destruction of cell membranes of neurons. Violation of the integrity of neuronal membranes leads to increase concentration in blood neurospecific marker of brain cells damage – NSE, whose concentration in serum of newborns with hypoxic CNS lesions increases dramatically [18, 19].

Thus, as markers of severity of perinatal hypoxic-ischemic CNS lesions in premature infants during the neonatal period can use activity of such indicators as SDH, LDH and NSE.

CONCLUSIONS

1. As markers of severity of perinatal hypoxic-ischemic CNS lesions in premature infants during the neonatal period can use activity of such indicators as SDH, LDH and NSE.
2. Metabolic effect of hypoxia in premature infants manifested by severe inhibition of mitochondrial respiratory activity, which appears in the reduction of aerobic enzyme activity of SDH and activation serum LDH. During the neonatal period in infants with perinatal hypoxic- ischemic lesions of the CNS levels of the of NSE, SDH and LDG aren't normalized, that indicated on energy deficiency and requires the development of effective methods of correcting this condition.
3. Perinatal hypoxia in premature neonates causes significant alteration of neuronal membranes and increase concentration in blood such neurospecific protein as NSE, whose concentration correlates with the degree of severity of CNS injury.

REFERENCES

1. Nikonov V.V., Pavlenko A.J. Metabolic therapy of hypoxic conditions. Medicine of emergency conditions. 2009;3-4:22-23.
2. Gromada N.Ye. Immunological, structural and metabolic abnormalities in term infants with perinatal hypoxic injury of central nervous system, predicting outcomes, optimizing treatment: Abstract diss ... Doc. med. Science. special. 14.00.09 "Pediatrics". Ekaterinburg, 2009. 21 p.
3. Semyachkina A.N., Sukhorukov V.S. Violations of the processes of cellular bioenergetics and methods of their therapeutical correction in children with mucopolysacharidoses. Russian Journal of Perinatology and Pediatrics. 2005;1:18-21.
4. Sinchihin S.P., Sinchihina M.E., Navruzova Z.T. et al. The possibility of using immunochemical and morphological methods of research in obstetrics and perinatology. Astrakhan Medical Journal. 2008;1:85-96.
5. Graham R.M., Frazier D.P., Thompson J.W. A unique pathway of cardiac myocyte death caused by hypoxia-acidosis. J. Exp. Biol. 2004;207:3189-3200.
6. Feala J. D., Coquin L., Zhou D. Metabolism as means for hypoxia adaptation: metabolic profiling and flux balance analysis. BMC Systems Biology. 2009:91-99.
7. Blennow M., Savman K., Lives P. et al Brain-specific proteins in the cerebrospinal fluid of severely asphyxiated newborn infants. Acta Paediatr. 2001;90: 1171-1175.
8. Nagornaya N.V., Chetverik N., Fedorov A. et al. Cell energy metabolism in health and disease. The possibility of its evaluation. Child Health. 2008;6 (15):69-71.
9. Kretovich V.L. An introduction to enzymology. Moscow: Nauka, 1974. 143 p.
10. Brièrea J.J., Favierb J., Ghouzzia V.El. et al. Succinate dehydrogenase deficiency in human. Cell. Mol. Life Sci. 2005;62:2317-2324.
11. Senatorova A.S., Kondratova I.J. Disorders of energy metabolism in pediatric practice: a view of the future. Modern Pediatrics. 2009;1 (23):25-30.
12. Nazarenko G.I., Kiskun A.A. Clinical evaluation of laboratory results. Moscow: Medicine, 2000. 544 p.
13. Nagornaya N.V., Chetverik N., Dubovaya A. et al. Antioxidant status in children living in ecologically unfavorable conditions, and the possibility of correction. Child Health. 2010;1 (22): 66-70.
14. Bhandari U., Neeti J., Pillai K.K. Further Studies on Antioxidant Potential and Protection of Pancreatic β-Cells by Embelia ribes in Experimental Diabete. Experimental Diabetes Research. 2007;15:1503-1509.
15. Hwan D., Seo S., Kim Y. et al. Selenium acts as an insulin-like molecule for the down-regulation of diabetic symptoms via endoplasmic reticulum stress and insulin signaling proteins in diabetes-induced non-obese diabetic mice. J. Biosci. 2007;32(4):723-735.
16. Stanely R., Prince M., Menon V.P. Hypoglycaemic and other related actions of Tinospora cordifolia roots in alloxan-induced diabetic rats. Journal of Ethnopharmacology. 2000;70: 9-15.
17. Prema Karthik. The pathogenesis of late gestosis of pregnancy. International Journal of Medicine. 2010;1:62-66.
18. Hlynina T. Neuroprotective therapy in clinical and metabolic adaptation in neonates with hypoxic injury of central nervous system: Abstract. Dissertation ... Candidate med. science: special. 14.00.09 "Pediatrics". - Tomsk, 2008. 20 p.
19. Sinchihin S.P., Lechneva E.U. Prognostic value of determination prior to delivery of enzymatic activity of lymphocytes in pregnant women. Herald VolSMU. 2008;2 (26):68-70.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Viktoria A. Petrashenko
Ukraine, 40035, Sumy, Zalyvna str., 17, 58
tel: +380663234080
e-mail: vika.illiashenko@gmail.com

Received: 23.03.2019
Accepted: 11.07.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

COGNITIVE DISABILITY IN ESTROGENECTOMITED AND OLD RATS WITH DEVELOPMENT OF DIABETES MELLITUS

Inga R. Tymofiychuk[1], Ihor K. Churpiy[2], Tetiana P. Savchuk[1], Lilia D. Boreyko[1], Xenia V. Slobodian[1]
[1]HIGHER STATE EDUCATIONAL ESTABLISHMENT OF UKRAINE "BUKOVINIAN STATE MEDICAL UNIVERSITY", CHERNIVTSI, UKRAINE
[2]IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Introduction: In recent years, there have been many works devoted to the study of the effects of sex hormones on cognitive function. Clinical studies have found that in menopausal women, the tendency to develop type 2 diabetes will increase, the spatial and short-term working memory worsens, and there is a tendency to develop depression. The findings suggest that estrogens are involved in the mechanisms of insulin resistance in tissues, in the synthesis of mediators in the catecholaminergic systems of the brain, but many questions remain unresolved.

The aim: Therefore, the aim of our study was to establish the effect of estrogens on the indices of spatial memory in ovariectomized and old rats against the background of the development of experimental diabetes.

Materials and methods: The study was conducted on 30 adult rats females 4-5 months and 20 months. The study groups were ovariectomized and reproduced experimental type 2 diabetes mellitus with protamine sulfate. The study of spatial memory was carried out in an eight-sleeved radial labyrinth.

Results and conclusions: Ovariectomy caused the deterioration of spatial memory relative to the living control group, and diabetes mellitus aggravated pathological changes. The changes that occur after ovariectomy suggest estrogen involvement in the regulation of cognitive functions.

KEY WORDS: estrogens, cognitive functions, a spatial memory, diabetes mellitus

Wiad Lek 2019, 72, 8, 1517-1522

INTRODUCTION

SEX HORMONES AND COGNITIVE FUNCTION

In the scientific works of the last years appeared a lot of evidence that sex hormones take active part in the process of the neurogenesis, synaptogenesis have an impact on the energy balance of the neurons through regulation of functions of mitochondria. The high frequency of Alzheimer's disease (AD) in women during menopause makes scientists think about the influence of estrogens on the development of neurodegenerative diseases [1]. The main target of estradiol is the hypothalamus, forebrain, hippocampus, and nuclei of the seam. One finds estrogen synthase in the brain and there is anevidence that estrogens participate in the regulation of cognitive disorders, bipolar disorders, anxiety, Parkson disease, Alzheimer's disease. The decreased production of sex steroids is associated with impaired processes of forming long-term declarative memory, deterioration of spatial working memory and implementation of executive functions [2]. Cognitive function, which is supported by the prefrontal cortex may be especially dependent on estrogen [3]. Estrogens affect molecular mechanisms of synaptic plasticity. The imbalance of estrogen causes modulation effect, reflected in the changing of cognitive processes. Non-genomic action of estradiol is associated with effects on membrane receptors, which causes membrane hyperpolarisation of neurons, the activation of extracellular regulatory kinase, protein kinases A and C, calcium channels through the activation of the central regulatory pathways that regulate synaptic plasticity. Estradiol launches of NR-2 subunits of NMDA receptors in the hippocampus, which is important for the formation of new synapses [4]. The main manifestations of non-genomic actions of estradiol is its effect on the excitability of the cerebellum, cortex, striatum, hippocampus and hypothalamus, activation of ion channels, the activation of Inositol 3 phosphate, modulation of the entry and exit of calcium, protection of neurons from cellular toxins and free radicals, modulation of the activity of calcium/calmodulin, activation of kainat current in hippocampal neurons and release of neurotransmitters [5]. Estradiol has a significant influence on synaptic plasticity of neurons in the brain by affecting the functional activity of signal proteins synaptotagmin 1, synaptotagmin 4, synaptein are revealed in cyclical changes synaptotagmin 1 during ovarian cycle in hippocampal region CA_3. The deficit in steroidogenesis leads to the reduction and subsequent complete cessation of neurogenesis in the hippocampus, further remodeling of astroglia in the CA_1 region of the hippocampus, the dentate gyrus [2]. Estradiol provides modulation effect on neurocrine that belong to the family of nerve growth factor. Genomic holinergic and non-genomic mechanisms of estrogens have an effect on neuromediator systems, such as holinergic, adrenergic, serotonin-

ergic, dopaminergic, GABA-ergic, oppoidergic. Estrogen deficiency causes a deficit in the cholinergic system that, according to the latest research is considered as a possible factor in Alzheimer's disease. Experimental study has shown that estrogens reduce the toxicity of beta-amyloid to neurons by affecting on N-holinoreseptors. The positive effects of estrogen can de explained by antioxidant effects and influence on the expression of neurocrine factor [6]. Hysterectomy influences the noradrenergic system and causes a decrease in levels of noradrenaline, by activation of monoamine oxidase in the hypothalamus and amygdala. Affecting neurotransmitter systems estrogens can affect to act on the processes of learning and memory. There are well-known periods of a women's life associated with reduced levels of estrogen, when there is the greatest risk of cognitive disorders: puberty, pregnancy, premenopausal, menopause, postmenopausal. The experiments on the animals have shown that hysterectomy made it difficult to save the skills of passive and active avoidance [7].

THE ROLE OF SEX HORMONES IN THE DEVELOPMENT OF DIABETES MELLITUS AND COGNITIVE DYSFUNCTION

The incidence of diabetes mellitus is significantly increased in women over the age of 50, and possibly menopause has a definite effect on increasing its prevalence among women of the elderly [8]. The literature describes the regulatory role of the steroid hormones of the ovaries in the utilization of glucose in the brain, as well as the control of estrogens of neurological disorders induced by insulin hypoglycemia in ovariectomized rats [9].

One of the «targets» of hyperglycemia in the early stages of diabetes, is the central nervous system, which is clinically manifested by cognitive dysfunction.

Only recently has it been shown in patients with diabetes, there is an increased risk of not only mild cognitive impairment, but also the probability of progression to dementia. Monitoring of patients with type 2 diabetes (DM) found that they were 2 times more likely to develop AD [6].

The increase in neuro-specific proteins in the blood of patient with diabetes mellitus points to the damage to the nervous tissue and lets life-time assessment of the condition of the central nervous system and the dynamics of neurodegenerative processes be carried out. Increase neurospecific proteins in patients with diabetes does not itself mean that they are markers of cognitive dysfunction.

According to the study one of the main causes of cognitive dysfunction is the chronic hyperglycemia. Most of the indicators that determine cognitive dysfunction have been reduced in patients with diabetes mellitus 1, namely memory functions and attention. There was a significant difference in the level of neurospecific proteins, which correlated with decompensation of carbohydrate metabolism and the presence of cognitive deficits. The most frequent morphological change in the central nervous system was the presence of signs of atrophy of the gray matter of the frontal and parietal lobes, which correlated with hyperglycemia and cognitive dysfunction [3].

A relatively high density of insulin receptors was revealed in the regions of the brain (hippocampus, tonsils, cortex), which support the processes of memory and study. Thus, the brain's resistance to insulin is particularly clearly manifested in Alzheimer's disease and to a lesser extent with moderate cognitive impairment, as evidenced by studies of the hippocampus of patients who died of Alzheimer's disease [10, 11].

It was found that the activation of estrogen receptors: ERα and / or ERβ with their agonists improve memory in the tests of the water labyrinth of Morris and Y-labyrinth, increases neurogenesis of the hippocampus and prevents from apoptotic reactions of the hippocampus [12, 13]. It is important to note that treatment with agonists of pharmacological ER caused a significant increase in the expression of the ERα and ERβ membrane and subsequent activation of BDNF in the hippocampus of mice with type 2 diabetes. ERα and ERβ, cause cognitive impairment in patiants with 2 diabetes, and ER activation with ER agonists may be a new and promising strategy for the treatment of diabetic cognitive impairment [14, 15].

The literature about the influence of estrogens on prognosis of diabetes mellitus and cognitive functions in ovariectomized and elderly animals has shown that points of view are not properly studied.

THE AIM

The purpose of our work was to investigate the behavioral reactions of rats in the radial labyrinth, after ovariectomy and against the background of the development of experimental diabetes mellitus in 4-5 months old and old rats.

MATERIALS AND METHODS

The study was carried out on 30 adult, sexually mature female rats of 4-5 months weighing 200-220 grams and 20 months weighing 300-320 grams. Animals were kept in vivarium under normal light conditions, with free access to water and were on a standard diet. Animals were divided into several groups: a control group of 4-5 months which were subjected to false surgery, the study groups: 4-5 month ovariectomized, and with experimental diabetes mellitus, duration 2 months and a group of 20 months rats, which did not have estral cycles, and a group of old rats with experimental diabetes [16, 17].

Ovariectomy was performed using the method of Y.M. Kabak firstly cut of the skin in the lumbar region, then cut into the abdominal cavity, pulled out the ovary through the incision with the oviduct and the upper part of the uterus horn, and then removed and repeated this procedure on the other side [16]. Surgical intervention was performed under general anesthesia of calypsoil, 0.75 mg / kg of body weight was injected intraperitoneally.

Experimental type 2 diabetes mellitus was reproduced by intramuscular injection of 15 mg / kg of protamine sulphate twice daily for 2 weeks. After 2 and 4 weeks after the end of the administration of the protamine, blood serum was examined and the hyperglycemia was detected [18, 19, 20].

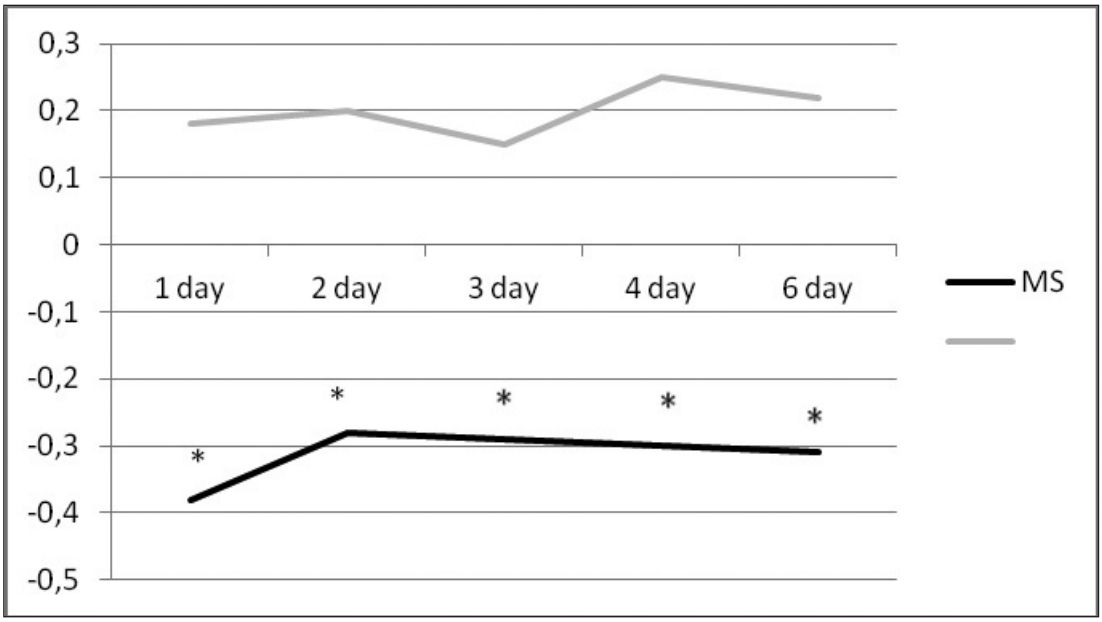


Fig. 1. The result of the neurobehavioral test « Eight-sleeved radial labyrinth» of ovariectomized animals at the age of 4-5 months: the average memory point depends on the number of correct and incorrect entries in the sleeves. Each point means the average of the memory score in the test phase in one day. * - here and in other studies comparing the group of examined animals with the control group to false-operated 4-5 months ($p \leq 0.05$).

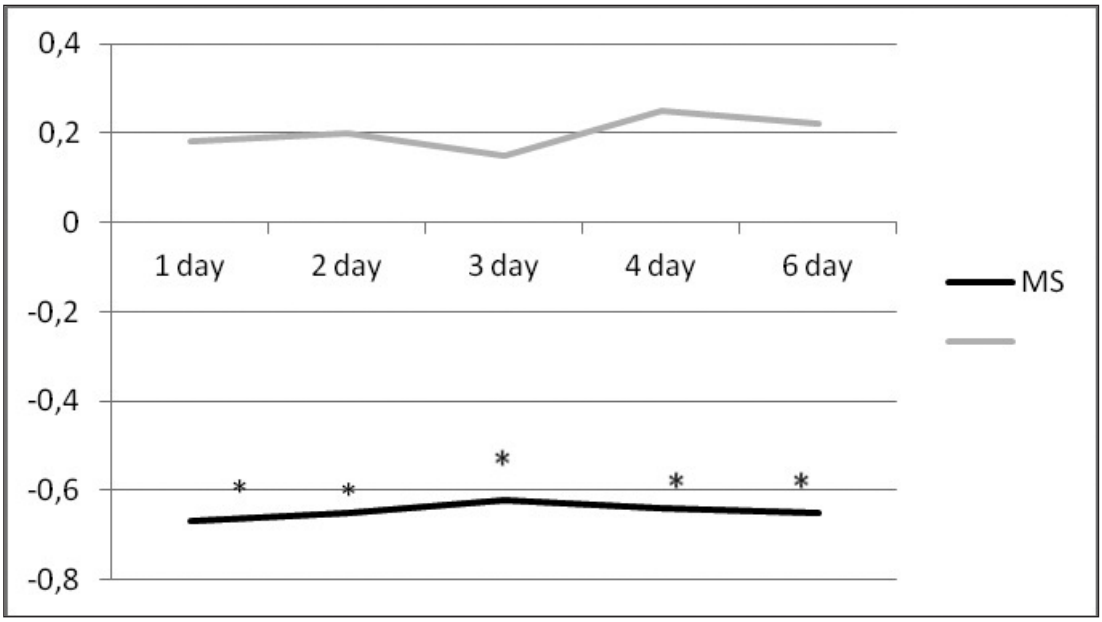


Fig. 2. The result neurobehavioral testing « Eight-sleeved radial labyrinth» the old animals at the age of 20 months.

The study of spatial memory was carried out in an octagonal radial labyrinth: the length of each of the sleeves was 86 cm, the width - 10 cm, the diameter of the central platform - 34 cm, the height of the walls - 20 cm and height above the floor - 50 cm. Each sleeve is separated from the playground guillotine doorways At the end of each sleeve there was a feeder with food supply, separated by guillotine doors. The test protocol consisted of 3 stages: Stage I - addictive, carried out on the first day to familiarize the animal with the conditions. It consisted of three phases of 5 minutes duration with a 30 second break. In each phase, the animal had the opportunity to explore the labyrinth without nutritional supply. Phase II consisted of a phase of training and testing phases, each of that lasted for 5 minutes with a 30 second rest. In the training phase, at the end of each sleeve, food reinforcements were placed, with four random sleeves closed. After, in the phase of the break, the animal was placed in the center of the labyrinth, with closed doors. In the testing phase, all eight doors were opened, pre-positioning food supply those sleeves that were previously blocked. In this case, the animal should have visited only those sleeves that were blocked during the training phase. The testing phase was estimated by the number of correct and incorrect inputs in the sleeves.

A mistake was considered to be the entrance to the sleeves, the feeder of which was opened in the training phase, as well as the repeated visits to the sleeves opened in the testing phase. Stage III - Stage II repetition after a break for 60 minutes. As a researched criterion, the average memory point (MS) was used.

The statistical processing of the obtained results was carried out using the Statplus Professional program, a collection of 5.9.8.5 / Core v.5.9.33 methods of nonparametric statistics. The data is presented in the form Me (Q1 ... Q3), where Me is the median, Q1 ... Q3 is the interquartal scale. The Mann-Whitney criterion was used to compare the indices in independent samples, and the comparison of dependent samples was performed using Wilkinson's criterion. The differences were taken to be significant at the significance level of $p \leq 0.05$ [18].

Animal studies have been carried out and in accordance with the principles of humanity laid down in the European Community Directive (2010/63 / EC).

RESULTS AND DISCUSSION

On the first day of testing, after the previous training, ovariectomized 4-month rats showed an average memory score of -0.38 (-0.42 ... -0.14), which statistically improved on the second day of testing ($p < 0.05$) and amounted to -0.28 (-0.41 ... -0.18) (Fig. 1). On the fourth day of testing, the ovariectomized animals performed statistically significantly ($p = 0.035$) more re-entries in the sleeve compared with the second day of testing, as evidenced by the average memory point in the second -0.29 (-0.43 ...- 0.12) and the fourth day -0.28 (-0.34 ... -0.16). On the fifth day of testing in ovariectomized animals the value of the average memory score from the fourth -0.30 (-0.38 ... -0.16) until the sixth day of the test -0.31 (-0.47. .. -0.12) did not change statistically significantly ($p < 0.05$).

At the age of 20 months, there was a general positive dynamics of the average memory score in the period from the first -0.32 (-0.38 ... -0.21) to the fourth day of testing -0.27 (-0.28 ... 0,14) at p = 0,028 (Fig. 2). The break for the fifth day of testing in the old rats did not statistically change the value of the average memory score from the fourth -0.32 (-0.38-0.14) to the sixth day of the test -0.33 (-0.39 ... 0,11).

Menopause is closely associated with memory loss and cognitive impairment. Estrogen plays an important role in neuroplasticity, for example, an increase in the population of the dendritic CA_1 segment in the hippocampus, prolonged potentiation and neurogenesis. In the experiments, the memory and density of dendritic spines are reduced in the prefrontal cortex and the hippocampus after ovariectomy; it is also settled that the ability to recognize the object decreases after 1 week of ovariectomy and spatial memory decreases after 4 weeks [20].

Studies have shown that physical activity and stress influences improve of spatial memory of ovariectomized rats and are associated with increased extragranular aromatisation. This, in turn, affects the expression of estrogen receptors [21]. The data we obtained on ovariectomized animals indicate that on the second day the cognitive function is somewhat improved compared with the first day of testing, which is associated with physical activity in the labyrinth. There is a similar tendency in the old animals on the second day, but indicators of spatial memory are much worse.

Diabetes causes a significant deterioration in memory. So, on the first day of the test, after the previous training, 4-5 month diabetic rats showed an average memory point

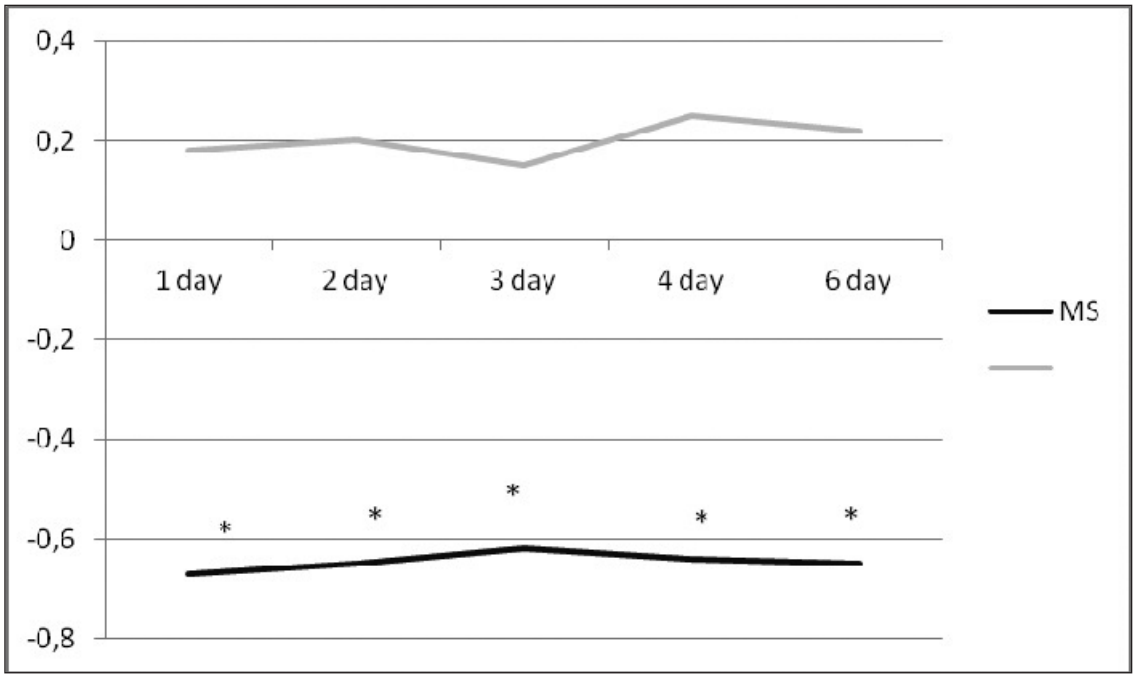


Fig. 3. The result of the neurobehavioral test «Eight-sleeved radial labyrinth» of ovariectomized 4-5 month old animals with diabetes mellitus.

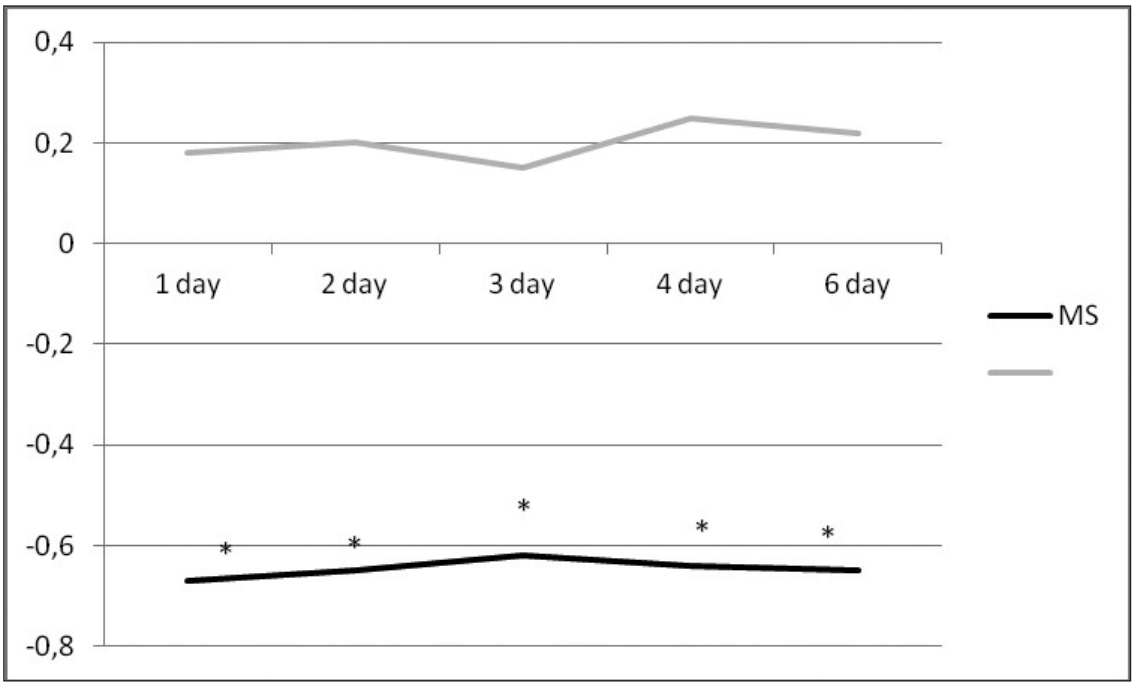


Fig. 4. The result of the neurobehavioral test «Eight-sleeved radial labyrinth» of old animals with diabetes mellitus.

of -0.62 (-0.63 ... -0.34), which on the second and further days of testing actually did not change (p <0,05) and ranged from -0,60 (-0,65 ... -0,28), to -0,65 (-0,63 ... -0,30) at the last test day (Fig. 3).

The combination of ovariectomy and diabetes has caused a significant deterioration of cognitive function. From the first day of testing, after a preliminary training period of 4-5 rats with diabetes and ovariectomy showed an average memory score of -0.67 (-0,73 ... -0,34), which on the second day of testing was statistically improved (p < 0.05) and amounted to -0,65 (-0,72 -0,24...), and in the further days of testing has not changed (p < 0.05) and ranged from -0,62 (-0.75 per -0,18...) to -0,65 (-0,63....-0,20) on the last day of testing (Fig.4).

DISCUSSION

Some authors claim that cognitive impairment in diabetes mellitus is clinically closer to cognitive impairment in vascular diseases of the brain and develops due to the macro- and microvascular complications of DM 2. Deterioration of verbal memory and optic-spatial functions are described, in other studies, as the main constituents of cognitive impairments in DM 2 which is more characteristic of AD, and not for vascular dementia. Data of neuroimaging of the life-time of the brain show different variants of changes: a combination of global atrophy, subcortical atrophy, leukoareosis and lacunar, but small heart attacks, isolated heart attacks of the hippocampus, which can cause an «alzheimer-like» clinic with vascular etiology of lesions [19]. In diabetes, neurotropic factors and transcription factors induce structural changes in the neurons of the hippocampus, which are critical for memory and learning. Neurotrophic factors such as neurotrophin 3 (NT-3) and the neurotrophic factor (BDNF) from the brain are powerful regulators of neuronal and synaptic excitability, and when these factors are active, synaptin I and associated with protein growth 43 (GAP-43) plays an important role in neuronal and synaptic plasticity.

In patients with type 2 diabetes, cognitive dysfunction has been detected in the form of memory reduction, attention, and optic-spatial activity interrelated with chronic hyperglycemia. With an increase in the level of HbA1c in patients with type 2 diabetes, BDNF (brain neurotrophic factor) in blood plasma decreases and decreases in cognitive function [4, 22].

CONCLUSIONS

Our data allow us to draw conclusions about the influence of sex hormones on cognitive functions. Ovariectomy caused a deterioration of spatial memory, and the combination of ovariectomy and diabetes deepened the pathological process. In older animals, the study revealed a decrease in spatial memory, and diabetes further aggravated the cognitive function.

Changes that occur after ovariectomy indicate involvement of estrogen in the regulation of cognitive functions in the process and indicate the possibility of using estrogens in the treatment of neurodegenerative changes in premature and age-related menopause and in the against the background of concomitant pathological processes.

REFERENCES

1. Kareva E.N., Oleynikova O.M., Panov V.O., Shimanovskiy N.L., Skvortsova V.I. Estrogeny i golovnoy mozg. Vestnik Rossiyskoy akademii meditsinskikh nauk. 2012; 2: 48–59.
2. Tapilskaya N.I., Glushakov R.I. Endokrinologiya kognitivnykh rasstroystv: vozmozhnosti menopauzalnoy gormonalnoy terapii. Meditsinskiy alfavit. Sovremennaya ginekologiya. 2017; 2 (10): 30–34.

3. Gatskikh I.V., Veselova O.F., Petrova M.M., Shalda T.P., Adamyan R.A., Kalashnikova K.M. Rol mozgovogo neyrotroficheskogo faktora (BDNF) v diagnostike kognitivnoy disfunktsii u patsiyentov s sakharnym diabetom 2 tipa. V mire nauchnykh otkrytiy. 2016; 1(73): 10–23. 4. Sokolova L.K., Pushkarev V.M., Pushkarev V.V., Kovzun E.I., Tronko N.D. Rol metformina v patogeneze i lechenii kognitivnoy disfunktsii (obzor literatury). Problemy endokrinnoy patologii Sakharnyy diabet i kognitivnyye narusheniya. 2018; 2: 75–92.
5. Matveyeva M.V., Samoylova Yu.G., Zhukova N.G., Oleynik O.A., Rotkank M.A. Taupatiya i kognitivnyye narusheniya pri eksperimentalnom sakharnom diabete. Sakharnyy diabet. 2017;20(3):181-184. doi: 10.14341/2072-0351-5842
6. Sosina V.B., Zakharov V.V., Strokov I.A., Vakhnina N.V. Kognitivnyye narusheniya pri sakharnom diabete. Nevrologiya. neyropsikhiatriya. psikhosomatika. 2017;9(1):90–95.
7. Akker L.V.. Stefanovskaya O.V., Leonova N.V., Khamadianova S.U. Sakharnyy diabet i klimaks: sovremennyye vozmozhnosti zamestitelnoy gormonalnoy terapii. Meditsinskiy vestnik Bashkortostana. 2008; 3(5): 21–24.
8. Timofiychuk IR., Savchuk TP., Shvets VI., Slobodyan KV., Slobodyan OM. Effect of estrogens on the fluorescence intensity of catecholamines in the limbic-hypothalamic structures of the brain. Georgian medical news. 2018; 9(282): 150-156.
9. Chavushyan V.A., Simonyan K.V., Galstyan A.M., Meliksetyan I.B. Issledovaniye neyroprotektornoy i insulinotropnoy aktivnosti gidroponicheskogo Teucrium Polium L v usloviyakh ovariektomii. Morfologiya. 2012; Tom VI (21):58-69.
10. Gorina Ya. V., Iptyshev A. M., Lopatina O. L., Komleva Yu. K., Chernykh A. I., Salmina A. B. Analiz prostranstvennoy pamyati u NLRP3 - nokautnykh zhivotnykh. Sibirskoye meditsinskoye obozreniye. 2017;(6):50-56. Doi: 10.20333/2500136-2017-6-50-56
11. De Felice FG, Ferreira ST. Inflammation, defective insulin signaling, and mitochondrial dysfunction as common molecular denominators connecting type 2 diabetes to Alzheimer disease. Diabetes. 2014;(63):2262-72. Doi: 10.2337/db13-1954
12. Yuko Hara, Elizabeth M. Waters, Bruce S. McEwen, and John H. Morrison. Estrogen Effects on Cognitive and Synaptic Health Over the Lifecourse. Physiol Rev. 2015;95(3): 785–807. Published online 2015 Jun 24. Doi: [10.1152/physrev.00036.2014]
13. Stephanie V. Koebele, Sarah E. Mennenga, Ryoko Hiroi, alicia M. Quihuis, Lauren T. Hewitt, Mallori L. Poisson, christina George, Loretta P. Mayer, Cheryl A. Dyer, Leona S. Aiken, laurence M. Demers, Catherine Carson, Andheather A. Bimonte-Nelson. Cognitive Changes Across the Menopause Transition: A Longitudinal Evaluation of the Impact of Age and Ovarian Status on Spatial Memory. Horm Behav. 2017; 87: 96–114. Doi: [10.1016/j.yhbeh.2016.10.010]
14. Miriam T. Weber, PhD, Pauline M. Maki, PhD, andMichael P. McDermott, Cognition and mood in perimenopause: A systematic review and meta-analysis. J Steroid Biochem Mol Biol. 2014; 0: 90–98. doi: [10.1016/j.jsbmb.2013.06.001]
15. Tang SS, Ren Y, Ren XQ, Cao JR, Hong H, Ji H, Hu QH. ERα and/or ERβ activation ameliorates cognitive impairment, neurogenesis and apoptosis in type 2 diabetes mellitus mice. Exp Neurol. 2018;7;311:33-43. doi: 10.1016/j.expneurol.2018.09.002.
16. Kabak Ya.M. Praktikum po endokrinologii. - M.: Izd-vo MGU. 1968. - S. 25-28 17. Milyutina Yu.P., Korenevskiy A.V., Stepanov M.G., Pustygina A.V., Oparina T.I., Zaloznyaya I.V., Arutyunyan A.V. Vliyaniye gipergomotsisteinemii na katekholaminergicheskoye zveno gipotalamicheskoy regulyatsii reproduktivnoy funktsii krys. Zhurnal akusherstva i zhenskikh bolezney. 2012; 1(LI):41-46
18. Vorhees C, Williams M. Assessing Spatial Learning and Memory in Rodents. ILAR Journal. 2014;(55):310-32. DOI: 10.1093/ilar/ilu013.
19. SalkovicMPetrisic M. What have we learned from the streptozotocininduced animal model of sporadic Alzheimer's disease, about the therapeutic strategies in Alzheimer's research. J Neural Transm. 2013; 1(120):233–252.
20. Vit V.V., Tsiselskaya O.Yu., Tsiselskiy Yu.V., Levitskiy A.P. Patologicheskiye izmeneniya setchatki glaza krys pri eksperimentalnom sakharnom diabete 2 tipa i ikh korrektsiya oralnymi gelyami s biologicheski aktivnymi veshchestvami. Oftalmologiya. 2013; 4(10):49-52
21. Kaidah S, Soejono K, Partadiredja G. Exercise improves hippocampal estrogen and spatial memory of ovariectomized rats. Bratisl Lek Listy. 2016;117(2):94-9
22. Pandey SP, Singh HK, Prasad S. Alterations in Hippocampal Oxidative Stress, Expression of AMPA Receptor GluR2 Subunit and Associated Spatial MemoryLoss by Bacopa monnieri Extract (CDRI-08) in Streptozotocin-Induced Diabetes Mellitus Type 2 Mice. 2015; ;10(7):e0131862. doi: 10.1371/journal.pone.0131862.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Inha Tymofiychuk
Higher State Educational Institution of Ukraine
"Bukovinian State Medical University"
Komarova str. 30/59, Chernivtsi, Ukraine
tel:+380965608470
e-mail: inga10051973@ukr.net

Received: 25.01.2019
Accepted: 19.06.2019

ORIGINAL ARTICLE
PRACA ORYGINALNA

LOCAL RECURRENCES AFTER THE TREATMENT OF SOFT TISSUE MALIGNANT FIBROUS HISTIOCYTOMA (UNCLASSIFIED PLEOMORPHIC SARCOMA) OF THE LIMBS

Oleksandr O. Lytvynenko[1], Volodymyr F. Konovalenko[2], Anton Yu. Ryzhov[3]
[1]NATIONAL CANCER INSTITUTE OF THE MOH OF UKRAINE, KYIV, UKRAINE
[2]R.E. KAVETSKY INSTITUTE OF EXPERIMENTAL PATHOLOGY, ONCOLOGY AND RADIOBIOLOGY, KYIV, UKRAINE
[3]TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV, KYIV, UKRAINE

ABSTRACT

Introduction: The treatment of patients with malignant fibrous histiocytoma as well as other soft tissue sarcomas is not sufficiently effective up to date, and has largely changed and reflects the alterations, occurred in oncology as a whole. The number of amputation decreased over the last 10-15 years. Some researchers associate the improvement of treatment outcomes with the development of combined and complex methods.

The aim of the study is an improvement of the results of treatment of patients with soft tissue malignant histiocytoma on the basis of determination of factors, influencing local recurrence development.

Materials and methods: The basis of our study was a comprehensive analysis of examination and treatment results of 130 patients with MFH of the soft tissue of limbs, of them in 84 patients (64.6%) the recurrences developed. The group included 45 (53.6%) males and 39 (46.4%) females. The major part of patients – 82.1% (60 patients) were older than 40 years.

Results and conclusions: The number of recurrences after the treatment in general surgical facilities is 86.9%, whereas in the patients after the treatment in the specialized oncological facilities this figure is twice lower (40%). The characteristic of the medical facility where the patient receives his/her primary treatment largely affects the development of local recurrences, patients' quality of life and overall survival rates.

The surgical method remains the leading modality in the treatment of MFH of ST. Wide and radical excision of tumors in the specialized oncological facilities allows achieving better survival outcomes of the patients.

KEY WORDS: malignant fibrous histiocytoma, treatment, recurrence, survival rate

Wiad Lek 2019, 72, 8, 1523-1526

INTRODUCTION

The treatment of patients with malignant fibrous histiocytoma (MFH) as well as other soft tissue sarcomas (STSs) is not sufficiently effective up to date, and has largely changed and reflects the alterations, occurred in oncology as a whole. The number of amputation decreased over the last 10-15 years to 5-18% (V.E. Milov, 2005; A.D. Burov, 2014; E. Stoeckleet. al. 2009). Some researchers associate the improvement of treatment outcomes with the development of combined and complex methods (N.S. Petrochenko, 2015; N.V. Dengina, 2015; J.P. Derooseet.al., 2012; N.J. Look Honq et.al., 2013). The rate of local recurrencies after surgical treatment, on average, is up 50% (D.V. Martynkov et. al., 2011; A. Belal et.al., 2002).

The development of local recurrences significantly complicates a problem of STS patients' treatment (D.A. Burov et. al., 2009). Local STS recurrence occurring depends not only on biological characteristics of the tumor (tumor grade, size, localization) but also on a degree of radicality of the primary surgical treatment. After local tumor excision as an independent treatment method, the rate of local recurrences is from 38.8 to 90% (M.D. Aliev et. al., 2005; Doganavsargil et. al., 2009). STS removal within its pseudocapsule is accompanied by tumor relapses in about 90% of patients, at local excision (with small sections of healthy surrounding tissues) 40% rate of recurrences is seen (B.T. Baizakov, O.K. Kulakeev, 2010), whereas the wide radical tumor excision (within anatomy structures or group of muscles, or amputation) is followed by local recurrences in 10 – 20% of cases (M.D. Aliev et. al., 2005; C.D.M. Fletcher, 2002).

THE AIM

The aim of the study is an improvement of the results of treatment of patients with soft tissue malignant histiocytoma on the basis of determination of factors, influencing local recurrence development.

MATERIALS AND METHODS

The basis of our study was a comprehensive analysis of examination and treatment results of 130 patients with MFH of the soft tissue of limbs, of them in 84 patients

(64.6%) the recurrences developed. The group included 45 (53.6%) males and 39 (46.4%) females. The major part of patients – 82.1% (60 patients) were older than 40 years.

Surgical treatment as an independent treatment was performed in the scope of organ-saving operation – tumor excision with resection of adjacent anatomical structures, with substitution of bones and arteries defects, and myoplasty, whenever required. The combined treatment included pre-surgical and post-surgical radiation therapy (RT), combined with surgical intervention. The complex treatment involved surgical intervention, RT and polychemotherapy (PCT) in the pre-operational period (system and intra-arterial) and PCT in the adjuvant regimen.

RESULTS AND DISCUSSION

The development of local recurrences was registered over the period from 2 months to 7 years and 8 months after the primary surgical treatment. A substantial number of patients, having recurrences of the disease – 57.1% were older than 51 years. In 42.8% the data about the stage of the primary process were absent in 42.8%, and in 57.1% the stage was determined. From this 57.1% of patients with determined category T, in 50% it corresponded to the category T2 (14.3% of patients had category T2a, and 35.7% - T2b). The category No corresponded to all patients, regardless of category T. High-grade tumors were in 85.7%, moderate – in 14.3% of patients.

One local recurrence was registered in y 42.8% of patients, two recurrences were diagnosed in 14.3%, three recurrences – in 7.1 of patients, four – in 14.3%, five – also in 14.3%, and six recurrences were determined in 7.1% of patients.

Among the patients with diagnosed local recurrences 7.1% started treatment with pre-operational RT, 35.7% received post-operational PCT, 7.1% received CT alongside with RT, and 57.1% started treatment with tumor removal that was not accompanied by other methods. All the patients started treatment in general hospitals.

In patients who had process localization in the forearm, the recurrences occurred in 60% of cases, among them 10% of tumors were of low grade and in 50% of high grade. Two recurrences were diagnosed in 50% of patients, three – in 33.3%, and six – in 16.7% of patients.

In 60% of patients, the treatment was started with surgical intervention as an independent treatment method in the scope of tumor removal. All the patients received treatment in general hospitals.

Forty percent of patients as the first step received combined treatment in the specialized oncologic clinics. Disease recurrences were not found in this group of patients. The complex treatment, as the first step, was not conducted for this group of patients.

A single type of treatment – only surgical tumor removal – was received by 22.2% of patients, however, for each patient it was provided twice due to the development of local recurrences. The rest 77.7% of the patients received combined and complex treatment; among them, 33.3% obtained combined treatment, and 44.4% - complex treatment. The sequence of the applied treatment methods was not repeated, excluding 22.2% of patients that had only surgical neoplasm excision. These data demonstrate the planning of individual approaches to each patient's treatment program, considering all examination data and the methods of treatment, which have been previously received by the patient (before the admission to the specialized clinics), the time between treatment steps, the presence and size of the recurrence, and tumor grade.

In 67.7% of patients with femoral MFH of ST local recurrences occurred, which have developed over the period from 10 days to 6 years and 3 months after treatment; of them, 64.6% started their treatment at the place of residence. The major part of the patients – 81.8% that had sarcoma recurrences were older than 40 years. One recurrence of the disease was diagnosed in 50% of patients, two – in 31.8% of patients, three – in 9.1%, four – in 44.5%, and five also in 4.5%. Data about the staging of the primary process in patients with recurrences are absent in 59.1% of patients; in 40.9% the stage was determined. Of them, the category T1b was diagnosed in 2.3%, T2b in 38.6% of patients. All the patients of this group corresponded to the category No. In patients with local recurrences, metastatic lesions of other organs were determined in y 2.3% of cases. The majority of patients had high-grade malignancy – in 77.3%, moderate - in 15.9%, and low-grade malignancy was seen in 6.8% of patients. The share of patients which treatment started from the surgical method – tumor removal that was not accompanied by other methods was 45.5%. Of them, in 90.9% of patients disease recurrences developed. In 38.6% of patients, the treatment started from the combined method, in 29.5% surgical removal was supplemented by RT. Before the surgical intervention, the RT was given to 4.5% of patients. The complex method at the beginning of treatment was applied in 15.9% of patients. The local recurrences development was seen in all patients' subgroups that received surgical, combined and complex treatment.

Not always, the surgical removal of the tumor recurrence occurred without injuring of great hip vessels. In our study, in 38.6% of cases, there was the involvement of great hip vessels in the area where the recurrence growth occurred. Tumor removal was provided with artery resection, followed by defect replacement with a vascular endoprosthesis.

RT in different combinations with surgical intervention and chemotherapy was applied in 81.8% of patients that received treatment for local recurrence. In 18.2% of patients that were treated for MFH recurrences pulmonary metastatic lesions developed.

In 72.7% of patients with tibial MFH of ST local disease recurrences developed in different periods – from 1 month to 5 years and 6 months.

The data about primary process staging in patients with local recurrences were absent in 31.2% of patients – these were patients who started their treatment in general hospitals. In the rest 68.7% of patients, the stage of the disease was determined. The category T2b was in all 68.7% of

Table I. Total survival of patients with MFH, having disease recurrences, depending on treatment method.

Interval beginning \ Treatment method	Surgical		Combined		Complex	
	Number at the beginning	Survival rate %	Number at the beginning	Survival rate %	Number at the beginning	Survival rate %
0	12	100.0±0.0	33	100.0±0.0	36	100.0±0.0
12	11	91.6±7.9	28	87.6±5.7	32	91.5±4.6
24	10	83.3±10.7	23	72.0±7.9	21	62.4±8.2
36	9	78.9±11.8	18	59.2±8.7	18	53.5±8.5
48	8	74.3±12.8	16	55.8±8.8	14	47.2±8.6
60	7	69.3±13.7	13	53.9±8.9	14	45.5±8.6
72	5	58.6±15.2	12	51.7±9.1	11	42.0±8.6
84	4	46.9±16.0	11	49.5±9.2	11	40.1±8.6
96	4	41.0±16.0	9	44.7±9.4	8	38.0±8.7
108	3	30.8±14.9	8	39.8±9.6	7	35.5±8.8
120	2	24.6±14.2	8	37.3±9.6	5	30.0±9.0

Table II. Overall survival of primary MFH patients and MFH patients with recurrences and recurrent irrespective of the treatment applied.

Survival	Patients with recurrences			Primary patients		
Interval beginning	Number at the beginning	Cumulative survival rate	Standard error of cumulative survival	Number at the beginning	Cumulative survival rate	Standard error of cumulative survival
0	82	1.000000	0.000000	26	1.000000	0.000000
12	54	0.689441	0.051573	19	0.875000	0.067508
24	41	0.558119	0.056028	17	0.827703	0.078702
36	34	0.529126	0.056744	13	0.724240	0.097085
48	30	0.520858	0.057049	12	0.695270	0.101479
60	23	0.482978	0.058853	10	0.663667	0.106253
72	17	0.436980	0.061584	5	0.619423	0.116141
84	14	0.422884	0.062741	4	0.550598	0.138126
96	11	0.390354	0.065809	3	0.471941	0.156918
108	9	0.370836	0.068062	3	0.393284	0.165563
120	7	0.347659	0.071265	3	0.327737	0.161852

patients. The category No corresponded to all the patients of the subgroup. The category Mo corresponded to 56.2% of patients that had the data about disease staging. In total, the category Mo corresponded to 87.5% of the patients. Pulmonary metastatic lesions were in 12.5% of the patients. High-grade tumors were in all patients without exceptions.

In half of the patients (50%) the treatment was started with surgical method – tumor removal – that was not supplemented by any other treatment methods. Of them, in 37.5% of patients, the treatment was started at the place of residence. The combined treatment was given to 31.2% of patients; in 25% of cases tumor removal was accompanied by RT.

The development of local recurrences of tibial MFH of ST in all patients' subgroups was determined irrespective of the applied treatment method.

Our data suggest that in patients with high-grade tibial MFH of ST, in other words, with tumors of a high degree of malignancy, receiving only surgical organ-saving treatment, local recurrences develop in all the patients. Application of combined and complex treatment modalities does not improve the outcomes. The majority of patients received several treatment methods in various sequences.

Out of 84 of patients with recurrences, 81 patients were managed under the program of radical treatment, and 3 patients – under the program of palliative treatment. Therefore, we studied long-term outcomes in 81 patients.

We examined and analyzed long-term results of treatment of MFH patients in terms of overall survival using Kaplan-Meier analysis (Table I; Table II).

The analysis of indicators shown in Table I suggests the futility of complex treatment procedures since the trend of worsening of these rates comparing with the application of combined procedures was discovered. Three-year and five-year survival rates are 60.2±7.0% and 54.4±7.3% versus 54.0±6.7%, respectively, that are in line with other authors' findings (15).

The data of Table II strongly demonstrate the need for the patients with MFH to receive medical care in the specialized oncological hospitals as at that the survival rates increase significantly.

Basing on the analysis of the outcomes of MFH patients treatment, one of the major factors, negatively affecting the dynamics of the indices, is a characteristic of medical facility where the patient received his/her primary treatment; at that, the number of recurrences in patients that underwent surgery in general surgical facilities reaches 86.9% that thereafter, according to literature data (16), significantly worsens the quality of life and overall survival rates.

The indices of immediate and long-term results of MFH patients treatment worsen significantly following application of inadequate primary treatment – local recurrences are seen in 64.6% of cases, and overall survival rates of the patients reduce significantly – three-year survival rate - by 19.5% (52.9±5.7%), and five-year survival rate - by 22.1% (48.3±5.9%) versus (72.4±9.7%) and (66.4±10.6%), P< 0.05, respectively.

CONCLUSIONS

1. The surgical method remains the leading modality in the treatment of MFH of ST. Wide and radical excision of tumors in the specialized oncological facilities allows achieving better survival outcomes of the patients.
2. Application of the combined treatment (surgical intervention + radiation therapy) leads to the trend of recurrences reduction and improvements of overall survival rates of the patients.
3. Among the factors, considerable affecting the development of the recurrences of MFH of ST are T-criterion, tumor grade and molecular profile of the tumor.

REFERENCES

1. Milov V.E. Treatment of patients with malignant fibrous histiocytoma (MFH) of soft tissues and prognostic factors. Extended abstract of cand. med. sci. dissertation. Moskva, 2005.22p. (In Russian).
2. Burov D.A. Prognostication factors for local recurrences in patients with soft tissue sarcomas. Extended abstract of cand. med. sci. dissertation. Moskva, 2014.105p. (In Russian).
3. Stoeckle E., Coindre J.M., Kantor G. et.al. Evaluating surgery quality in soft tissue sarcoma. Recent Results, Cancer Res. 2009;179:229-242.
4. Petrochenko N.S. Isolated regional perfusion in the combined treatment of patients with soft tissue sarcomas of limbs. Sarcomas of bones, soft tissue and skin tumors. 2015;1:26-31 (In Russian).
5. Dengina N.V. About role and potentials of radiation therapy in the treatment of soft tissue sarcomas. Sarcomas of bones, soft tissue and skin tumors. 2015;4:52-62 (In Russian).
6. Deoose J.P, Van Geel A.N., Burper J.W. Eqqermont A.M., et al. Isolated limb perfusion with TNE- alpha and melphalan for distal parts of the limb in soft tissue sarcoma patients. J. Surg. Oncol. 2012;105:563-569.
7. Look Hong N.J., Hornicek F.J., Harmon D.C. et al. Neoadjuwant chemoradiotherapy for patients with high-risk extremity and truncal sarcomas: A. 10-year single institution retrospective study. Eur. J. Cancer. 2013;49(4):875-883.
8. Martynkov D.V., Kharatashvili T.K., Bokhian B.Yu., et al. Synchronous development of local recurrence in patients with soft tissue sarcomas. Sarcomas of bones, soft tissue and skin tumors. 2011; 4:51-55 (In Russian).
9. Belal A., Kandil A., Allam A. et.al. Malignant fibrous histiocytoma; a retrospective study of 109 cases. Am. J. Clin. Oncol. 2002;25(1):16-22.
10. Burov D.A., Bokhian B.Yu., Fedorenko A.A. et al. Diagnostic pitfalls in soft tissue sarcomas. Sarcomas of bones, soft tissue and skin tumors. 2009;1:48 – 52 (In Russian).
11. Aliev M.D., Mekhtieve N.I., BokhianB.Yu. Prognostication factors in soft tissue sarcomas. Voprosy oncologii (Oncology issues). 2005;51(3):288-291 (In Russian).
12. Doqanavsarqil B., Arqin M., Sezak M. et. al. Dedifferentiated chondrosarcoma of the thumb: a case report. Arch. Orthop. Trauma Surg. 2009;129(2):161-166.
13. Baizakov B.T. Surgical treatment of patients with recurrent soft tissue sarcomas. Modern problems of science and education. 2011;6 (In Russian).
14. Fletcher C.D.M., Unni K.K., Mertens F. Pathology and genetics of tumors of soft tissue and bone. World Health Organization: classification of tumours. – IARC Press, Lyon 2002;5:70.
15. Sandro Pasquali, Alessandro Gronchi. Neoadjuvant chemotherapy in soft tissue sarcomas: latest evidence and clinical implications / Ther Adv Med Oncol. 2017 Jun; 9(6): 415–429. . (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5455882/)
16. Jenna S. Bleloch, Reyna D. Ballim, Serah Kimani, Jeannette Parkes, Eugenio Panieri, TarrynWillmer, Sharon Prince. Managing sarcoma: where have we come from and where are we going? /Ther Adv Med Oncol. 2017 Oct; 9(10): 637–659. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5613860/)

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Oleksandr O. Lytvynenko
22 Liatoshynskogo str., 03191 Kyiv, Ukraine
tel: +3 0679600000
e-mail: litvinenko_san@ukr.net

Received: 05.04.2019
Accepted: 19.07.2019

PRACA POGLĄDOWA
REVIEW ARTICLE

WITAMINA D W PREWENCJI I LECZENIU ATOPOWEGO ZAPALENIA SKÓRY - FAKTY I SPEKULACJE

FACTS AND SPECULATIONS ON VITAMIN D IN PREVENTION AND TREATMENT OF ATOPIC DERMATITIS

Anna Lis-Święty[1], Dorota Milewska-Wróbel[2]
[1]KATEDRA I KLINIKA DERMATOLOGII, WYDZIAŁ LEKARSKI W KATOWICACH, ŚLĄSKI UNIWERSYTET MEDYCZNY W KATOWICACH, KATOWICE, POLSKA
[2]PORADNIA DERMATOLOGII DZIECIĘCEJ, I-D MED S.C., BIELSKO-BIAŁA, POLSKA

STRESZCZENIE
Ostatnio eksperci z Polski przedstawili konsensus zawierający dostosowane do wieku i masy ciała zalecenia suplementacji witaminą D (Vitamin D, VD), dotyczące prewencji i leczenia jej niedoboru w ogólnej populacji i grupach ryzyka niedoboru (m.in. atopowym zapaleniu skóry). VD reguluje szereg procesów fizjologicznych w skórze, począwszy od wpływu na podział komórek, ich różnicowanie i apoptozę, do utrzymania funkcji bariery naskórkowej i układu immunologicznego. Z niedoborem VD może wiązać się wzrost występowania i ciężkości atopowego zapalenia skóry. Co więcej, w licznych badaniach wykazano, że suplementacja VD zmniejsza nasilenie atopowego zapalenia skóry. Wyniki przeglądów systematycznych i metaanaliz pozostają jednak kontrowersyjne.

SŁOWA KLUCZOWE: atopowe zapalenie skóry, witamina D, prewencja , leczenie

ABSTRACT
Recently, age- and weight-specific recommendations for prevention, supplementation and treatment of vitamin D (VD) deficiency in the general population and in groups at increased risk of the deficiency (i.a. atopic dermatitis) has been presented by the Polish Experts group. VD regulates many physiological processes in the skin ranging from cellular proliferation, differentiation, and apoptosis to barrier maintenance and immune functions. VD deficiency has been associated with increased incidence and severity of atopic dermatitis. In addition, VD supplementation was shown to decrease atopic dermatitis severity in several studies, but the findings obtained in systematic reviews and meta-analyses are controversial.

KEY WORDS: atopic dermatitis, calcitriol, prophylaxis, therapy

Wiad Lek 2019, 72, 8, 1527-1531

WSTĘP

Witamina D (VD – *Vitamin D*) obejmuje grupę kalcyferoli – rozpuszczalnych w tłuszczach sekosteroidów, spośród których praktyczne znaczenie ma przede wszystkim cholekalcyferol (witamina D3) syntetyzowany w skórze pod wpływem promieniowania ultrafioletowego B lub dostarczany do organizmu drogą pokarmową. Z cholekalcyferolu powstaje dwustopniowo (w wątrobie, nerkach) aktywny biologicznie 1a,25-dihydroksycholekalcyferol (kalcytriol), uważany powszechnie za hormon ze względu na oddziaływanie na różne tkanki i narządy, gdzie wiąże się z receptorami (VDR – *Vitamin D receptor*), przyczyniając się do regulowania czynności licznych genów. Podstawowymi narządami docelowymi działania VD są jelito, kości i nerki. Współdziałając z parahormonem, VD zapewnia prawidłowe stężenie jonów wapniowych i fosforanowych w surowicy – zwiększa wchłanianie wapnia w jelicie, jego uwalnianie z kości oraz absorpcję w nerkach. Poza klasyczną funkcją – utrzymaniem homeostazy wapniowo-fosforanowej, do głównych działań VD należy także regulacja odpowiedzi immunologicznej, zarówno wrodzonej, jak i nabytej. Ekspresję VDR stwierdza się w m.in. w aktywowanych limfocytach T i B, neutrofilach, monocytach, makrofagach, komórkach dendrytycznych. W efekcie VD może odgrywać istotną rolę w procesach autoimmunizacji, nowotworzenia oraz kontroli stanu zapalnego. Dlatego też od kilkunastu lat rozważa się jej udział w rozwoju szeregu schorzeń, takich jak: choroby sercowo-naczyniowe, cukrzyca, stwardnienie rozsiane, depresja, otępienie, nowotwory, choroby autoimmunologiczne, alergiczne, infekcyjne. VD wydaje się mieć także znaczenie w etiopatogenezie chorób skóry, szczególnie łuszczycy i atopowego zapalenia skóry (AZS). Mogłaby stanowić opcję terapeutyczną w tych dermatozach.

Celem pracy jest krytyczny przegląd dotychczasowych ustaleń naukowych dotyczących przesłanek skuteczności stosowania VD w prewencji i leczeniu AZS.

DYSKUSJA

MECHANIZM DZIAŁANIA WITAMINY D W ATOPOWYM ZAPALENIU SKÓRY

AZS jest przewlekłym schorzeniem zapalnym charakteryzującym się dysfunkcją bariery naskórkowej, dysregulacją układu immunologicznego i zaburzeniem mechanizmów naturalnej obrony przeciwdrobnoustrojowej. Niedobór VD ma wpływ na wszystkie wyżej wymienione zjawiska. Wykazano bezpośredni związek VD z funkcją bariery naskórkowej polegający na kontroli proliferacji, różnicowania i apoptozy keratynocytów, zwiększeniu tworzenia profilagryny i blaszek lipidowych (lamelli) [1]. Potwierdzono szereg efektów VD w zakresie odpowiedzi immunologicznej i zapalnej. VD hamuje różnicowanie, dojrzewanie i aktywność komórek dendrytycznych, oddziałuje supresyjnie na komórki Th1, Th2, Th17, limfocyty B (zmniejszenie produkcji IgE) [2]. Pobudza natomiast komórki NK i limfocyty T regulatorowe, zwiększa wydzielanie interleukiny 10 przez mastocyty [2]. VD stymuluje produkcję peptydów przeciwdrobnoustrojowych, takich jak: katelicydyny, β-defensyny i LL-37 oraz pobudza komórki wrodzonej odpowiedzi immunologicznej – makrofagi i monocyty - zwiększając ich chemotaksję i fagocytozę [3]. Do nowszych, bardzo ciekawych odkryć należy opisanie wpływu zmienności genetycznej VDR na mikrobiom jelitowy [4].

WITAMINA D A RYZYKO ROZWOJU AZS

Niektóre duże badania populacyjne wskazują, że ryzyko rozwoju AZS może być wyższe u osób z suboptymalnym lub niskim stężeniem VD. Większa zachorowalność na AZS występuje w wyższych szerokościach geograficznych, gdzie ekspozycja na UV jest mniejsza i produkcja VD niższa [2]. Część badań dostarcza jednak odmiennych danych sugerując, że podwyższone stężenie VD w surowicy może także wpływać na wzrost ryzyka AZS [1, 3]. Niskie stężenie VD u ciężarnej może wiązać się ze zwiększonym ryzykiem AZS u dziecka, z drugiej strony suplementacja VD w ciąży może podwyższać zachorowalność na AZS u niemowląt [2]. Oceny stężenia VD w krwi pępowinowej i jego związku z AZS dają sprzeczne i niskiej wartości wyniki [5, 6]. Istotnych informacji mogłyby dostarczyć obserwacje uwarunkowań genetycznych związanych z metabolizmem VD i funkcją VDR. Badanie metodą randomizacji Mendla nie potwierdziło jednak wpływu wariantów genetycznych stwierdzanych w populacji na wystąpienie AZS. Analizowano występowanie 4 alleli powiązanych z obniżonym stężeniem VD: rs2282679 w GC, rs12785878 w sąsiedztwie DHCR7, rs10741657 w sąsiedztwie CYP2R1, i rs6013897 w CYP24A1 [7]. W populacji tureckiej i niemieckiej wykazano natomiast znaczenie polimorfizmów genu VDR w rozwoju i ciężkim przebiegu AZS: BsmI (Turcja) oraz BsmI, ApaI i TaqI (Niemcy) [8].

NIEDOBÓR WITAMINY D U CHORYCH NA AZS

Obecnie uważa się, że niedobór witaminy D jest problemem ogólnoświatowym i w Polsce jest również bardzo rozpowszechniony sięgając 90% populacji [9]. Jednak według niektórych badaczy wykrywanie tak często występującej hipowitaminozy D jest kontrowersyjne, a definicja niedoboru VD nie została ustalona ostatecznie [10]. Niższe stężenia VD stwierdza się w miesiącach zimowych (listopad –marzec) oraz w obszarach miejskich [11]. Na stężenie VD w surowicy wpływa też szereg czynników indywidualnych, m.in. fototyp skóry, płeć, wiek, wskaźnik masy ciała (BMI), styl życia, spożywanie alkoholu, palenie papierosów, polimorfizm VDR [12]. Znacząco obniżone stężenie VD w porównaniu do grupy kontrolnej zdrowych osób obserwowano w większości chorób zapalnych skóry [13]. U chorych na AZS, zdaniem części autorów, niedobór VD należy raczej odnieść do innych towarzyszących przyczyn, takich jak: ciemny fototyp skóry, wysokie BMI [14, 15] czy też mała aktywność fizyczna [7]. W dużym badaniu populacyjnym NIKI, pomimo często występującego suboptymalnego stężenia (34%) lub niedoboru VD (17%), stężenie parathormonu było podwyższone tylko u kilku dzieci, co przemawia za zbyt niskim progiem odcięcia i wykrywaniem obniżonego stężenia VD w przypadkach bez znaczenia klinicznego. Co ciekawe, w przeciwieństwie do wcześniejszych badań [16, 17], w wyżej wymienionym badaniu stężenie VD u dzieci chorych na AZS lub astmę było wyższe niż u dzieci zdrowych [18]. Badanie nastolatków – w próbie reprezentatywnej dla Korei, po uwzględnieniu wieku, płci, miejsca zamieszkania, BMI i próbek pobranych w miesiącach zimowych – nie wykazało związku pomiędzy stężeniem VD a AZS i astmą [11]. Metaanaliza obejmująca badania porównujące stężenie VD w surowicy dorosłych pacjentów chorujących na AZS ze zdrowymi ochotnikami nie wykazała statystycznie istotnych różnic [16]. W Polsce podobne wyniki uzyskali również Samochocki i wsp. [19]. Badanie przeprowadzone w Holandii sugeruje, że u dorosłych chorych na AZS ryzyko niedoboru VD jest najwyższe u pacjentów opornych na leczenie (76%), wymagających przewlekłego stosowania miejscowych kortykosteroidów. Autorzy zalecają w tej grupie chorych oznaczanie stężenia VD w surowicy [20]. Według zaleceń polskiej grupy ekspertów ryzyko deficytów witaminy D dotyczy zarówno astmy oskrzelowej, jak i AZS – u pacjentów z tymi schorzeniami powinno się zatem oznaczyć stężenie VD [9]. Autorzy rekomendacji przyjmują następującą klasyfikację hipowitaminozy D: stężenie suboptymalne >20–30 ng/ml, niedobór znaczny >10–20 ng/ml, niedobór ciężki 0–10 ng/ml.

STĘŻENIE WITAMINY D A CIĘŻKOŚĆ AZS

Piśmiennictwo obejmuje szereg doniesień wskazujących na związek pomiędzy nasileniem AZS a stężeniem VD. W łagodnych przypadkach AZS stężenie VD jest wyższe niż w przypadkach o umiarkowanym lub ciężkim przebiegu. Wykazano odwrotną korelację pomiędzy stężeniem VD a nasileniem AZS ocenianym przy pomocy SCORAD [18, 21, 22,]. Jednocześnie niskim stężeniom VD towarzyszyło wysokie stężenie IgE [21, 23]. Podobną korelację obserwowano również pomiędzy odsetkiem komórek CD8+ [24]. Niektóre prace, pomimo często obserwowanego w AZS

niedoboru VD, nie potwierdzają jednakże takich związków [25,14]. W badaniach Mesquita Kde [26] analizujących korelacje stężenia VD i nasilenia AZS (SCORAD) zaleca się uwzględnienie wpływu natężenia promieniowania ultrafioletowego (Ultraviolet, UV) w określonym miejscu i czasie – indeksu UV. Autorzy zwrócili uwagę, że wartości SCORAD były zależne od stężenia IgE i indeksu UV. Na stężenie VD poza indeksem UV wpływały takie czynniki, jak fototyp skóry i wiek pacjentów [26]. Opublikowane ostatnio badanie oceniające oddziaływanie fototerapii UVB na komórki T regulatorowe we krwi, stężenie cytokin w skórze i stężenie VD w surowicy, wykazało wzrost jej stężenia bez towarzyszących zmian w zakresie parametrów immunologicznych [27]. Korzystny efekt UV w AZS prawdopodobnie nie zależy od VD, lecz może być związany z wpływem antydrobnoustrojowym i przeciwzapalnym. W świetle niektórych doniesień wydaje się, że korelacja nasilenia AZS ze stężeniem VD w surowicy może wynikać z jej wpływu na funkcję bariery naskórkowej. U dorosłych Koreańczyków nie stwierdzono różnic w stężeniu VD pomiędzy grupą łagodnego a grupą umiarkowanego i ciężkiego AZS, ale zaobserwowano związek niskich stężeń VD z alergią na roztocza kurzu domowego [28]. Podobną zależność opisali wcześniej tureccy badacze u dzieci chorych na AZS uczulonych na alergeny wziewne i pokarmowe [29]. Badanie norweskich dzieci do 2 r.ż. nie wykazało jednak zależności ciężkości AZS od stężenia VD przy uwzględnieniu mutacji w genie filagryny [30].

SUPLEMENTACJA DOUSTNA WITAMINĄ D W AZS

Przegląd systematyczny z 2018 r. uwzgledniający 6 prac oceniających wpływ suplementacji VD na poprawę objawów AZS nie dostarczył jednoznacznych wyników. Doniesienie wskazuje na potencjalną korzyść stosowania VD w AZS, ale dowody są niskiej jakości. Autorzy podkreślają nieuwzględnienie w badaniach dodatkowych czynników mogących wpływać na wyniki, na takich jak: wiek, sezon roku, szerokość geograficzna, dawka VD i czas trwania suplementacji [31]. Wyniki opublikowanych ostatnio 2 randomizowanych kontrolowanych prób również budzą kontrowersje. W badaniu przeprowadzonym w Meksyku po 3 miesiącach stosowania VD w dawce 5000 IU/dziennie, jako leczenia wspomagającego w AZS, efekt oceniono jako korzystny, ale doniesienie dotyczy małej grupy chorych w różnym wieku 2–54 lat [32]. Według doniesienia z Kanady, suplementacja VD w dawce 2000 IU/dziennie nie przyczyniła się do znaczącej poprawy AZS u dzieci, chociaż odnotowano korelację pomiędzy stężeniem VD a ciężkością choroby [33]. Część publikacji zwraca uwagę na potencjalne mechanizmy uzasadniające podaż VD w AZS. Drozdenko i wsp. oraz Di Filippo i wsp. stwierdzili, że podczas stosowania VD u pacjentów chorujących na AZS dochodzi do zmniejszenia produkcji cytokin przez limfocyty T, m.in. interleukiny 17 i interferonu-γ [34, 35]. Według Udompataikul i wsp. poprawie AZS po suplementacji VD towarzyszyło zmniejszenie kolonizacji *Staphylococcus aureus* [36]. Efekt ten mógł być zależny od niedostatecznej produkcji peptydów antydrobnoustrojowych przy niedoborze VD. Albenali i wsp. wykazali, że podawanie VD u chorych na AZS spowodowało zwiększenie stężenia LL-37 [37]. Nowsze dane sugerują wpływ suplementacji VD na zmniejszenie aktywacji komórek dendrytycznych i poprawę mechanizmów tolerancji [38].

Informacje na temat skuteczności suplementacji VD w celu pierwotnej prewencji AZS są ograniczone i niejasne. Chociaż raportowano związek pomiędzy niskim stężeniem VD w surowicy a rozwojem chorób alergicznych, brak dowodów, że jest to zależność przyczynowo-skutkowa. Ostatni przegląd systematyczny badający ten problem w różnych grupach populacyjnych (kobiety ciężarne, karmiące, niemowlęta) nie potwierdził profilaktycznego działania VD w AZS [39]. Aktualnie wśród kobiet ciężarnych prowadzone są dwa badania interwencyjne dotyczące oceny ochronnego wpływu VD na rozwój astmy (clinicaltrial.gov; identifier, NCT00856947 and NCT00920621).

Mimo wątpliwości dotyczących związku między niedoborem witaminy D a AZS lub ryzykiem jego rozwoju, także wobec skali problemu niedoboru tej witaminy w populacji ogólnej i u chorych, w Polsce rekomenduje się wdrożenie opracowanego schematu profilaktyki i leczenia niedoboru witaminy D do codziennej praktyki lekarzy i dietetyków klinicznych [9]. W AZS, które stanowi grupę ryzyka niedoboru VD, wskazana jest ocena zaopatrzenia w VD na podstawie oznaczeń jej stężenia w surowicy (stężenia kalcytriolu) [9]. Autorzy przyjmują następujące przedziały określające stężenie VD podczas suplementacji: optymalne >30–50 ng/ml (zalecane jest kontynuowanie dotychczasowego postępowania), >50–75 ng/ml (zalecane jest zmniejszenie dawki o 50%), >75–100 ng/ml (należy zalecić wstrzymanie podaży VD na okres 1–2 miesięcy), stężenie wysokie >50–100 ng/ml (należy dokonać korekty postępowania), stężenie toksyczne >100 ng/ml (należy bezwzględnie przerwać suplementację VD) [9]. Według obszernej metaanalizy badań prowadzonych w AZS, objawy uboczne suplementacji VD nie były zgłaszane lub miały charakter o mniejszym znaczeniu bez poważnych konsekwencji [40].

LECZENIE ZEWNĘTRZNE WITAMINĄ D W AZS

Miejscowe preparaty VD nie są rekomendowane do leczenia AZS. Co więcej, aplikacja cholekalcyferolu lub jego analogu MC903 na skórę indukuje zmiany morfologiczne i zapalenie skóry przypominające pod względem zaburzeń immunologicznych (wzrostu ekspresji TSLP, IL-4) zmiany odpowiadające zaostrzeniu AZS [41, 42].

PODSUMOWANIE

Powszechnie spotykany w populacji ogólnej i u chorych na AZS niedobór VD wynika prawdopodobnie z jej niedostatecznego spożycia z pokarmami i niskiej ekspozycji słonecznej, rzadziej z powodu schorzeń zmniejszających absorpcję VD lub stanów zaburzających jej konwersję do aktywnej postaci. Grupa polskich ekspertów z udziałem

konsultantów krajowych i prezesów towarzystw naukowych zaleca profilaktyczne dawkowanie witaminy D w populacji ogólnej lub w grupach ryzyka (w tym AZS), które powinno być zindywidualizowane w zależności od wieku, masy ciała, nasłonecznienia (pory roku), diety i trybu życia [9]. Wymagane są jednak dalsze badania w celu poprawy strategii wykrywania, prewencji i leczenia hipowitaminozy D w AZS.

PIŚMIENNICTWO

1. Mesquita Kde C, Igreja AC, Costa IM. Atopic dermatitis and vitamin D: facts and controversies. An Bras Dermatol. 2013;88(6):945-953.
2. Umar M, Sastry KS, Al Ali F et al. Vitamin D and the Pathophysiology of Inflammatory Skin Diseases. Skin Pharmacol Physiol. 2018;31(2):74-86.
3. Palmer DJ. Vitamin D and the Development of Atopic Eczema. J Clin Med. 2015;4(5):1036-1050.
4. Wang J, Thingholm LB, Skiecevičienė J et al. Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. Nat Genet. 2016;48(11):1396-1406.
5. Li MM, Lu CY, Wang XM.Predictive value of cord blood 25(OH)D3 for early infantile atopic dermatitis]. Zhongguo Dang Dai Er Ke Za Zhi. 2018;20(4):303-307.
6. Blomberg M, Rifas-Shiman SL, Camargo CA Jr et al. Low Maternal Prenatal 25-Hydroxyvitamin D Blood Levels Are Associated with Childhood Atopic Dermatitis.J Invest Dermatol. 2017;137(6):1380-1384.
7. Manousaki D, Paternoster L, Standl M et al. Vitamin D levels and susceptibility to asthma, elevated immunoglobulin E levels, and atopic dermatitis: A Mendelian randomization study. PLoS Med. 2017;14(5):e1002294.
8. Kılıç S, Sılan F, Hız M, et al. Vitamin D Receptor Gene BSMI, FOKI, APAI, and TAQI Polymorphisms and the Risk of Atopic Dermatitis. J Investig Allergol Clin Immunol. 2016;26(2):106-110.
9. Rusińska A, Płudowski P, Walczak M et al. Zasady suplementacji i leczenia witaminą D - nowelizacja 2018 r. Stan Med – Pediatr. 2018;15:531-552, 555-559.
10. Antonucci R, Locci C, Clemente MG et al. Vitamin D deficiency in childhood: old lessons and current challenges. J Pediatr Endocrinol Metab. 2018;31(3):247-260.
11. Byun EJ, Heo J, Cho SH et al. Suboptimal vitamin D status in Korean adolescents: a nationwide study on its prevalence, risk factors including cotinine-verified smoking status and association with atopic dermatitis and asthma. BMJ Open. 2017;7:e016409.
12. Kechichian E, Ezzedine K. Vitamin D and the Skin: An Update for Dermatologists. Am J Clin Dermatol. 2018;19(2):223-235.
13. Amon U, Baier L, Yaguboglu R et al. Serum 25-hydroxyvitamin D levels in patients with skin diseases including psoriasis, infections, and atopic dermatitis. Dermatoendocrinol. 2018;10(1):e1442159.
14. Darji 1, Tobin C, Bryan ZT et al. Vitamin D Deficiency and Atopic Dermatitis: Consider Disease, Race, and Body Mass. Skinmed. 2017;15(6):415-420.
15. D'Auria E, Barberi S, Cerri A et al. Vitamin D status and body mass index in children with atopic dermatitis: A pilot study in Italian children. Immunol Lett. 2017;181:31-35.
16. Kim MJ, Kim SM, Lee YW et al. Vitamin D status and efficacy of Vitamin D supplementation in atopic dermatitis: A systematic review and meta-analysis. Nutrients. 2016;8. pii: E789.
17. Yang HK, Choi J, Kim WK et al. The association between hypovitaminosis D and pediatric allergic diseases: A Korean nationwide population-based study. Allergy Asthma Proc. 2016;37(4):64-69.
18. Reinehr T, Langrock C, Hamelmann E et al. 25-Hydroxvitamin D concentrations are not lower in children with bronchial asthma, atopic dermatitis, obesity, or attention-deficient/hyperactivity disorder than in healthy children. Nutr Res. 2018;52:39-47.
19. Samochocki Z, Bogaczewicz J, Jeziorkowska R et al. Vitamin D effects in atopic dermatitis. Am Acad Dermatol. 2013;69(2):238–244.
20. Van der Schaft J, Ariens LF, Bruijnzeel-Koomen CA, de Bruin-Weller MS. Serum vitamin D status in adult patients with atopic dermatitis: Recommendations for daily practice. J Am Acad Dermatol. 2016;75(6):1257-1259.
21. Su O, Bahalı AG, Demir AD et al. The relationship between severity of disease and vitamin D levels in children with atopic dermatitis. Postepy Dermatol Alergol, 2017;34(3):224-227.
22. Sharma S, Kaur T, Malhotra SK , et al. Correlation of Vitamin D3 Levels and SCORAD Index in Atopic Dermatits: A Case Control Study. J Clin Diagn Res. 2017;11(7):WC01-WC03.
23. Molloy J, Koplin J, Allen K et al. Vitamin D insufficiency in the first 6 months of infancy and challenge-proven IgE-mediated food allergy at 1 year of age: a case-cohort study. Allergy. 2017;72(8):1222-1231.
24. Lipińska-Opałka A, Wawrzyniak A, Lewicki S et al. Evaluation of Immune Indices and Serum Vitamin D Content in Children with Atopic Dermatitis. Adv Exp Med Biol. 2017;1020:81-89.
25. Robl R, Uber M, Abagge KT et al. Serum Vitamin D Levels Not Associated with Atopic Dermatitis Severity. Pediatr Dermatol. 2016;33(3):283-288.
26. Mesquita Kde C, Igreja AC, Costa IM. Ultraviolet index: a light in atopic dermatitis and vitamin D research? An Bras Dermatol. 2016;91(1):34-39.
27. Simonsen S, Bonefeld CM, Thyssen JP et al. Increase in Vitamin D but not Regulatory T Cells following Ultraviolet B Phototherapy of Patients with Atopic Dermatitis. Acta Derm Venereol. 2019;99(2):139-145.
28. Jang YH, Sim HB, Moon SY et al. House Dust Mite Sensitization Is Inversely Associated with Plasma 25-Hydroxyvitamin D3 Levels in Patients with Severe Atopic Dermatitis.Ann Dermatol. 2017;29(4):400-406.
29. Akan A, Azkur D, Ginis T et al. Vitamin D level in children is correlated with severity of atopic dermatitis but only in patients with allergic sensitizations. Pediatr Dermatol. 2013;30(3):359–363.
30. Berents TL, Lødrup Carlsen KC, Mowinckel P et al. Vitamin D levels and atopic eczema in infancy and early childhood in Norway: a cohort study. Br J Dermatol. 2016;175(1):95-101.
31. Huang CM, Lara-Corrales I, Pope E. Effects of Vitamin D levels and supplementation on atopic dermatitis: A systematic review. Pediatr Dermatol. 2018;35(6):754-760.
32. Sánchez-Armendáriz K, García-Gil A, Romero CA et al. Oral vitamin D3 5000 IU/day as an adjuvant in the treatment of atopic dermatitis: a randomized control trial. Int J Dermatol. 2018;57(12):1516-1520.
33. Lara-Corrales I, Huang CM, Parkin PC et al. Vitamin D Level and Supplementation in Pediatric Atopic Dermatitis: A Randomized Controlled Trial. J Cutan Med Surg. 2019;23(1):44-49.
34. Drozdenko G, Heine G, Worm M. Oral vitamin D increases the frequencies of CD38+ human B cells and ameliorates IL-17-producing T cells. Exp Dermatol. 2014;23(2):107-12.
35. Di Filippo P, Scaparrotta A, Rapino D et al. Vitamin D supplementation modulates the immune system and improves atopic dermatitis in children. Int Arch Allergy Immunol 2015;166(2):91–96.
36. Udompataikul M, Huajai S, Chalermchai T et al. The Effects of Oral Vitamin D Supplement on Atopic Dermatitis: A Clinical Trial with Staphylococcus aureus Colonization Determination. J Med Assoc Thai. 2015;98:S23-30.

37. Albenali LH, Danby S, Moustafa M et al.Vitamin D and antimicrobial peptide levels in patients with atopic dermatitis and atopic dermatitis complicated by eczema herpeticum: A pilot study. J Allergy Clin Immunol. 2016;138(6):1715-1719.e4.
38. Cristi F, Perez-Mateluna G, Vera-Kellet C et al. Vitamin D modulates the allergic phenotype of dendritic cells in children with atopic dermatitis. Exp Dermatol. 2019;9:doi: 10.1111/exd.13873.
39. Yepes-Nuñez JJ, Brożek JL, Fiocchi A et al. Vitamin D supplementation in primary allergy prevention: Systematic review of randomized and non-randomized studies. Allergy. 2018;73(1):37-49.
40. Kim G, Bae JH. Vitamin D and atopic dermatitis: A systematic review and meta-analysis. Nutrition. 2016;32(9):913-20.
41. Moosbrugger-Martinz V, Schmuth M, Dubrac S. A Mouse Model for Atopic Dermatitis Using Topical Application of Vitamin D3 or of Its Analog MC903. Methods Mol Biol. 2017;1559:91-106.
42. Maarouf M, Vaughn AR, Shi VY. Topical micronutrients in atopic dermatitis-An evidence-based review. Dermatol Ther. 2018;31(5):e12659.

Konflikt interesów
Autorzy deklarują brak konfliktu interesów

AUTOR KORESPONDUJĄCY
Anna Lis-Święty,
Katedra i Klinika Dermatologii, Śląski Uniwersytet Medyczny w Katowicach, ul. Francuska 20/24, 40-027 Katowice, Polska
e-mail: alis-swiety@sum.edu.pl

Nadesłano: 09.04.2019
Zaakceptowano: 28.05.2019

 © Wydawnictwo Aluna

PRACA POGLĄDOWA
REVIEW ARTICLE

THE ESTIMATION OF ECONOMIC EFFECTIVENESS OF PREVENTIVE MEASURES OF NON-INFECTIOUS DISEASES

Borys Palamar[1], Tetiana Gruzeva[2]
[1]RESEARCH INSTITUTE OF THE NATIONAL UNIVERSITY OF PHYSICAL EDUCATION AND SPORTS OF UKRAINE, KYIV, UKRAINE
[2]O.O. BOHOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

Introduction: The world today is characterized by the globalization of processes affecting social development. The globalization of preventive work today necessarily involves inter-sectorial interaction. Such a huge work with the preventive aim allows to comprehensively influencing the positive effect on the prevention of negative manifestations in the health of both human and the whole population. That means, that we can talk about a preventive strategy, which should be inherent in total character. The Public Health Service should have a state approach. Many countries confirmed this, such as: USA, Sweden, France, Germany, Great Britain, etc. Prevention, in the broadest sense of the word, must take place, of course, both at the local level and at the interregionale one, and if we talk about a global approach, then we also can speak about the international level. The economic effect of such work is clearly demonstrated as a consequence of prophylactic programs on infectious diseases.

The aim of this article is to justify the economic effectiveness of the prevention of non-communicable diseases and to determine the criteria for calculating the economic forecast.

Materials and methods: The research was conducted with the help of theoretical and statistical methods, by studying scientific sources and systematic analysis and generalization of the experience of finding economic efficiency of preventive measures for non-communicable diseases.

Review and conclusions: The analysis of the considered methods of economic evaluation of preventive measures of non-infectious diseases made it possible to draw the following conclusions, the effectiveness of the health care system and individual treatment and prevention institutions should be considered in a complex way from the point of view of medical, social and economic efficiency. Economic efficiency, as a rule, is the result of medical and social efficiency. When calculating the cost-effectiveness of treatment and prevention measures, the whole complex of losses and costs associated with illnesses should be taken into account, in particular: direct costs directly related to prevention and treatment; Indirect losses associated with the illness, due to the unprocessed part of the gross domestic product, paid by sick leave letters.

KEY WORDS: economic efficiency, medical efficiency, social efficiency, non-infectious diseases/non-communicable diseases, risk factors

Wiad Lek 2019, 72, 8, 1532-1541

INTRODUCTION

Reducing the incidence of NCDs in Ukraine is a priority task in the health sector. One of the important tools in this work is to improve preventive measures.

Social-preventive health care includes medical, sanitary, technical, hygienic and socio-economic measures [1].

The current and most important socio-economic and medical tasks of the state to date are the creation of a prevention system, which should include an individual and a social component.

Taking into account that the major problem of Ukrainian health care is the NCD, the issue of prevention of this pathology is becoming more acute. The risk factors that are causing NCD are known and studied today. The first place among the NCD is arterial hypertension (AH). Both the pathology itself and its complications are complicated hypertensive crisis, stroke, myocardial infarction, left ventricular septum, hypertensive kidney, angiopathy, aneurysm of the aorta, aortic valve deficiency, hypertensive encephalopathy require significant economic costs for treatment. According to many studies Babadzhanova A.[2], Kislyak O.[3], Morozova T.[4] low rates of controlled hypertension are found out in ambulatory conditions, within 7-15%. As a result, the high frequency of hospitalization due to complications of hypertension and the growth of economic costs for treatment. One of the main causes of uncontrolled hypertension is the irregular ingestion of antihypertensive drugs. Patients either do not take medications or violate the recommended regimen of their use [5]. It is clear that with an increase in the frequency of admissions increases the economic index.

Arterial hypertension among civil servants (CS) ranks first in the structure of the prevalence of circulatory system diseases (CSD) and is the leading cause of complications such as hypertensive crisis, stroke and myocardial infarction (Yezhel N., Dyachuk D., Pischikov V.)[6].

According to research by scientist Zozulia I. S. [5] acute cerebrovascular accident (ACA) and acute coronary syndrome (ACS) has a prevalence of 1.3% and 1.2% among the Ukrainian population, which leads to significant economic losses.

The economic efficiency of preventive measures consists in the implementation of innovative healthcare projects that ensure the needs of the population in preserving and strengthening health, reducing the morbidity, premature mortality, especially in working age, thus contributing

to the preservation and restoration of labor resources, increase of labor productivity and the growth of the gross national product and, thus, are definitely an economic component [7].

Economic efficiency is the sum of reducing the cost of treatment, sick pay and loss of production as a result of improving the health of the population - reducing morbidity, premature mortality, disability, etc.

It is common, that the economic efficiency is a consequence of social and medical efficiency. In some cases, therapeutic and preventive measures, having medical and social efficiency, deprive the economic effect. For example, these are expensive newest methods of treatment that preserve and lengthen the lives of the elderly, but do not justify themselves from an economic point of view. The economic justification for preventive measures does not require a compulsory positive economic effect in the presence of medical and social efficiency. The calculation of the required volume of material and financial resources, as well as their effective use, is an economic justification. In addition, the coefficient of economic efficiency of preventive measures determines the amount of savings per unit of expenditure.

Enough effective, from an economic point of view, are the preventive measures in the early childhood that provide health for future generations, preventive measures to reduce mortality in working age, etc.

However, one must understand where economic efficiency should be in the priority, and where not, since, the main goal of health care is to provide public health, rather than to achieve profitable activity. For example, measures to prolong life expectancy can not be called cost-effective, as it increases the economic burden on the able-bodied population to retain pensioners.

Estimates of the cost-effectiveness of treatment and prophylactic measures are indicative because they are based on average statistics and assumptions.

For the economic justification and determination of the economic efficiency of preventive measures in the health care it is necessary to calculate losses from morbidity, mortality, disability, etc. in the basic and current periods.

In the context of reforming the healthcare sector, when the transition from state funding to health care facilities to financing the provision of medical care to the population based on its needs, the scientific problem and the development of measures to preserve and strengthen the health of the population of the preventive orientation. Formation of an approach in carrying out preventive measures for elimination of the negative influence of social determinants of health, creation of conditions for preservation and strengthening of health of the population, formation of a responsible attitude of each person to personal health allows to reduce expenses on health protection both on a national scale, and in personal one. It is well-known that prevention of non-infectious diseases is 2.7 times less costly than treatment of patients. At the same time, a healthy lifestyle is 5 times more effective than the medical and diagnostic activity of the health care sector, connected with the preservation and strengthening of public health.

The socio-economic consequences of premature mortality are not only a reduction of the years of potential life and an increase in the amount of irrecoverable losses due to death, but also significant economic losses. Due to the premature mortality of the population of Ukraine, only about 4 million years of potential life are lost every year, and the volume of underproduced national product in recent years ranged from 47.9 to 89.1 billion UAH.

The rationality of the health system, in the current context of the European region, is to improve its performance and reduce costs by increasing the volume of investments involved in health promotion and disease prevention, as well as improving the effectiveness of treatment. and the rehabilitation of those affected by the disease. Consequently, conducting modern innovative scientific developments on the prevention of non-communicable diseases and the application of the results of scientific developments in the practice of health care is not only relevant, but also cost-effective.

THE AIM

To substantiate economic efficiency and to simulate the economic forecast in the implementation of multifactorial prevention of non-communicable diseases among affiliated contingents in a multidisciplinary health care institution.

MATERIALS AND METHODS

The research was conducted using theoretical and statistical methods, by studying and analyzing the experience of finding economic effectiveness of preventive measures of non-infectious diseases.

REVIEW

The study was conducted on an example of hypertension disease (HD) and its complications. The subject of the study were patients with a diagnosis of hypertension, which was treated in the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs.

Thus, according to the data (Table I), by the classes and individual diseases in the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs 14895 patients with hypertension were registered in 2015. 8403 and 13105 patients were subject to dispensary examinations and were under clinical supervision at the end of the reporting period, respectively. In 2016, 13896 patients were registered. 7516 patients were subject to dispensary examinations; 13318 patients were under observation. In 2017, 13639 patients were registered, of which 7084 were subject to dispensary examinations, and 13110 patients were under a dispensary supervision at the end of the reporting period.

During the analysis of the dispensary group of patients with hypertension (arterial hypertension) who were registered with the therapists, it was established that according to the data (Table II), in the State Scientific Institution

Table I. Registration by disease classes (2015 – 2017)

Name of classes and individual diseases	The code (rubric) according to MKH-10	2015			2016			2017		
		Registered diseases - total	Subject to dispensary inspections at the end of the reporting period	Under the dispensary supervision at the end of the reporting year	Registered diseases - total	Subject to dispensary inspections at the end of the reporting period	Under the dispensary supervision at the end of the reporting year	Registered diseases - total	Subject to dispensary inspections at the end of the reporting period	Under the dispensary supervision at the end of the reporting year
Hypertonic disease (all forms)	I10-I13; I20.X.7-I25.X.7;I60.X.7-I69.7	14895	8403	13105	13896	7516	13318	13639	7084	13110
including: hypertonic disease (without mention of ICD and vascular lesions)	I10-I13	2615	1112	1411	2373	1159	1498	1730	677	1466
Secondary hypertension	I15	55	10	0	41	20	0	42	9	0
Congestive heart disease	I20- I25	14154	8666	14720	13219	7503	14988	13412	7319	14811
including hypertonic disease	I207-I257	11655	7181	11512	11133	6256	11653	11369	6252	11503
acute and repeated myocardial infarction	I21-I22	31	10	0	40	7	0	22	5	0
including majestic (transmural)	I21.0-3,I22.0,1,8	4	3	0	16	2	0	2	1	0
cerebrovascular disease	I60- I69	5385	433	1961	4265	494	1695	4405	463	1680
of them with hypertonic disease	I60.7-I69.7	625	110	182	390	101	167	540	155	141
insults (all forms)	I60- I64	369	54	111	282	53	77	314	35	76
of them with hypertonia	I60.7-I64.7	252	37	108	207	36	74	241	31	73

Table II. Complications of hypertonic disease 2015-2017

№	Nosology	2015	2016	2017
1	Patients with hypertension (arterial hypertension) "D" accounting	1273	1484	1393
2	Examined during the reporting period	858	1238	892
3	Coronary heart disease with HD	11238	11308	10818
4	Examined during the reporting period	8542	9459	7235
5	Post-infarction cardiosclerosis with HD	No data	480	365
6	Examined during the reporting period	No data	418	257
7	Cerebrovascular disease with HD	1691	1544	1529
8	Examined during the reporting period	1021	932	923
9	Amount of patients with AH who had complications of hypertensive crisis	No data	No data	No data
10	Number of patients who had complications Total acute violations of cerebrovascular circulation with hypertension including hemorrhagic stroke	139/11	91/8	98/7
11	Amount of patients with complications of myocardial infarction Total / including with HD	80/79	63/62	68/68

"Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs during 2015-2017 years at the dispensary record was 1273; 1484; 139 patients who had a diagnosis of hypertonic disease (arterial hypertension) without concomitant pathology.

The high percentage of patients surveyed according to the records that were on the "D" accounting at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs, which is 67.4% in 2015, 83.42% in 2016 and 64.03% in 2017, of patients with hypertension and coronary heart disease with hypertension 76% in 2015, 83.64% in 2016, 66.87% in 2017, suggests proper dispensary control of this group of patients. However, as can be seen from the data of the record of complications, the course of hypertension in the form of hypertensive crisis in patients was not conducted.

In the analysis of complications, the course of hypertension in the form of hypertensive crisis, in patients with hypertension 1 st. and hypertonic disease of the 2-nd, which are on the "D" accounting at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs, found that such complications by district therapists during 2015-2017 years recorded a small amount.

Thus, out of 150 patients, only 15 (10%) were diagnosed with complications. One complication for 3 years was recorded in 12 patients (8%), two complications per year were recorded in 1 patient (0.6%), three complications were observed, also in 1 patient (0.6%): one in 2015 and two in 2016, and 5 complications in one patient (0.6%): two in 2015, one in 2016, two in 2017. It is noteworthy that the patients received preventive measures (according to doctors) is very small. Or once, or not at all. 83 patients did not receive the preventive measures, which is 55.33%. Three patients did not permanently receive drugs.

In 2017, in accordance with the regulatory documents on conducting medical examinations of the population, the plan for the provision of preventive medical examination services was approved at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs. The result of the analysis of services of preventive medical examination received and calculated using the database management program of the multidisciplinary medical institution ESCULAP. In analyzing the provision of preventive medical care services (data obtained only in 2017), it is clear that there is no accurate monitoring of the prophylactic process. The indicative plan for monitoring a patient from primary, secondary (specialized) and tertiary (highly specialized) medical care providers according to the unified protocol is presented in Table III.

According to the local protocol, Annex 4, which regulates the actions of the district therapist, regarding the dispensary supervision of patients at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs, the cost of dispensary supervision is 980.84. (Table IV).

The calculation was made in accordance with the price list of the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs, which is calculated in accordance with "Methodology for calculating the cost of providing medical services in state and communal health care facilities" in 2015.

The treatment of all patients was carried out in accordance with a local protocol, which was developed on the basis of unified clinical protocols of the Ministry of Health - "Local protocol of medical aid" Arterial hypertension 110-115", approved in the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs December 11, 2015.

The calculation of the cost of treatment and examination was calculated according to the protocol.

Table III. Tentative patient monitoring plan (according to the unified clinical protocol of primary, secondary (specialized) and tertiary (highly specialized) medical aid)

Name of the examination	Multiplicity	Cost (UAH)
Clinical examination	1 time per year if necessary	178,72
General blood test	1 time per year	15+10
General urine test	1 time per year	28,00
Glycemia on an empty stomach	1 time per year	27,00
ZHS	1 time per year	64,36
Creatinine	1 time per year	129,00
Electrocardiography	1 time per year if necessary	131,40
Conduct a risk assessment for SCORE	At initial treatment for patients with low and moderate risk - 1 time per 5 years	Not calculated
		Total: 583.48 UAH

Table IV. Annex 4. Plan of dispensary supervision of patients with arterial hypertension by a district therapist

Name of the examination	Multiplicity	Cost (UAH)
Clinical examination*	1 time per year if necessary	178,72
General blood test	1 time per year	15+10
General urine test	1 time per year	28,00
Glycemia on an empty stomach	1 time per year	27,00
ZHS	1 time per year	64,36
Creatinine	1 time per year	129,00
ALT	1 time per year	60,42
AST	1 time per year	60,35
Electrocardiography	1 time per year if necessary	131,40
Consultation of the cardiologist	Patients with high and very high risk – 1 time per year is obligatory, others - if necessary	276,59
		Total: UAH 980.84

* Clinical review includes:
1. Blood pressure measurement (PM)
2. Definition of Body Mass Index (MI)
3. Determination of waist circumference (WC)
4. Assessment of the status of tobacco smoke
5. Assessment of the level of physical activity
6. Assessment of compliance with the principles of healthy eating
7. Assessment of a heavily family history of cardiovascular disease (CVD) and diabetes mellitus (DM) - at the initial treatment
8. Information on planned therapy - a list of drugs, doses, frequency of admission and notes on the administration of prescribed drugs by the patient

According to the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs, the cost of treatment for arterial hypertension under the supervision of a physician and a cardiologist is 1,366.7 UAH. If an emergency doctor leaves for the challenge of complications of hypertension - the cost is increased by 814.42 UAH.

Medicinal cost of treatment for arterial hypertension, according to the local protocol is presented in table V.

Treatment on stationary beds at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs costs according to table VI.

In 2017, 26 patients (28 patients were prescribed) with a diagnosis of arterial hypertension were treated in a therapeutic hospital with therapeutic beds. The cost of treatment for one disease was, on average, 2497.54 UAH. The number of bed days from 1 to 11, on average, is 5.9 bed-days. 38 patients with the complication of hypertension were treated and 15 were re-treated. In total 53 cases. The average cost of treatment was 2682.66 UAH. The average bed day was 4.97 days. With the aggravation of hypertension, 23 patients were treated; the average cost of treatment was 2430.41 UAH. In analyzing the implementation of the plan of preventive actions in these patients, it was found that in any case they were not fulfilled in 100%.

Table V. Medicinal cost of treatment for arterial hypertension, according to a local protocol

№	List of medicines	Cost (UAH)		
		Total	Website of Ministry of Healthcare	Commercial price
	List of medicines (permanent)			
1	Bisoprolol 5mg №30 – 1t	0,42	12,54	
2	Enap 5mg №20 – 2t	3,3	33,06	
3	Konkor 5mg №50 -1t	3,09	154,44	
4	Nebilet 5mg №14 – 1t	6,71	93,96	
5	Koronal 5mg №30 -1t	0,77	23,24	
6	Arifon retard 1,5mg №30 -1t	3,24		97,14
7	Lozap 50mg №90 -1t	2,25	202,65	
8	Prestarium 5mg №30 – 1t	4,76		142,94
9	Lerkamen 10mg №60 -1t	4,12	247,3	
10	Berliprul 10mg №30 – 2t	3,26	49,14	
11	Amlodupin 5mg №30 – 1t	0,56	16,67	
12	Bi-pretarium 5/5 №30 – 1t	4,64		139,26
13	Azumex 5mg №30 – 1t	2,58	82,71	
14	Truplixam 10/2,5/10 №30 – 1t	7,35		220,53
15	Aspirin 100mg №28 – 1t	2,3	64,35	
16	Ramipril 5mg №30 – 1t	11,3		339
17	Deokor 160mg №30 – 1t	3,31	99,15	
18	Agen 5 tabl.5mg №30 – 1t	1,65		49,63
19	Korvitol 100mg №50 – 2t	4,74	118,45	
20	Zanidip 10mg №28 – 1t	4,74		132,95
21	Ko-renitek 20mg+12,5 №28 – 1t	3,9	109,05	
22	Rozykard 10mg №30 – 1t	2,13		127,69
23	Vazar 80mg №30 – 1t	3,29		98,56
24	Torvakard 10mg №90 – 1t	3,17	285,46	
25	Arifam 1,5 №30 – 1t	4,19		125,37
26	Koriol 6,25mg №28 – 1t	5,42	151,82	
27	Atoris 10mg №30 – 1t	6,38	191,35	
28	Ekvator 20mg+5mg №30 – 1t	5,72		171,71
29	Valsartan 80mg №80 – 1t	2,79		222,93
30	Krestor 5mg №28 – 1t	6,58	184,28	
31	Hipotel 40mg №28 – 1t	3,05	85,29	
		121,72	***4072,62***	
№	List of medicines	Cost (UAH)		
		Total	Website of Ministry of Healthcare	Commercial price
	List of medicines (course of treatment)			
1	Tiatriazolin 25mg/ml d/13ah. 2ml №10	219,78	109,89	
2	Predyktal MR 35mg №60	166,9		166,9
3	Trudyktal 20mg №60	91,83	91,83	
4	Venosmin 500mg №60	327	327	
5	Tivormin 42mg/ml d/in. 100ml	99,07	99,07	
6	Advokard №30	131,74		65,87
7	Magnium sulfat 25mg/13ahfor.5ml №10	15,16	15,16	
8	Dimedrol d/in.1% amp. 1ml №10	17,3	17,3	
9	Analgin 500mg d/in. 2ml №10	28,3	28,3	
10	Vestibo 24mg №60	198	198	
11	Sermion 10mg №50	3403,84	30,44	
12	Noobyt 0,25 №20	184,49	131,78	
13	Kratal №60	168,3	168,3	
14	Glicused 100mg №50	58,06	48,4	
15	Klopidogrel 75mg №30	67,45	67,45	
16	Mexukor 100mg №20	745,11		82,79
17	Lekoptin 40mg №50	53,05	44,21	
		5975,38	***1762,69***	
№	List of medicines (for HC)	Cost (UAH)		
		Total	Website of Ministry of Healthcare	Commercial price
1	Kaptopres №20 – 1t	2	40,04	
2	Anaprulin 10mg tabl. №50 – 1t	0,8	39,75	
3	Advokard №30	131,74		65,87
		134,54	***145,66***	

Table VI. Price for treatment on the stationary bed of a day stay at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs

№	Name of the service	Index	Price (UAH)	VAT	Price with VAT	Course of treatement	Total price
1	Single room in a therapeutic hospital with infarct beds	Bed-day	144,17	28,83	173,00	10 days	1730,00
2	Single room in a therapeutic hospital with infarct beds (12 hours)	Bed-day	97,50	19,50	117,00	10 days	1170,00
3	Single room in a therapeutic hospital with infarct beds (6 hours)	Bed-day	72,50	14,50	87,00	10 days	870,00

Table VII. Calculation of the cost of emergency medical aid

№	Ambulance	Name	Service/ procedure	Price (UAH)	VAT	Price with VAT (UAH)
1	Ambulance – 1	Servicing patients with brigade of Ambulance (1 doctor, 2 junior specialists with medical education)	Service	678,68	135,74	814,42
2	Ambulance – 2	Medical services in transportation of patients	Service	242,76	48,55	291,31
3	Ambulance – 3	Measurement of the level of glucose in the capillary blood at home by the brigade of Ambulance	Procedure	91,46	18,29	109,75
4	Ambulance – 4	Determination of cardiomarriers of myocardial damage (troponin) at home by the brigade of Ambulance	Procedure	171,19	34,24	205,43

An analysis of the cost of treatment for patients with myocardial infarction on inpatient beds revealed the average cost of treatment case of 4413.97 UAH. The cost of treatment of acute cerebrovascular accident on the stationary bed was on average 5077.3 UAH.

When calculating the cost of treatment for complications of hypertension, hemorrhagic stroke and acute myocardial infarction treated permanently, the calculation of the cost of emergency medical care should be added table VII.

The salaries of employees in calculating the cost of treatment for arterial hypertension and its complications were not separately calculated, as the calculations were carried out using the database management program of the multidisciplinary medical institution "Esculap", where the salary was taken into account.

The cost of examinations, studies and consultations of patients with arterial hypertension and its complications calculated according to the approved method at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs and calculated by the program Esculap are presented in tables VIII and IX.

DISCUSSION

The calculation of the economic effect should be made by comparing the cost of treatment of nosology with complications and without the absence of preventive measures, and the cost of treatment of nosology with complications and without the use of preventive measures. To determine the cost and economic effect of treating arterial hypertension with complications and without using a multifactorial model of prevention, a form of calculation has been developed and proposed in accordance with table X.

According to the proposed methodology:

1. Fill in the data table.
2. Calculate the cost of treating all cases of hypertension; multiplying the column 2x3, the result is entered in column 4.
3. Make a prediction of reducing the number of complications for each group of patients and find the cost of treatment according to the same algorithm.
4. Find the difference between the estimated cost of treatment and the actual one.

The result will be the economic effect of implementing a multifactorial model of prevention.

Taking into account that the concept of economic efficiency of prevention of chronic non-infectious diseases is complex and is characterized by three aspects:

1. Medical efficiency;
2. Social efficiency;
3. Economic efficiency

To determine efficiency, as such, it is possible to propose the following:

to determine **the medical efficiency** by the duration of treatment (the faster the patient will recover, the greater the

Table VIII. Assessment of diseases

№	Services	2015		2016		2017	
		Amount	Price (UAH)	Amount	Price (UAH)	Amount	Price (UAH)
	Laboratory						
1	Total blood analysis on a hematologic analyzer (18 indicators)	1762	38764	1940	42680	1804	39688
2	Total urine analysis	1762	52860	1940	58200	1804	54120
3	Blood analysis on sugar (venous blood)	1762	46693	1940	51410	1804	47806
4	Determination of total cholesterol	1762	47574	1940	52380	1804	48708
5	Determination of triglycerides	1762	53741	1940	59170	1804	55022
6	Determination of creatinine	1762	47574	1940	52380	1804	48708
	Total of the mentioned group	10572	287206	11640	316220	10824	294052
	Research						
7	Electrocardiography (12)	881	96910	970	106700	902	99220
8	Doppler examination of the vessels of the neck in pulsed mode	881	258133	970	284210	902	264286
	Total of the mentioned group	1762	355043	1940	390910	1804	363506
	Consultations						
9	Repeated consultation of a Terapist of the Highest Category	1762	134793	1940	148410	1804	138006
10	Primary Consultation of a Terapist of the Highest Category	881	165628	970	182360	902	169576
	Total of the mentioned group	2643	300421	2910	330770	2706	307582
	Total Price	14977	942670	16490	1037900	15334	965140

effectiveness of the treatment), as well as by the monitoring the complications (the less complications, the greater the medical effectiveness of treatment and prevention measures;

to determine **the social effectiveness** of which is to prevent and reduce morbidity, reduce the number of premature deaths, increase life expectancy, improve the quality of medical care to be monitored (measured) by the following indicators:

- the level of morbidity of the population,
- average life expectancy,
- the magnitude of mortality (especially of children),
- the number of disabled and premature deaths.

Taking into account that indicators of social effectiveness are applied not to each individual patient (as indicators of medical efficiency), but to certain contingents of the population the magnitude of the social effect of certain medical and preventive measures can be determined by the formula:

Ec = AoTn – (A1+A2+…+An),

where

Ec - the value of the social effect, expressed in reducing the number of diseases for a certain period of time;

Ao - number of diseases in the base period (before conducting of treatment and preventive measures);

A1, A2 ..., An - the number of diseases for each period (for a period it is possible to take 1 year or more) carrying out of medical and preventive measures;

Tn - the number of periods of medical and preventive measures.

• **Economic efficiency** (the result of social and medical effectiveness) of preventive activities includes three components:

- reducing the cost of treatment;
- reduction of expenses for payment of disability letters;
- reduction of production losses (reduction of morbidity, premature mortality, disability).

The calculation of the economic effectiveness of treatment and prevention measures can be carried out according to the formula:

Ee = P1 – P2,

where

Ee - the economic efficiency of medical preventive measures in hryvnias;

P1 - losses from morbidity, mortality, disability, etc. for the period preceding the current (base period);

P2 - losses from morbidity, mortality, disability, etc. during the period of medical and preventive measures (current period).

The amount of savings per unit cost, as **the coefficient of economic efficiency**, can be calculated by the formula:

Ke = (P1-P2) : Rl,n

where

Ke - coefficient of economic efficiency;

RL,n - funds spent on medical and preventive measures.

Table IX. Assessment of diseases

№	Services	2015		2016		2017	
		Amount	Price (UAH)	Amount	Price (UAH)	Amount	Price (UAH)
	Laboratory						
1	Total blood analysis on a hematologic analyzer (18 indicators)	5284	116248	4872	107184	3524	77528
2	Total urine analysis	5284	158520	4872	146160	3524	105720
3	Blood analysis on sugar (venous blood)	5284	140026	4872	129108	3524	93386
4	Determination of total cholesterol	5284	142668	4872	131544	3524	95148
5	Determination of triglycerides	5284	161162	4872	148596	3524	107482
6	Determination of creatinine	5284	142668	4872	131544	3524	95148
	Total of the mentioned group	31704	861292	29232	794136	21144	574412
	Research						
7	Electrocardiography (12)	2642	290620	2436	267960	1762	193820
8	Echocardiography with Doppler analysis	339	112548	199	66068	137	45484
9	Doppler examination of the vessels of the neck in pulsed mode	2642	774106	2436	713748	1762	516266
	Total of the mentioned group	5623	1177274	5071	1047776	3661	755570
	Consultations						
10	Primary consultation of the endocrinologist of the Highest Category	0	0	1	161	2	322
11	Repeated consultation of a Terapist of the Highest Category	5284	404226	4872	372708	3524	269586
12	Primary Consultation of a cardiologist of the highest category + Electrocardiography	1623	449571	1291	357607	685	189745
13	Primary Consultation of a Terapist of the Highest Category	2642	496696	2436	457968	1762	331256
	Total of the mentioned group	9549	1350493	8600	118844	5973	790909
	Total Price	46876	3389059	42903	3030356	30778	2120891

CONCLUSIONS

According to the analysis, the cost of preventive measures in the form of a complex of medical and organizational measures for the prevention of cardiovascular disease in a health facility (HF) at the level of primary care, according to the unified clinical protocol of the primary, secondary (specialized) and tertiary (highly specialized) medical treatment, which is calculated according to «Methodology for calculating the cost of providing medical services in state and communal health care institutions», is 1 082.76 UAH. The complex of preventive measures at the level of secondary and tertiary medical care is 1014.2 UAH. An analysis of the indicative plan for monitoring the patient from primary health care physicians (according to the unified clinical protocol of primary, secondary (specialized) and tertiary (highly specialized) medical care), according to the calculations according to the given method, allows to reduce the cost to 583,48 UAH, at the State Scientific Institution "Scientific-Practical Center for Prophylactic and Clinical Medicine" of the State Department of Affairs - 980,84 UAH.

Comparing the cost of preventive measures with direct costs for the treatment of the disease and its complications, it can be concluded that direct costs for treatment are significantly higher than for preventing the disease or preventing complications.

The effectiveness of medical care and prevention, as well as the improvement of the health of the population, depend on the observance of medical technologies, including compliance with the standards of examination and treatment of patients with cardiovascular pathology. The improvement of the quality of medical care, based on the analysis of cost minimization and economic forecast, is facilitated by the organizational and methodological methods employed among physicians.

Taking into account that the scientific analysis of preventive actions is based on the efficiency and cost, according to the given research it is possible to recommend dynamic observation (monitoring) for the patients of the «intervention group». Control over

Table X. Final table of cost of treatment (UAH) of arterial hypertension with complications and without the complications with the use of multi-factor model of prevention

№	Nosology	Cost of treatment of hypertension with and without complications in the absence of application of the model of multifactorial prevention			Cost of treatment of hypertension with and without complications in applying multivariate prophylaxis model (prognosis value)		
		Cost of treatment is one case	Number of cases of diseases	Total treatment costs (2x3)	Number of cases of diseases	Total treatment costs (2x3)	Difference in the cost of treatment (4-6)
0	1	2	3	4	5	6	7
1	AH without complications						
2	AH with complications (HC)						
3	Hypertensive Disease + Acute cerebrovascular accident including hemorrhagic stroke						
4	Hypertensive Disease + myocardial infarction						

the physical and emotional state of the patient - the final indicators of reducing the number of complications, reducing the number of episodes of pain; intermediate results and indicators - the number of crises, the number of episodes of pain, the progress of angiopathy and other complications. Monitoring results that may be related to the quality of life of patients should also include the ability to work well, the ability to self-service. If several results or side effects of treatment are achieved, it is economically necessary to evaluate all of them, both positive and negative. The indicators that are «inconceivable» for the patient should be monitored and appropriately assessed - the results of the analyzes on an example of hypertension (arterial hypertension), are cholesterol, lipidograms, and other indicators. The choice of indicators and evaluation criteria in each case should depend on the goals.

The second group of achievement should aim at monitoring the activities of the medical institution. Indicators - the time of service, procedures; the number of patients who receive a service or procedure for a specific time, the number of patients covered by the specified service or procedure at a certain time. Development and compilation of clinical guidelines or protocols to improve the coordination of doctors' work. Determining the priorities of providing medical care and prevention.

The standardization of methodological and organizational approaches in the assessment of preventive measures should be carried out on the basis of the study of indicators of the effectiveness, which characterize the level of achievement of goals and indicators of defects of activity, when the developed standards are not observed, which adversely affects the final results. To monitor the clinical efficacy and productivity of the treatment, diagnostic and preventive process, indicators reflecting the quality of health care should be used, the assessment of these indicators in the dynamics must be conducted quarterly and included in the annual report section of the economic efficiency of activities.

REFERENCES

1. Voronenko Yu., Paschenko V. Financial management with the basics of health economics: a teaching manual. Interregional Publishing Center "Medinform", 2011. p.499.
2. Babadzhanova A., Kadomtsev L. Adherence to the treatment of patients with arterial hypertension. Management of the health care institution. 2013;5:28
3. Kislyak O. A. Treatment of arterial hypertension in patients at high risk: the choice of an angiotensin-converting enzyme inhibitor. News of medicine and pharmacy. 2009;5-6 (272-273):14-16
4. Morozova T., Yudin I. Modern strategy of favoring treatment of patients with arterial hypertension: fixed combinations of drugs. Consilium Medicum. 1 (12):22-25
5. Zozulya A. I., Zozulya I. S. The problem of cerebrovascular diseases in Ukraine and the world and its perspectives. Works of sciences co. NMAPO named after Shupika. 2014; 23 (1):422
6. Dyachuk D. D., Yashchenko Yu. B., Melnyk N. Yu., Zabolotna I. E., Iskrenko I. M. Methodical recommendations for conducting medical and sociological supervision of obesity in children, 2017.
7. Palamar B. I., Gruzeva T. S. Criteria of economic effectiveness of preventive measures of chronic non-infectious disease. Wiad Lek. 2018;4:897-906

№ of the state registration 0114U002118. "Development of criteria for the economic evaluation of the effectiveness of multifactorial prevention of major non-communicable disease (NCD)".

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Borys Palamar
st. Mykola Krasnova 54/2,
03115, Kiev, Ukraine
tel: +380672387654
e-mail: palamar.bi@ukr.net

Received: 03.02.2019
Accepted: 04.06.2019

 © Wydawnictwo Aluna

PRACA POGLĄDOWA
REVIEW ARTICLE

NARAŻENIE CZŁOWIEKA NA BIOLOGICZNIE AKTYWNE HETEROCYKLICZNE AMINY AROMATYCZNE POWSTAJĄCE PODCZAS TERMICZNEJ OBRÓBKI ŻYWNOŚCI WYSOKOBIAŁKOWEJ

HUMAN EXPOSURE TO BIOLOGICALLY ACTIVE HETEROCYCLIC AROMATIC AMINES ARISING FROM THERMAL PROCESSING OF PROTEIN RICH FOOD

Beata Janoszka, Agnieszka Nowak, Magdalena Szumska, Ewa Śnieżek, Krystyna Tyrpień-Golder
KATEDRA I ZAKŁAD CHEMII, WYDZIAŁ LEKARSKI Z ODDZIAŁEM LEKARSKO DENTYSTYCZNYM W ZABRZU, ŚLĄSKI UNIWERSYTET MEDYCZNY W KATOWICACH, ZABRZE, POLSKA

STRESZCZENIE

Częste spożywanie termicznie przetwarzanego mięsa zostało zaliczone przez Międzynarodową Agencję Badań nad Rakiem do czynników bezpośrednio kancerogennych dla ludzi. Podczas obróbki termicznej żywności wysokobiałkowej tworzą się w niej mutagenne i kancerogenne, a także neurotoksyczne heterocykliczne aminy aromatyczne. Badania epidemiologiczne potwierdzają, że narażenie na niektóre z tych związków może zwiększyć ryzyko wystąpienia u człowieka zmian nowotworowych, zwłaszcza jelita grubego. Najwięcej amin heterocyklicznych zawierają produkty mięsne smażone i grillowane, a najmniej – potrawy gotowane oraz o słabym stopniu spieczenia. Zastosowanie przypraw i dodatków roślinnych o właściwościach przeciwutleniających pozwala uzyskać dania o zmniejszonej zawartości tych ksenobiotyków. Można ograniczyć narażenie organizmu człowieka na aminy heterocykliczne poprzez równoczesne spożywanie wraz z potrawami mięsnymi warzyw z rodziny roślin krzyżowych oraz produktów zwierających błonnik. Uwalniane z roślin krzyżowych izotiocyjaniany mogą hamować procesy aktywacji metabolicznej amin heterocyklicznych, natomiast błonnik adsorbuje cząsteczki tych związków w przewodzie pokarmowym. Uświadomienie społeczeństwa o powstawaniu podczas intensywnej obróbki cieplnej żywności wysokobiałkowej mutagennych i kancerogennych związków, w tym heterocyklicznych amin aromatycznych, jak również upowszechnienie wiedzy na temat warunków przyrządzania potraw o obniżonej zawartości takich związków mogłoby stać się jednym z elementów profilaktyki nowotworów w Polsce.

SŁOWA KLUCZOWE: heterocykliczne aminy aromatyczne, produkty mięsne, narażenie z diety, metabolizm, nowotwór

ABSTRACT

Frequent consumption of thermally processed meat was classified by the International Agency for Research on Cancer to directly carcinogenic agents for humans. During the heat treatment of high protein food, mutagenic and carcinogenic, as well as neurotoxic heterocyclic aromatic amines are formed. Epidemiological studies confirm that exposure to some of these compounds may increase the risk of cancer in humans, especially the colon cancer. Most heterocyclic amines contain fried and grilled meat products, and the lowest content of these compounds can be found in boiled and slightly baked dishes. The use of spices and vegetable additives with antioxidant properties allows to obtain dishes with reduced content of these xenobiotics. An effective way to reduce human exposure to cancerogenic amines may be simultaneous consumption, together with meat dishes, products containing fiber which can adsorb molecules of heterocyclic amines in the gastrointestinal tract, as well as enrichment of the diet in the crucifers plants, as isothiocyanates released from them can inhibit the metabolic activation processes of heterocyclic amines. Raising the public awareness of the formation of mutagenic and carcinogenic compounds, including heterocyclic aromatic amines, during the intensive heat treatment of high protein food, as well as the dissemination of knowledge on the conditions regarding the preparation of dishes with reduced content of such compounds could become one of the components of cancer prevention programs in Poland.

KEY WORDS: heterocyclic aromatic amines, meat products, dietary exposure, metabolism, tumors

Wiad Lek 2019, 72, 8, 1542-1550

WSTĘP

Badania epidemiologiczne wskazują na istotny wpływ diety na stan zdrowia człowieka i jego podatność na choroby cywilizacyjne, w tym nowotworowe i neurodegeneracyjne [1, 2]. Do głównych czynników wzrostu częstości zachorowań na nowotwory zalicza się dietę obfitą w produkty wysokobiałkowe [3–5]. W 2015 roku spożywanie przetworów mięsnych, między innymi konserwowanych, wędzonych i poddanych obróbce termicznej, zostało zaliczone przez Międzynarodową Agencję Badań nad Rakiem (IARC – *International Agency for Research on Cancer*) do 1 grupy kancerogenności, czyli do czynników bezpośrednio kancerogennych dla człowieka, a częste spożywanie czerwonego mięsa – do grupy 2A, czyli do czynników prawdopodobnie kancerogennych [6].

Procesy przetwarzania mięsa, takie jak konserwowanie i obróbka termiczna, przyczyniają się do zwiększenia jego trwałości i poprawy walorów smakowych, jednak prowadzą również do powstania związków o działaniu rakotwórczym, między innymi nitrozoamin i wielopierścieniowych węglowodorów aromatycznych (WWA) [6]. Badania rozpoczęte w latach 70. ubiegłego wieku wykazały, że w produktach o dużej zawartości białka poddawanych procesom termicznym, takim jak: gotowanie, pieczenie, wędzenie, smażenie i grillowanie, tworzą się mutagenne i kancerogenne związki z grupy heterocyklicznych amin aromatycznych (HAA) [7-10]. Niektóre z nich wykazują również aktywność neurotoksyczną [2].

CEL PRACY

Celem pracy jest przedstawienie − na podstawie przeglądu piśmiennictwa − danych na temat stopnia narażenia organizmu człowieka na mutagenne i kancerogenne związki z grupy heterocyklicznych amin aromatycznych, ich aktywności biologicznej oraz wyników najnowszych badań epidemiologicznych dotyczących poszukiwania zależności pomiędzy dietą a rozwojem nowotworów u człowieka. Omówione zostaną ponadto warunki powstawania HAA i możliwości ograniczenia ich syntezy w żywności, a także składniki diety pozwalające zminimalizować wchłanianie tych związków w przewodzie pokarmowym.

DYSKUSJA

KLASYFIKACJA HETEROCYKLICZNYCH AMIN AROMATYCZNYCH ORAZ REAKCJE ICH POWSTAWANIA

Heterocykliczne aminy aromatyczne powstające w żywności wysokobiałkowej są organicznymi związkami azotu zbudowanymi z 2 lub 3 skondensowanych pierścieni, z których jeden jest aromatyczny, a pozostałe heterocykliczne. Za wyjątkiem trzech związków z tej grupy, wszystkie posiadają egzocykliczną grupę aminową ($-NH_2$) [8, 9].

HAA dzieli się na polarne, które tworzą się w temperaturze od 100°C do 250°C oraz niepolarne, powstające w wyższych temperaturach. W tabeli I przedstawiono podział, nazwy, skróty nazw oraz struktury amin najczęściej oznaczanych w żywności.

Heterocykliczne aminy aromatyczne zaliczane do związków polarnych powstają pod wpływem wysokiej temperatury, zgodnie z mechanizmem reakcji Maillarda, z naturalnie występujących w żywności α-aminokwasów, cukrów (glukozy, fruktozy i rybozy) oraz kreatyny [8−10]. Niepolarne HAA tworzą się głównie w wyniku rozkładu termicznego tryptofanu i kwasu glutaminowego [10]. Cząsteczka tryptofanu zawiera strukturę heterocykliczną indolu występującą w wielu niepolarnych HAA, w tym w norharmanie i harmanie [9, 10].

CZYNNIKI WPŁYWAJĄCE NA TWORZENIE SIĘ HAA W ŻYWNOŚCI WYSOKOBIAŁKOWEJ PODDAWANEJ OBRÓBCE TERMICZNEJ I SPOSOBY OGRANICZENIA ICH SYNTEZY

Rodzaj i stężenie heterocyklicznych amin aromatycznych powstających w żywności zależą od wielu czynników, przede wszystkim jednak od wysokości temperatury, czasu ogrzewania i sposobu obróbki cieplnej. Zależą też od rodzaju mięsa i zawartości w nim aminokwasów, cukrów i kreatyny, a także od rodzaju tłuszczów i dodatków użytych do przygotowania potrawy [8−12]. Heterocykliczne aminy aromatyczne tworzą się już po kilku minutach od chwili rozpoczęcia smażenia mięsa i występują nawet w daniach o nieznacznym stopniu spieczenia [8−11]. Istotny wpływ na tworzenie się tych związków ma również kształt, wielkość i grubość porcji mięsa, od których zależy intensywność ogrzewania potrawy [8−10]. Częste odwracanie porcji mięsa i jej krótkotrwałe smażenie z każdej strony pozwala uzyskać produkt o mniejszej zawartości amin heterocyklicznych niż w porcjach rzadko odwracanych, o dużej powierzchni kontaktu z źródłem ciepła. Najwięcej HAA tworzy się w potrawach smażonych oraz grillowanych, w których mięso ma bezpośredni kontakt z płomieniem [8−12]. Niewielkie ilości amin heterocyklicznych powstają natomiast w daniach mięsnych gotowanych lub duszonych, szczególnie w żywności gotowanej w szybkowarze [9].

Skutecznym sposobem obniżenia wydajności procesu syntezy HAA może być stosowanie dodatków do żywności o właściwościach przeciwutleniających, zawierających między innymi flawonoidy oraz witaminy C i E [10, 13]. Ich naturalnym źródłem są liczne przyprawy i dodatki do mięs, na przykład cebula, czosnek, papryka, pieprz, zioła, ekstrakty owocowe, wino i piwo [9, 10, 13]. Hamujący wpływ tych dodatków polega prawdopodobnie na dezaktywacji rodników, będących pośrednimi produktami syntezy amin heterocyklicznych w reakcji Maillarda.

ZAWARTOŚĆ HAA W POTRAWACH MIĘSNYCH SPORZĄDZONYCH WEDŁUG POLSKICH PRZEPISÓW KULINARNYCH

Badania dotyczące heterocyklicznych amin aromatycznych zainicjowane w Japonii [7, 14], a następnie prowadzone w wielu krajach Europy i w Stanach Zjednoczonych, doprowadziły do określenia struktury ponad 30 amin, których stężenia w różnych potrawach z mięsa i ryb wahają się od 0 do kilkudziesięciu ng w 1g produktu [9, 15, 16].

Dotychczas opublikowano nieliczne prace na temat oznaczania heterocyklicznych amin aromatycznych w daniach przygotowanych w Polsce [11−13]. Wynika z nich, że potrawy mięsne sporządzone w sposób często stosowany w polskich gospodarstwach domowych zawierają związki z grupy amin polarnych (IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP) i niepolarnych (α- i β-karboliny) na poziomie stężeń kilku ng/g produktu [11−13]. Najwięcej tych związków oznaczono w kotletach mielonych z mięsa wieprzowo-wołowego, smażonych na patelni. Całkowita zawartość pięciu wyżej wymienionych polarnych amin heterocyklicznych wynosiła w nich 77,4 ng/g. Potrawy z czerwonego mięsa (karczek i filety wieprzowe) grillowane na ruszcie ogrodowym opalanym węglem drzewnym zawierały około 50 ng tych związków w 1g produktu. Należy dodać, że stężenie polarnej aminy IQ, którą IARC

zaklasyfikowała do grupy kancerogenności 2A, wynosiło ponad 6 ng/g [11]. Znacznie mniejsze ilości amin heterocyklicznych zawierały potrawy sporządzone poprzez grillowanie w piekarniku elektrycznym, w którym ruszt umiejscowiony był nad mięsem (kotlety mielone: 12,3 ng/g; pieczeń ze schabu: 3,5 ng/g; karczek pieczony na warstwie soli: 2 ng/g), a także poprzez pieczenie i duszenie potraw z dodatkiem wody (zrazy z wołowiny: 1,9 ng/g; zrazy z wieprzowiny: 17,4 ng/g). Mniejsza była też zawartość amin w daniach z mięsa białego (grillowane piersi z drobiu: 16,2 ng/g; indyk pieczony: 11,3 ng/g) [11].

Badania polegające na porównaniu stężeń amin heterocyklicznych w daniach mięsnych sporządzonych bez dodatków oraz z dodatkiem popularnych w Polsce przypraw, cebuli lub czosnku, wykazały, że te bogate w przeciwutleniacze składniki roślinne wywierają hamujący wpływ na powstawanie HAA. Z kolei analiza próbek mięs i próbek tworzących się sosów dowiodła, że znaczna część amin (około 49–59% całkowitej ilości) powstających podczas smażenia mięsa przenika do sosu. Zaobserwowano również, że w obecności cebuli lub czosnku proces gromadzenia się amin w sosach zachodzi bardziej intensywnie [13].

AKTYWNOŚĆ MUTAGENNA I KANCEROGENNA HETEROCYKLICZNYCH AMIN AROMATYCZNYCH

Heterocykliczne aminy aromatyczne należą do jednych z najbardziej biologicznie aktywnych związków zidentyfikowanych dotychczas w żywności [9, 14]. Ich mutagenność oznaczona przy użyciu testu Amesa względem szczepów TA98 i TA100 bakterii *Salmonella typhimurium* (Tab. II) jest wielokrotnie wyższa od aktywności mutagennej benzo[a]pirenu, wielopierścieniowego węglowodoru aromatycznego, który IARC zaliczyła do związków rakotwórczych dla człowieka (grupa kancerogenności 1) [17].

W tabeli II nie uwzględniono harmanu i norharmanu, gdyż związki te nie wykazują mutagenności w teście Amesa. Mogą jednak nasilać aktywność innych amin, dlatego są określane jako związki „komutagenne" (z ang. *co-mutagen*) [14]. Badania wykazały, że harman i norharman reagują z aminami aromatycznymi stosowanymi między innymi jako barwniki (np. anilina) tworząc produkty, których aktywność względem szczepów bakterii *Salmonella typhimurium* TA98 jest bardzo wysoka. Przypuszcza się, że występujące w żywności związki z grupą aminową, takie jak aminy biogenne lub pozostałości leków weterynaryjnych również mogą nasilać mutagenne własności amin heterocyklicznych [14].

Długoterminowe badania na zwierzętach doświadczalnych, którym w pokarmie podawano aminy heterocykliczne wykazały, że związki te mogą indukować powstawanie nowotworów wątroby, żołądka, jelita cienkiego, okrężnicy, gruczołu sutkowego i gruczołu *Zymbala* oraz naczyń krwionośnych (Tab. II) [14]. Na podstawie zgromadzonych danych Międzynarodowa Agencja Badań nad Rakiem zaliczyła aminę IQ (Tab. I) do związków prawdopodobnie kancerogennych dla człowieka (grupa 2A) oraz kilka innych amin heterocyklicznych (wszystkie wymienione w Tabeli I, za wyjątkiem DiMeIQx i β-karbolin) do związków przypuszczalnie kancerogennych (grupa 2B) [18].

PRZEMIANY METABOLICZNE HAA

Przemiany metaboliczne zaabsorbowanych w jelitach heterocyklicznych amin aromatycznych prowadzą do ich bioaktywacji oraz detoksykacji. Aktywacja metaboliczna zachodzi przy udziale katalitycznym izoenzymów cytochromu P450 (głównie CYP1A2) wchodzącego w skład systemu oksydacyjno-redukującego S-9 ssaków [14, 19].

Schemat przemian metabolicznych tych związków na przykładzie MeAαC przedstawiono na rycinie 1 [20]. W początkowym etapie aktywacji egzocykliczna grupa aminowa ($-NH_2$) ulega N-hydroksylacji do hydroksyaminowej (-NHOH). Następnie przy udziale enzymów z grupy N-acetylotransferaz (NAT) i sulfotranferaz (SULT) powstają wysoce aktywne metabolity (estry i siarczany), które mogą łączyć się z deoksyguanozyną cząsteczek DNA lub z cząsteczkami białek, tworząc trwałe addukty [20, 21]. Addukty te mogą wywoływać błędy w replikacji DNA, co prowadzi do wystąpienia mutacji, a w dalszej kolejności do rozwoju nowotworu [19, 20]. Nowsze badania wykazały, że po oddysocjowaniu z metabolitu grupy acetylowej lub siarczanowej powstaje silnie reaktywny jon nitroniowy (z grupą ^{+}HN), również mogący utworzyć trwałe połączenia z deoksyguanozyną cząsteczki DNA [9, 21]. Addukty amin heterocyklicznych z DNA lub białkami zidentyfikowano w komórkach śluzówki jelita cienkiego, okrężnicy i odbytnicy, wątroby, trzustki, płuc, gruczołów sutkowych oraz prostaty u zwierząt doświadczalnych [21], a także w ludzkim materiale pooperacyjnym pochodzącym z okrężnicy, pęcherza moczowego, piersi i prostaty [22–24].

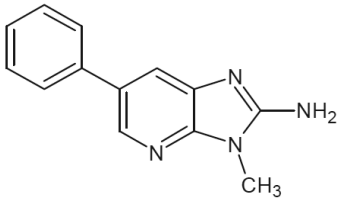
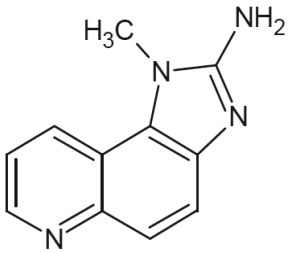
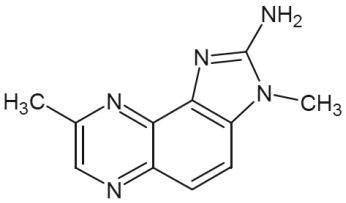
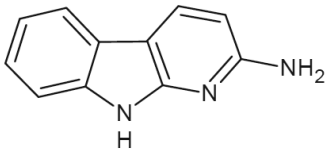
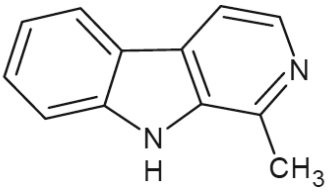
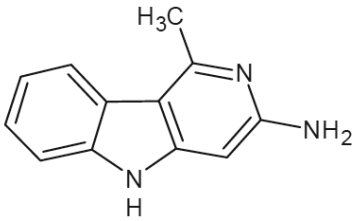
Równocześnie z aktywacją HAA zachodzi w wątrobie ich metaboliczna detoksykacja (Ryc. 1), która polega na utlenieniu, przy udziale izoenzymów cytochromu P450, pierścienia aromatycznego cząsteczki do pochodnych hydroksylowych, a następnie na katalizowanej przez enzymy (SULT i UDP) reakcji ich sprzęgania z grupami siarczanowymi i glukuronowymi. Możliwa jest też bezpośrednia N-glukuronidacja egzocyklicznej grupy aminowej. Powstałe w wyniku detoksykacji polarne metabolity zostają wydalone z organizmu wraz z żółcią lub z moczem [20].

Równowaga pomiędzy reakcjami bioaktywacji i detoksykacji oraz genetycznie uwarunkowane różnice w aktywności enzymów biorących udział w przemianach metabolicznych amin heterocyklicznych mogą mieć wpływ na ryzyko rozwoju zmian nowotworowych u ludzi. U osób, u których aminy ulegają szybkiej bioaktywacji do N-acetylowych pochodnych występuje większe ryzyko zachorowania na nowotwory jelita grubego [25, 26].

NEUROTOKSYCZNOŚĆ AMIN HETEROCYKLICZNYCH

Wyniki badań naukowych wskazują, że narażenie na niektóre ksenobiotyki pochodzące z żywności może być jednym z czynników wpływających na rozwój drżenia samoistnego oraz choroby Parkinsona [2, 27–29]. Do związków o właściwościach neurotoksycznych zalicza się aminy heterocykliczne z grupy γ-karbolin (Trp-P-1

Tabela I. Ogólna klasyfikacja heterocyklicznych amin aromatycznych i wzory wybranych związków występujących w termicznie przetwarzanej żywności wysokobiałkowej [9,10].

Nazwa związku (skrót nazwy)	Wzór*)
Związki polarne	
Pochodne imidazopirydyny 2-amino-1-metylo-6-fenyloimidazo[4,5-b]pirydyna (PhIP)	
Pochodne imidazochinoliny 2-amino-3-metyloimidazo[4,5-f]chinolina (IQ) 2-amino-3,4-dimetyloimidazo[4,5-f]chinolina (MeIQ)	
Pochodne imidazochinoksaliny 2-amino-3,8-dimetyloimidazo[4,5-f]chinoksalina (MeIQx) 2-amino-3,4,8-trimetyloimidazo[4,5-f]chinoksalina (DiMeIQx)	
Związki niepolarne	
Pochodne pirydoindoli: α-karboliny - 2-amino-9H-pirydo[2,3-b]indol (AαC) -2-amino-3-metylo-9H-pirydo[2,3-b]indol (MeAαC)	
β-karboliny - 1-metylo-9H-pirydo[3,4-b]indol (harman) - 9H-pirydo[3,4-b]indol (norharman)	
γ-karboliny - 3-amino-1-metylo-5H-pirydo[4,3-b]indol (Trp-P-2) - 3-amino-1,4-dimetylo-5H-pirydo[4,3-b]indol (Trp-P-1)	

*) w tabeli przedstawiono wzory związków, których skróty nazw podkreślono

Tabela II. Mutagenność wybranych heterocyklicznych amin aromatycznych i benzo(a)pirenu (B(a)P)*) względem szczepów bakterii *Salmonella typhimurium* oraz kancerogenne działanie względem zwierząt doświadczalnych [14, 17, 18].

Związek	Mutagenność (liczba rewertantów/ μg związku)		Kancerogenność		
	TA98	TA100	Testowane zwierzę	Stężenie w diecie [ppm]	Umiejscowienie nowotworu
IQ	433000	7000	szczury	300	wątroba, jelito cienkie i grube, skóra, gruczoł Zymbala
MeIQ	661000	30000	szczury	300	jelito cienkie, gruczoł Zymbala, skóra, jama gębowa, sutek
MeIQx	145000	14000	szczury	400	wątroba, gruczoł Zymbala, skóra
PhIP	1800	120	szczury	400	jelito grube, sutek, okrężnica
Trp-P-1	39000	1700	szczury	150	wątroba
Trp-P-2	104000	1800	myszy	200	wątroba
AαC	300	20	myszy	800	wątroba, naczynia krwionośne
MeAαC	200	120	myszy	800	wątroba, naczynia krwionośne
B(a)P*)	320	600	myszy i szczury	100	płuca, wątroba, skóra, sutek

*) B(a)P to kancerogenny wielopierścieniowy węglowodór aromatyczny

i Trp-P-2) oraz β-karbolin (harman i norharman). Poza termicznie przetwarzaną żywnością wysokobiałkową harman i norharman występują naturalnie w niektórych roślinach stosowanych w medycynie ludowej, a także w tytoniu i dymie papierosowym [2]. Karboliny pełnią rolę inhibitorów enzymu monoaminooksydazy. Inhibitor ten spowalnia metabolizm związków monoaminowych, m.in. serotoniny i dopaminy, powodując wzrost poziomu tych neuroprzekaźników w mózgu [2, 27]. Wysokie, w porównaniu do grupy kontrolnej, stężenia β-karbolin oraz ich metabolitów oznaczono we krwi obwodowej pacjentów, u których zdiagnozowano chorobę Parkinsona [2, 27] oraz drżenie samoistne [28]. Wyższe niż w próbkach kontrolnych stężenie harmanu oznaczono również w pośmiertnie pobranych próbkach tkanki nerwowej z kory móżdżku pochodzącej od chorych z drżeniem samoistnym [29]. Neurotoksyczna aktywność harmanu wynika prawdopodobnie z jego strukturalnego i funkcjonalnego podobieństwa do 1-metylo-4-fenylo-1,2,3,6-tetrahydropirydyny (MPTP), związku o udowodnionym wpływie na powstawanie choroby Parkinsona [2, 29].

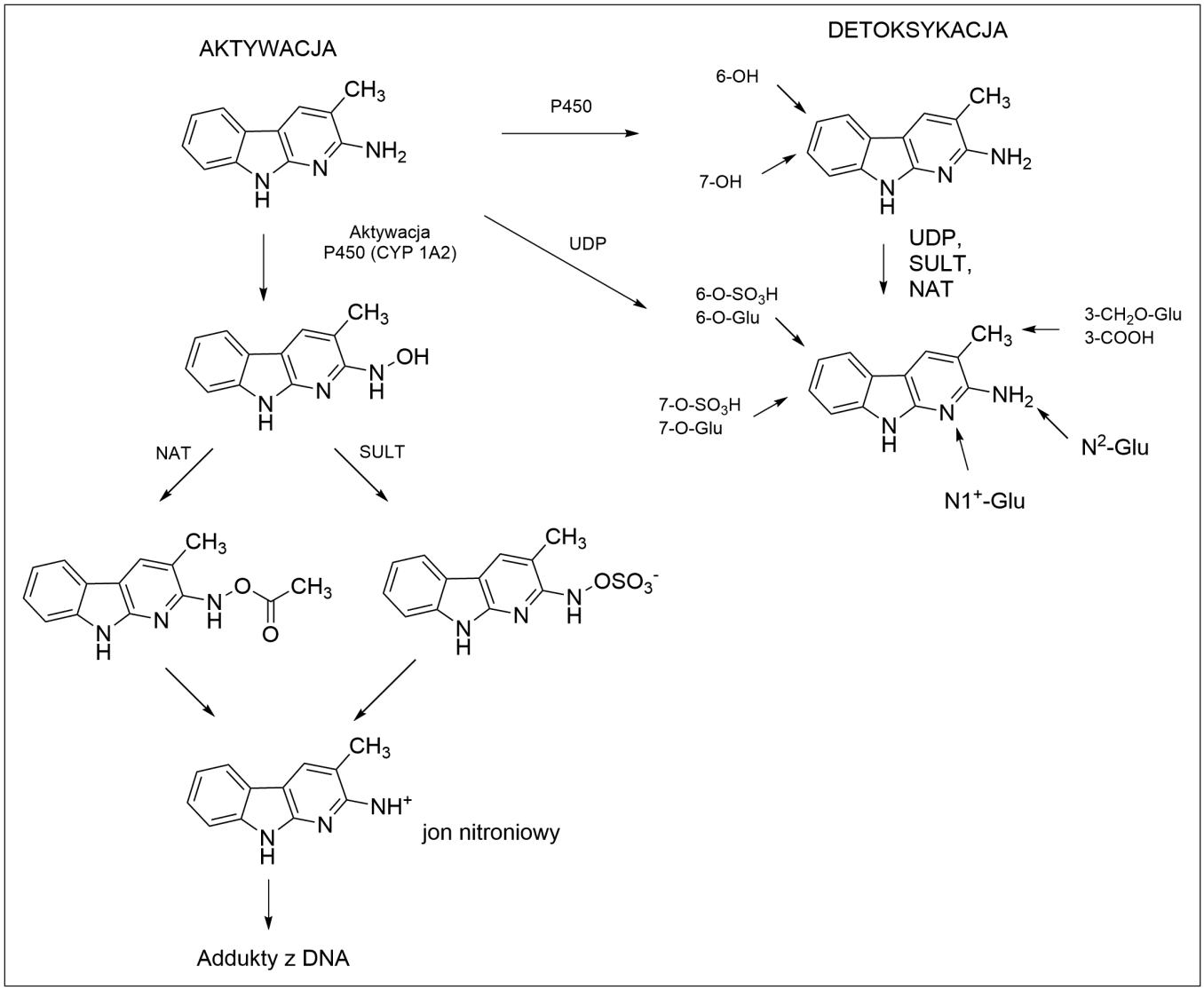
Badania *in vitro* dowodzą, że również polarna amina heterocykliczna PhIP oraz jej metabolit OH-NH-PhIP wykazują działanie toksyczne względem neuronów dopaminergicznych [2, 30].

OCENA STOPNIA NARAŻENIA CZŁOWIEKA NA HETEROCYKLICZNE AMINY AROMATYCZNE I WYNIKI BADAŃ EPIDEMIOLOGICZNYCH

Narażenie człowieka na HAA określane jest najczęściej poprzez ilość ng lub μg tych związków spożytych w ciągu doby [9, 15]. Wyniki badań przeprowadzonych w Europie (Szwecja, Szwajcaria, Niemcy, Hiszpania, Chorwacja), Japonii, Chinach, Nowej Zelandii i Stanach Zjednoczonych wykazały, że narażenie to może wynosić od kilku ng do ponad 4350 ng HAA na dobę, przy czym najwyższe wartości dotyczyły osób zamieszkujących Stany Zjednoczone i Chorwację [9, 31, 32]. W badaniach przeprowadzonych w grupach liczących kilkaset do kilku tysięcy osób wykorzystywano ankiety zawierające szereg pytań dotyczących upodobań kulinarnych, sposobu obróbki termicznej potraw, a także częstości spożywania dań wysokobiałkowych oraz wielkości porcji. Do niektórych ankiet dołączono także fotografie potraw mięsnych o różnym stopniu spieczenia [15, 16]. Następnie, w oparciu o wyniki oznaczeń amin heterocyklicznych w produktach preferowanych w danym regionie, możliwa była ocena narażenia człowieka na ksenobiotyki z grupy HAA.

W Polsce badania takie nie zostały dotychczas przeprowadzone z uwagi na brak wystarczającej liczby danych o zawartości amin heterocyklicznych w potrawach (z mięsa i ryb) przygotowywanych w sposób tradycyjny dla kuchni polskiej. Obliczono jedynie, że poprzez spożycie 100-gramowej porcji mięsa przyrządzonego w warunkach domowych, człowiek może wprowadzić do organizmu od 0,2μg do 7,7μg tych związków. Podobny poziom narażenia – 7 μg/dobę – oznaczono w Szwecji, analizując zwyczaje żywieniowe mieszkańców tego kraju [15]. Trudno ocenić czy spożycie w ciągu doby kilku μg amin heterocyklicznych jest bezpieczne dla człowieka, gdyż dotychczas nigdzie na świecie nie ustalono poziomu dopuszczalnego narażenia na HAA.

Prace badawcze przeprowadzone w Stanach Zjednoczonych wykazały, że wynikające z diety narażenie na dwie aminy, MeIQx i PhIP, wynoszące średnio 570 ng/dzień (z czego 474 ng przypadało na PhIP) uwarunkowane było przede wszystkim częstym spożywaniem produktów smażonych i grillowanych, natomiast w niewielkim stopniu zależało od rodzaju mięsa czy ryb [32]. Badania *in vitro* z użyciem



Ryc. 1. Schemat przemian metabolicznych heterocyklicznych amin aromatycznych na przykładzie MeAαC, opracowany na podstawie [20]. Znaczenie skrótów:
SULT - sulfotransferazy;
NAT - acetylotransferazy;
UDP - glukuronylotransferazy;
Glu - grupa glukuronowa.

ludzkich komórek nabłonka okrężnicy dowiodły, że ilości amin, które wywołują nowotwór przy bezpośredniej aplikacji na komórki nabłonka są znacznie większe od wynikającego z diety narażenia organizmu człowieka na HAA. Po przeliczeniu, odpowiadają one ekspozycji na 938 μg MeIQx oraz 190 μg PhIP. Autorzy pracy [32] podkreślają jednak, że badania na hodowlach komórkowych pomijają procesy przemian metabolicznych HAA, a ponadto nie uwzględniają faktu, że treści pokarmowe mogące zawierać aminy heterocykliczne pozostają przez długi czas w kontakcie z dużą powierzchnią ścian jelita [32, 33]. Należy też dodać, że niektóre osoby, na przykład palące papierosy, mogą być dodatkowo narażone na aminy heterocykliczne, zwłaszcza na harman i norharman występujące naturalnie w tytoniu. Potwierdziły to badania przeprowadzone przez *National Health and Nutrition Examination Survey* (NHANES) na grupie kilku tysięcy kobiet i mężczyzn w wieku od 6 do 65 lat [34].

Celem badań epidemiologicznych dotyczących heterocyklicznych amin aromatycznych było poszukiwanie zależności pomiędzy spożywaniem termicznie przetwarzanych produktów wysokobiałkowych o znanej zawartości HAA, a występowaniem raka u osób objętych badaniami. Wykazały one, że związki z grupy polarnych heterocyklicznych amin aromatycznych spożywane z mięsem poddanym obróbce termicznej mogą zwiększać ryzyko wystąpienia u człowieka nowotworów jelita grubego (okrężnicy), żołądka, płuc, trzustki, piersi i prostaty [1, 4, 35].

Obszerny przegląd piśmiennictwa dotyczącego kilkudziesięciu badań epidemiologicznych przeprowadzonych na świecie w latach 1997–2015 oraz sporządzona na ich podstawie metaanaliza kilkudziesięciu tysięcy przypadków potwierdziła występowanie istotnej zależności pomiędzy rozwojem u człowieka gruczolaków jelita grubego a narażeniem na PhIP ($p<0,001$), MeIQx i DiMeIQx

(p=0,001). Badania wykazały również znamienną statystycznie zależność pomiędzy rozwojem raka jelita grubego u człowieka a narażeniem na MeIQx (p=0,004), DiMeIQx (p=0,014) oraz na „ogólną aktywność mutagenną żywności" (p>0,001). Warto podkreślić, że nie stwierdzono takiej zależności dla kancerogennego węglowodoru − benzo(a) pirenu [36].

Badania epidemiologiczne wykazały również występowanie dodatniej korelacji pomiędzy dietą charakterystyczną dla krajów wysokorozwiniętych a rozwojem nowotworów jelita grubego (p<0,0001), zwłaszcza dystalnego odcinka okrężnicy (p=0,0004) i odbytnicy (p=0,01). Dieta taka charakteryzuje się częstym spożywaniem węglowodanów i produktów mięsnych o znacznym stopniu spieczenia [5].

Szczegółowe omówienie licznych badań prowadzonych na świecie od lat 90. ubiegłego stulecia w zakresie poszukiwania zależności pomiędzy spożywaniem przetworzonego mięsa a ryzykiem zachorowania na raka zawiera monografia Międzynarodowej Agencji Badań na Rakiem [6]. Wynika z nich, że codzienne spożywanie 50-gramowej porcji termicznie przetwarzanego mięsa może zwiększyć ryzyko rozwoju raka jelita grubego o 18%.

CZYNNIKI WPŁYWAJĄCE NA OGRANICZENIE NARAŻENIA NA HETEROCYKLICZNE AMINY AROMATYCZNE

MODYFIKACJA SPOSOBÓW PRZYGOTOWANIA POTRAW WYSOKOBIAŁKOWYCH

Najprostszym sposobem ograniczenia narażenia na HAA jest świadoma zmiana przyzwyczajeń kulinarnych prowadząca do przygotowywania i spożywania dań o mniejszej zawartości amin, czyli potraw gotowanych, duszonych, smażonych w niższej temperaturze, o niewielkim stopniu spieczenia. Można również ograniczyć narażenie na HAA poprzez unikanie spożywania dużych ilości sosów [13]. Rozpowszechnienie wiedzy na temat warunków przyrządzania potraw wysokobiałkowych o obniżonej zawartości heterocyklicznych amin aromatycznych stworzyłoby możliwość ograniczenia narażenia współczesnego człowieka na te ksenobiotyki. W Szwajcarii już około 20 lat temu naukowcy upowszechnili wśród mieszkańców tego kraju wyniki swoich badań na temat HAA w żywności oraz przygotowali ulotki ze wskazówkami, w jaki sposób przyrządzać potrawy mięsne by ograniczyć w nich syntezę tych związków i zmniejszyć narażenie na nie organizmu człowieka [16].

W Polsce od wielu lat, zarówno wśród kobiet, jak i wśród mężczyzn, obserwowany jest systematyczny wzrost liczby zgonów spowodowanych przez nowotwory złośliwe jelita grubego [37]. Być może upowszechnienie wiedzy na temat czynników prowadzących do tworzenia się związków kancerogennych w żywności, podobnie jak to miało miejsce w Szwajcarii, mogłoby stać się jednym z elementów profilaktyki nowotworowej w Polsce i pozwoliłoby zahamować ten niekorzystny trend w naszym kraju.

WZBOGACENIE DIETY W SKŁADNIKI WPŁYWAJĄCE NA METABOLIZM HAA W ORGANIZMIE CZŁOWIEKA

Skutecznym sposobem ograniczenia narażenia na HAA może być zmniejszenie ich biodostępności poprzez równoczesne spożywanie wraz z przetworami mięsnymi składników adsorbujących w przewodzie pokarmowym cząsteczki amin heterocyklicznych. Własności takie wykazują produkty o dużej zawartości błonnika (np. ziemniaki i otręby pszenne) [9,10]. Ponadto dieta bogata w owoce, a także szpinak oraz rośliny krzyżowe (brokuły, kapusta włoska, kalafior) może chronić przed mutagennym działaniem heterocyklicznych amin aromatycznych poprzez aktywację enzymów cytochromu P450 biorących udział w procesie metabolicznej detoksykacji tych związków [9, 10, 38]. Badania przeprowadzone w grupie 146 osób stosujących dietę bogatą w produkty roślinne oraz w produkty zawierające heterocykliczne aminy aromatyczne wykazały, że zmiany w cząsteczkach DNA oznaczane poprzez stężenie 8-okso-2'-deoksyguanozyny (8-OHdG), były większe u osób deklarujących w ankiecie spożywanie niewielkiej ilości owoców i jarzyn oraz dużo termicznie przetworzonych produktów wysokobiałkowych [39]. Stosowanie do przygotowania potraw wysokobiałkowych składników pochodzenia roślinnego, na przykład cebuli, czosnku oraz przypraw pozwala uzyskać nie tylko poprawę ich smaku, ale i dodatkowe korzyści. Dodatki roślinne zawierają bowiem liczne składniki o działaniu prozdrowotnym i przeciwnowotworowym: makro- i mikroelementy, witaminy oraz związki polifenolowe. Badania *in vitro* wykazały, że flawonoidy − rutyna i kwercetyna, które występują na przykład w cebuli, redukują stres oksydacyjny wywołany przez heterocykliczne aminy aromatyczne (IQ i PhIP) w limfocytach pochodzących od osób zdrowych i osób z nowotworem jelita grubego [40].

PODSUMOWANIE

Częste spożywanie mięsa i ryb o znacznym stopniu termicznego przetworzenia stanowi dla człowieka źródło narażenia na ksenobiotyki z grupy heterocyklicznych amin aromatycznych. Wiele z tych związków wykazuje silną aktywność mutagenną i kancerogenną, a nawet neurotoksyczną. Poprzez stosowanie prostych zabiegów kulinarnych można zminimalizować syntezę heterocyklicznych amin w żywności. Wzbogacenie diety w produkty roślinne o dużej zawartości błonnika i składników przeciwutleniających pozwala z kolei ograniczyć ich wchłanianie w przewodzie pokarmowym oraz uaktywnić metaboliczne procesy detoksykacji muta- i kancerogennych amin. Zgodnie z aktualnymi zaleceniami żywieniowymi [41] warzywa i owoce powinny być podstawą diety człowieka, gdyż ich regularne spożywanie należy do istotnych czynników chroniących przed rozwojem wielu chorób cywilizacyjnych. Równocześnie zalecane jest ograniczenie spożycia mięsa, zwłaszcza czerwonego i jego przetworów, do ilości mniejszej niż 0,5 kg tygodniowo, ponieważ według Międzynarodowej Agencji Badań nad Rakiem większe

spożycie tych produktów prowadzi do wzrostu zachorowań na niektóre nowotwory [6, 41]. Popularyzacja wiedzy na temat narażenia człowieka na kancerogenne składniki diety oraz możliwości ograniczenia ich szkodliwego wpływu na organizm mogłaby stać się jednym z elementów profilaktyki nowotworowej w Polsce.

PIŚMIENNICTWO

1. Abid Z, Cross AJ, Sinha R. Meat, dairy, and cancer. Am J Clin Nutr. 2014;100(1):386S–393S. doi: 10.3945/ajcn.113.071597.
2. Agim ZS, Cannon JR. Dietary factors in the etiology of Parkinson's disease. Biomed Res Int. 2015;2015:672838. doi: 10.1155/2015/672838.
3. Boada LD, Henríquez-Hernández LA, Luzardo OP. The impact of red and processed meat consumption on cancer and other health outcomes: Epidemiological evidences. Food Chem Toxicol. 2016;92:236-244. doi: 10.1016/j.fct.2016.04.008.
4. Ananthakrishnan AN, Du M, Berndt SI, Brenner H et al. Red meat intake, NAT2, and risk of colorectal cancer: a pooled analysis of 11 studies. Cancer Epidemiol Biomarkers Prev. 2015;24(1):198-205. doi: 10.1158/1055-9965.EPI-14-0897.
5. Mehta RS, Song M, Nishihara R et al. Dietary patterns and risk of colorectal cancer: analysis by tumor location and molecular subtypes. Gastroenterology. 2017;152(8):1944-1953.e1. doi: 10.1053/j.gastro.2017.02.015.
6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2015: Lyon, France). Red meat and processed meat. Lyon: IARC Monographs No.114; 2018.
7. Nagao M, Honda M, Seino Y, Yahagi T, Sugimura T. Mutagenicities of smoke condensates and the charred surface of fish and meat. Cancer Lett. 1977;2(4-5):221-226.
8. Jägerstad M, Skog K, Arvidsson P, Solyakov A. Chemistry, formation and occurrence of genotoxic heterocyclic amines identified in model systems and cooked foods. Z Lebensm- Unters- Forsch. 1998;207(6):419-427. doi: 10.1007/s002170050355.
9. Gibis M. Heterocyclic aromatic amines in cooked meat products: causes, formation, occurrence, and risk assessment. Compr Rev Food Sci Food Saf. 2016;15(2):269-302. doi: 10.1111/1541-4337.12186.
10. Meurillon M, Engel E. Mitigation strategies to reduce the impact of heterocyclic aromatic amines in proteinaceous foods. Trends Food Sci Technol. 2016;50:70-84. doi.org/10.1016/j.tifs.2016.01.007
11. Warzecha L, Janoszka B, Błaszczyk U, Stróżyk M, Bodzek D, Dobosz C. Determination of heterocyclic aromatic amines (HAs) content in samples of household-prepared meat dishes. J. Chromatogr. B – Analyt Technol Biomed Life Sci. 2004;802(1-2):95-106.doi: 10.1016/j.jchromb.2003.09.027.
12. Waszkiewicz-Robak B, Szterk A, Kaczmarczuk P. Bezpieczeństwo zdrowotne mięsa wołowego poddanego obróbce termicznej. Postępy Techniki Przetwórstwa Spożywczego. 2014;1:69-76.
13. Janoszka B. Heterocyclic amines and azaarenes in pan-fried meat and its gravy fried without additivities and in the presence of onion and garlic. Food Chem. 2010;120:463-473. doi: 10.1016/j.foodchem.2009.10.039.
14. Sugimura T, Wakabayashi K, Nakagama H, Nagao M. Heterocyclic amines: mutagens /carcinogens produced during cooking of meat and fish. Cancer Sci. 2004;(95):290-299. doi; 10.1111/j.1349-7006.2004.tb03205.x.
15. Augustsson K, Skog K, Jägerstad M, Steineck G. Assessment of the human exposure to heterocyclic amines. Carcinogenesis. 1997;18(10):1931-1935. doi: 10.1093/carcin/18.10.1931.
16. Zimmerli B, Rhyn P, Zoller O, Schlatter J. Occurrence of heterocyclic aromatic amines in the Swiss diet: analytical method, exposure estimation and risk assessment. Food Addit Contam. 2001;18(6):533-551. doi: 10.1080/02652030119545.
17. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2005: Lyon, France). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. Lyon: IARC Monographs No. 92; 2010.
18. IARC Working Group on the Evaluation of Carcinogenic Risk to Humans (1993: Lyon, France). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. Lyon: IARC Monographs No. 56; 1993.
19. Turesky RJ, Le Marchand L. Metabolism and biomarkers of heterocyclic aromatic amines in molecular epidemiology studies: lessons learned from aromatic amines. Chem Res Toxicol. 2011;24(8):1169-1214. doi: 10.1021/tx200135s.
20. Frederiksen H. Two food-borne heterocyclic amines: metabolism and DNA adduct formation of amino-a-carbolines. Mol Nutr Food Res. 2005;49(3):263-273. doi: 10.1002/mnfr.200400061.
21. Turesky RJ, Konorev D, Fan X, Tang Y, Yao L, Ding X, Xie F, Zhu Y, Zhang QY. The effect of cytochrome P450 reductase deficiency on 2-amino-9H-pyrido[2,3-b]indole metabolism and DNA adduct formation in liver and extrahepatic tissues of mice. Chem Res Toxicol. 2015;28(12):2400-2410. doi: 10.1021/acs.chemrestox.5b00405.
22. Jonsson C, Stål P, Sjöqvist U, Akerlund JE, Löfberg R, Möller L. DNA adducts in normal colonic mucosa from healthy controls and patients with colon polyps and colorectal carcinomas. Mutagenesis. 2010;25(5):499-504. doi: 10.1093/mutage/geq033.
23. Guo J, Villalta PW, Weight CJ, Bonala R, Johnson F, Rosenquist TA, Turesky RJ. Targeted and untargeted detection of DNA adducts of aromatic amine carcinogens in human bladder by ultra-performance liquid chromatography-high-resolution mass spectrometry. Chem Res Toxicol. 2018;31(12):1382-1397. doi: 10.1021/acs.chemrestox.8b00268.
24. Xiao S, Guo J, Yun BH, Villalta PW, Krishna S, Tejpaul R, Murugan P, Weight CJ, Turesky RJ. Biomonitoring DNA adducts of cooked meat carcinogens in human prostate by nano liquid chromatography-high resolution tandem mass spectrometry: identification of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine DNA Adduct. Anal Chem. 2016;88(24):12508-12515. doi: 10.1021/acs.analchem.6b04157.
25. Kobayashi M, Otani T, Iwasaki M et al. Association between dietary heterocyclic amine levels, genetic polymorphisms of NAT2, CYP1A1, and CYP1A2 and risk of stomach cancer: a hospital-based case-control study in Japan. Gastric Cancer. 2009;12(4):198-205. doi: 10.1007/s10120-009-0523-x.
26. Cascella M, Bimonte S, Barbieri A et al. Dissecting the mechanisms and molecules underlying the potential carcinogenicity of red and processed meat in colorectal cancer (CRC): an overview on the current state of knowledge. Infect Agent Cancer. 2018;13(3). doi: 10.1186/s13027-018-0174-9
27. Louis ED, Michalec M, Jiang W, Factor-Litvak P, Zheng W. Elevated blood harmane (1-methyl-9H-pyrido[3,4-b]indole) concentrations in Parkinson's disease. Neurotoxicology. 2014;40:52-56. doi: 10.1016/j.neuro.2013.11.005.
28. Louis ED, Benito-León J, Moreno-García S et al. Blood harmane (1-methyl-9H-pyrido[3,4-b]indole) concentration in essential tremor cases in Spain. Neurotoxicology. 2013;34:264-268. doi: 10.1016/j.neuro.2012.09.00.
29. Louis ED1, Factor-Litvak P, Liu X, Vonsattel JP, Galecki M, Jiang W, Zheng W. Elevated brain harmane (1-methyl-9H-pyrido[3,4-b]indole) in essential tremor cases vs. controls. Neurotoxicology. 2013;38:131-135. doi: 10.1016/j.neuro.2013.07.002.

30. Griggs AM, Agim ZS, Mishra VR et al. 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is selectively toxic to primary dopaminergic neurons in vitro. Toxicol Sci. 2014;140(1):179-189. doi: 10.1093/toxsci/kfu060.
31. Klapec T, Periš, Quick estimation of dietary exposure to heterocyclic aromatic amines and acrylamide in a Croatian female population, J Food Nutr Res. 2014;53(3):251–259.
32. Pouzou JG, Costard S, Zagmutt FJ. Probabilistic assessment of dietary exposure to heterocyclic amines and polycyclic aromatic hydrocarbons from consumption of meats and breads in the United States. Food Chem Toxicol. 2018;114:361-374. doi: 10.1016/j.fct.2018.02.004.
33. Carthew P, DiNovi M, Setzer RW. Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic: example: CAS No: 105650-23-5 PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine). Food Chem Toxicol. 2010;48 Suppl 1:S98-105. doi: 10.1016/j.fct.2009.10.035.
34. Jain RB. Concentrations of selected heterocyclic aromatic amines among US population aged ≥ 6 years: data from NHANES 2013–2014. Environ Sci Pollut Res. 2018;25(20): 19859-19874. doi: 10.1007/s11356-018-2210-0.
35. Barbir A, Linseisen J, Hermann S, Kaaks R, Teucher B, Eichholzer M, Rohrmann S. Effects of phenotypes in heterocyclicaromatic amine (HCA) metabolism-relatedgenes on the association of HCA intake with the risk of colorectal adenomas. Cancer Causes Control. 2012;23(9):1429-1442. doi: 10.1007/s10552-012-0017-8.
36. Chiavarini M, Bertarelli G, Minelli L, Fabiani R. Dietary intake of meat cooking-related mutagens (HCAs) and risk of colorectal adenoma and cancer: a systematic review and meta-analysis. Nutrients. 2017;9(5):514; doi:10.3390/nu9050514.
37. http://onkologia.org.pl/nowotwory-zlosliwe-jelita-grubego-c18-21/ [Dostęp 20.05.2019]
38. Lewandowska A, Przychodzeń W, Kusznierewicz B, Kołodziejski D, Namieśnik J, Bartoszek-Pączkowska A. Isothiocyanates may chemically detoxify mutagenic amines formed in heat processed meat. Food Chem. 2014;15;157:105-110. doi: 10.1016/j.foodchem.2014.01.082.
39. de Carvalho AM, Carioca AA, Fisberg RM, Qi L, Marchioni DM. Joint association of fruit, vegetable, and heterocyclic amine intake with DNA damage levels in a general population. Nutrition. 2016;32(2):260–264. doi: 10.1016/j.nut.2015.08.018.
40. Kurzawa-Zegota M, Najafzadeh M, Baumgartner A, Anderson D. The protective effect of the flavonoids on food-mutagen-induced DNA damage in peripheral blood lymphocytes from colon cancer patients. Food Chem Toxicol. 2012;50(2):124-9. doi: 10.1016/j.fct.2011.08.020.
41. Jarosz M, Wolnicka K, Sajór I, Wierzejska R. Zalecenia dotyczące żywienia i aktywności fizycznej. In Jarosz M. Normy żywienia dla populacji Polski. Warszawa: Instytut Żywności i Żywienia; 2017, 261-287.

Konflikt interesów:
Autorzy deklarują brak konfliktu interesów

AUTOR KORESPONDUJĄCY
Beata Janoszka
Katedra i Zakład Chemii, Wydział Lekarski z Oddziałem Lekarsko-Dentystycznym w Zabrzu, Śląski Uniwersytet Medyczny w Katowicach
ul. Jordana 19, 41-808 Zabrze
tel. 32 2755188
e-mail:bjanoszka@sum.edu.pl

Nadesłano: 31.05.2019
Zaakceptowano: 26.06.2019

PRACA POGLĄDOWA
REVIEW ARTICLE

ROLA TKANKI TŁUSZCZOWEJ ZE SZCZEGÓLNYM UWZGLĘDNIENIEM CYTOKIN W PATOGENEZIE CHORÓB NOWOTWOROWYCH

THE ROLE OF ADIPOSE TISSUE WITH PARTICULAR EMPHASIS ON CYTOKINES IN THE PATHOGENESIS OF NEOPLASTIC DISEASES

Jakub Warakomski, Lucyna Siemińska
KATEDRA PATOFIZJOLOGII I ENDOKRYNOLOGII WYDZIAŁU LEKARSKIEGO Z ODDZIAŁEM LEKARSKO-DENTYSTYCZNYM ŚLĄSKIEGO UNIWERSYTETU MEDYCZNEGO W ZABRZU, ZABRZE, POLSKA

STRESZCZENIE
Mechanizmy, które prowadzą do rozwoju nowotworów są niezwykle złożone. W pracy przedstawiono aktualny stan wiedzy na temat różnych patofizjologicznych powiązań pomiędzy tkanką tłuszczową a onkogenezą oraz wyniki niektórych badań klinicznych analizujących patomechanizmy chorób nowotworowych w otyłości.

SŁOWA KLUCZOWE: cytokiny, nowotwory, onkogeneza, tkanka tłuszczowa

ABSTRACT
Carcinogenesis leading mechanism are hugely complex. In paper there is show current state of knowledge about different pathophysiological links among adipose tissue, carcinogenesis and some clinical findings.

KEY WORDS: cytokines, tumors, carcinogenesis, adipose tissue

Wiad Lek 2019, 72, 8, 1551-1558

ETIOPATOGENEZA CHORÓB NOWOTWOROWYCH

W większości przypadków zachorowanie na chorobę nowotworową ma charakter sporadyczny, czyli jest wynikiem kumulacji uszkodzeń kwasu deoksyrybonukleinowego u danego osobnika oraz mutacji punktowych i chromosomowych, co prowadzi do niekontrolowanej proliferacji komórkowej. W kilku procentach zachorowanie wynika z dziedziczonej predyspozycji genetycznej. Dotyczy to przede wszystkim dziedzicznie uwarunkowanego raka piersi i jajnika (BRCA1, BRCA2) oraz jelita grubego (mutacje genów MSH2, MLH1, MSH6, APC, NOD2). Jednak nawet obecność genetycznych czynników rozwoju nowotworu nie musi determinować rozwinięcia się raka. Ważne jest również zainicjowanie procesu kancerogenezy na poziomie komórkowym, czyli między innymi aktywacja protoonkogenów i przekształcenie ich w onkogeny.

Istotną rolę w patogenezie chorób nowotworowych odgrywa układ odpornościowy. Według teorii immunoredagowania nowotworu wyróżnia się fazę eliminacji, w której układ odpornościowy próbuje eliminować komórki nowotworowe, następnie fazę równowagi, w której dochodzi do jednoczesnego usuwania komórek nowotworowych i ich wzrostu oraz fazę ucieczki, w której komórki nowotworowe wymykają się spod kontroli układu immunologicznego. Aktywność układu odpornościowego wyraża się poprzez produkcję przeciwciał oraz cytotoksyczność głównie komórek NK, CD8+ oraz CD4+. Limfocyty Th wydzielają cytokiny, które przyciągają i aktywują kolejne komórki układu odpornościowego: limfocyty B, makrofagi, NK i Tc. Pobudzone makrofagi mogą poprzez interferon (INF), czynnik martwicy nowotworów (TNF – Tumor Necrosis Factor i interleukinę 1 (IL-1) zabijać komórki nowotworowe lub hamować ich proliferację, ale mogą też produkować czynniki wzrostowe nowotworów: nabłonkowy czynnik wzrostu (EGF – *Epidermal Growth Factor*) , czynniki wzrostu fibroblastów (FGF – *Fibroblastal Growth Factor*), płytkopodobny czynnik wzrostu (PDGF – *Platelet- Derived Growth Factor*). Makrofagi wykazujące korzystne efekty przeciwnowotworowe nazywane są M1, ale w nacieku nowotworowym dominują makrofagi M2, sprzyjające proliferacji nowotworowej [1]. Produkują one różne chemokiny, z których część przyciąga komórki zapalne oraz hamuje powstawanie naczyń. Inne chemokiny mogą stymulować angiogenezę i proliferację komórek nowotworowych, a jeszcze inne hamują apoptozę i przyciągają do guza nowotworowego limfocyty Treg. W początkowej fazie rozwoju nowotworu proliferacja komórkowa toczy się bez udziału naczyń krwionośnych (jest to tzw. fenotyp

proliferacyjny). Gdyby nie dochodziło do zaopatrywania komórek nowotworowych w krew, wzrost guza byłby zahamowany przy objętości 1–2 mm^3. Jednakże w procesie kancerogenezy dochodzi do przełączenia fenotypu na angiogenny i komórki nowotworowe zaczynają produkować znaczne ilości substancji proangiogennych: w tym czynnik wzrostu śródbłonka naczyniowego (VEGF – *Vascular Endothelial Growth Factor*), FGF, PDGF, IL-6 i IL-8. W masie komórek nowotworowych dochodzi do rozwoju nowej sieci naczyń krwionośnych, tzw. neoangiogenezy. To zjawisko jest niekorzystne, ponieważ komórki nowopowstałego śródbłonka są morfologicznie niedojrzałe i mają zdolność do proliferacji i migracji, co sprzyja rozwojowi przerzutów. Pomimo neowaskularyzacji, w masie guza rozwija się hipoksja, co stymuluje wytwarzanie czynnika indukowanego hipoksją 1 (HIF-1 – *Hypoxia- Inducible Factor 1*). W wyniku uwalniania HIF-1 zwiększa się synteza VEGF, który wiąże się ze swoimi receptorami na powierzchni komórek. Uruchamiany jest szlak kinazy tyrozynowej co aktywuje kinazy, fosfolipazy, białka Ras, Scr – odpowiedzialne za niekontrolowaną proliferację i nowotworzenie naczyń. Kolejnym zjawiskiem wtórnym do hipoksji guza nowotworowego jest spadek ekspresji białek proapoptycznych (Bid, Bad, Bax) i wzrost ekspresji białek antyapoptycznych (Bcl-2, Bcl-XL). VEGF pobudza też aktywność enzymów odpowiedzialnych za niszczenie macierzy pozakomórkowej. Zmiany w podścielisku mogą prowadzić do zmiany metabolizmu komórek, w tym aktywacji glikolizy i wzmożonej produkcji mleczanu.

Wzrost ekspresji genów odpowiedzialnych za kancerogenezę jest w dużej mierze indukowany przez mikrośrodowisko guza, czyli macierz pozakomórkową oraz populację komórek zrębowych, takich jak: fibroblasty, makrofagi i inne. Mikrośrodowisko poprzez ekspresję cytokin, czynników wzrostu i proteaz może wpływać na zachowanie komórek nowotworowych. Ważna jest bezpośrednia interakcja komórek podścieliska z komórkami nowotworowymi, ponieważ to one mogą brać udział w pobudzaniu protoonkogenów. Aktywacja komórek nowotworowych przez fibroblasty podścieliska guza została wykazana w raku piersi, prostaty, wątroby i wielu innych [2; 3].

Udowodniono, że fibroblasty oraz metaloproteinazy podścieliska są zaangażowane w różne fazy progresji raka takie jak inwazja i przerzuty. Fibroblasty produkują związki promujące wzrost guza . Jednym z nich jest fibronektyna 1, która łączy się z białkami fibrylarnymi osocza, włóknami kolagenowymi i integrynami, ułatwiając adhezję i migrację komórek nowotworowych. Stwierdzono oddziaływanie podścieliska w zakresie genu FN1, który koduje fibronektynę w czerniaku i raku piersi [4].

OTYŁOŚĆ JAKO CZYNNIK RYZYKA CHORÓB NOWOTWOROWYCH

Wykazano ewidentne powiązania pomiędzy otyłością i 13 nowotworami. Są to nowotwory przewodu pokarmowego (przełyku, żołądka, jelita grubego, wątroby, nerek, trzustki, pęcherzyka żółciowego), narządu rodnego u kobiet (jajnika, endometrium), piersi, nerki, tarczycy i szpiczak mnogi. Sugerowane są związki otyłości z chłoniakiem nieziarniczym oraz rakiem prostaty [5; 6].

Najlepiej udokumentowany jest wpływ tkanki tłuszczowej na rozwój nowotworów przewodu pokarmowego. Chen i wsp. po przeanalizowaniu ponad 25 tys. przypadków pierwotnego raka wątroby wykazali, że ryzyko względne RR rozwoju raka dla otyłych mężczyzn wynosiło 1,91, a dla otyłych kobiet 1,55. Ryzyko to było niezależne od spożycia alkoholu, cukrzycy czy wirusowego zapalenia wątroby w wywiadzie [7].

Zespół badawczy z tego samego ośrodka, w innej prospektywnej analizie przeprowadzonej w oparciu o dane prawie 42 tysięcy pacjentów z rozpoznanym rakiem żołądka wykazał, że otyłość wiązała się z większym ryzykiem raka wpustu (RR 1,82), ale nie raka innej okolicy niż wpust (RR 1,0) [8].

W wieloośrodkowym badaniu EPIC (przeprowadzonym wśród ponad 368 tys. pacjentów wykazano, że ryzyko względne rozwoju raka jelita grubego u mężczyzn z górnego kwartyla BMI w porównaniu do dolnego kwartyla BMI wynosiło 1,55, a gdy porównywano górny i dolny kwartyl WHR to ryzyko względne RR wynosiło 1,51 [9].

W badaniu przeprowadzonym wśród ponad 424 tysięcy pacjentów z rejonów Azji, Australii i Nowej Zelandii, otyłość zwiększała ryzyko rozwoju raka jelita grubego o 50%, a raka odbytnicy o 68%. Ryzyko względne wystąpienia raka jajnika u kobiet otyłych, w porównaniu do kobiet z prawidłową masą ciała wynosiło 2,62, a raka szyjki macicy 4,21. Ryzyko RR raka prostaty wynosiło 1,45 [10]. W tej samej analizie ryzyko rozwoju raka piersi u otyłych kobiet było o 63% większe w porównaniu do szczupłych kobiet, ale inni autorzy piszą nawet o trzykrotnym wzroście ryzyka [11]. Udowodniono, iż nie tylko wskaźnik BMI, ale też obwód bioder korelują dodatnio z występowaniem raka sutka u kobiet [12]. Stwierdzono, że w przypadku raka jelita grubego, przełyku i nerki masa tkanki tłuszczowej trzewnej koreluje z gorszym rokowaniem [13].

O otyłości mówimy wówczas, jeśli nagromadzenie tkanki tłuszczowej w organizmie przekracza 20% całkowitej masy ciała u mężczyzn i 25% u kobiet. Najbardziej rozpowszechnionym sposobem oceny otyłości jest wyliczanie wskaźnika BMI (*Body Mass Index*). Wskaźnik ten uważany jest za patologiczny, gdy przekracza 30,0. Używany jest również wskaźnik WHR (*Waist-Hip Ratio*), czyli stosunek obwodu talii do bioder. Jako patologiczne uznawane są wartości > 1 u mężczyzn oraz > 0,8 u kobiet.

Wyróżniamy 3 rodzaje tkanki tłuszczowej: białą, brunatną i beżową. Różnią się rozwojem, budową, właściwościami i lokalizacją. Tkanka tłuszczowa brunatna i beżowa odgrywają rolę w termogenezie, natomiast biała jest magazynem energii gromadzonej jako trójglicerydy. Tkankę tłuszczową białą dzielimy na trzewną i podskórną. W promowaniu i rozwoju chorób nowotworowych istotną rolę odgrywa przede wszystkim tkanka tłuszczowa trzewna. Jest to złożony, metabolicznie aktywny narząd wydzielania wewnętrznego, produkujący szereg adipokin, cytokin zapalnych, chemokin oraz dopełniaczy [14]. Na proces

onkogenezy mają wpływ adipocyty i komórki układu immunologicznego, ale znaczącą rolę odgrywa też zrąb łącznotkankowy, naczynia krwionośne i komórki endotelium. Obecnie prowadzonych jest wiele badań molekularnych, które mają wyjaśnić znaczenie związków produkowanych przez tkankę tłuszczową: adipokin, czynników wzrostu, a także różnych postaci mikroRNA, które mogą nie tylko wywoływać, ale i istotnie uczestniczyć w progresji nowotworów złośliwych

TKANKA TŁUSZCZOWA A MIKRORNA

Tkanka tłuszczowa u ludzi jest istotnym źródłem krążącego mikroRNA. Adipocyty uwalniają do krwiobiegu egzosomy bogate w mikroRNA, które docierają do różnych tkanek i narządów (np. wątroby), regulując w nich ekspresję genów. MikroRNA to krótkie cząsteczki niekodującego RNA, które mogą oddziaływać nawet na 60% genów i na tysiące mRNA. MikroRNA wpływają na proces translacji, czyli syntezy białek na matrycy mRNA, regulują proliferacje, różnicowanie, metabolizm komórkowy, apoptozę i szlaki przekaźnictwa wewnątrzkomórkowego. Wykazano, że choroby nowotworowe charakteryzuje zaburzona ekspresja, struktura oraz zmieniona regulacja tkankowa mikroRNA. Coraz więcej doniesień dotyczy roli mikroRNA jako promotora kancerogenezy, zwłaszcza w raku wątroby, żołądka i płuc [15].

Cząsteczki mikroRNA mogą działać jako onkogeny lub jako geny supresorowe, ale mogą też pełnić obie funkcje. Często działają jako przełącznik pomiędzy proliferacją i apoptozą. W większości nowotworów w płynach ustrojowych obserwuje się wzrost poziomu mikroRNA, ale czasami stwierdza się istotnie obniżony poziom mikroRNA [16].

PRZEWLEKŁY STAN ZAPALNY

Mechanizmem wiążącym tkankę tłuszczową i onkogenezę jest przewlekły stan zapalny. Nadmiernemu rozplemowi tkanki tłuszczowej towarzyszy wzmożona aktywacja zapalna w obrębie tkanki tłuszczowej, czego wyrazem jest zwiększone gromadzenie markerów zapalenia: IL-6, IL-8, IL-1, TNF-α, MCP-1 (białko chemotaktyczne monocytów). Na powierzchni adipocytów znajdują się receptory TLR, których pobudzenie aktywuje mechanizmy odporności immunologicznej nieswoistej. Cytokiny prozapalne są produkowane przez hipertroficzne adipocyty oraz nagromadzone w tkance tłuszczowej makrofagi i inne komórki systemu immunologicznego. Badania eksperymentalne wykazały, że cytokiny oddziaływują na przekaźnictwo i metabolizm wewnątrzkomórkowy poprzez szlaki NFkB (transkrypcyjny czynnik jądrowy κB), STAT3 (białka przekazujące informację i aktywujące transkrypcję 3), JNK (N-końcowa kinaza c-Jun) [17].

Kinaza JNK prowadzi do fosforylacji IRS-1 i blokuje przewodzenie sygnałów insuliny. NF-kB jest dobrze poznanym czynnikiem transkrypcyjnym odpowiedzialnym za ekspresję genów, które regulują proliferację, wzrost komórek oraz apoptozę. Nadmierna lub nieprawidłowa aktywacja szlaku NF-kB prowadzi do zmiany metabolizmu wewnątrzkomórkowego, pobudza system immunologiczny i zwiększa sekrecję cytokin, co w efekcie może być przyczyną transformacji nowotworowej. Zaburzenia sygnalizacji białka NF-kB prowadzą do rozwoju guzów litych i nowotworów hematologicznych oraz sprzyjają przerzutom komórek nowotworowych [18].

Szlak NF-kBjest zaangażowany w proces nowotworowy raka jajnika, macicy (zarówno trzonu, jak i szyjki) sutka oraz prostaty [19]. Aktywacja NF-kB prowadzi do nasilenia progresji raka jajnika i prostaty. Hamowanie aktywności czynnika transkrypcyjnego NF-kB może mieć działanie terapeutyczne w onkologii.

Szlak STAT-3 stanowi szlak sygnałowy, który poprzez transkrypcje zmienia ekspresje genową odpowiedzialną za fizjologiczne oraz fizjopatologiczne procesy komórkowe, takie jak: zapalenie, proliferacja, angiogeneza i metastaza komórek nowotworowych. Ufosforylowany STAT-3 po przemieszczeniu do jądra stymuluje transkrypcję. Uważa się, iż białko może być celem terapii nowotworowej, zwłaszcza w nowotworach chemioopornych [20].

Jedną z przyczyn wzmożonej aktywacji zapalnej w tkance tłuszczowej jest niedotlenienie tkanki tłuszczowej. Hipoksja jest skutkiem relatywnie zredukowanego w stosunku do masy tkanki tłuszczowej przepływu krwi. U osób otyłych przepływ krwi w ml/min na 100 g tkanki tłuszczowej może być mniejszy nawet o 30–40% w porównaniu do osób szczupłych [21]. Dyfuzja tlenu zachodzi do 120 um, natomiast średnica adipocytów często przekracza ten dystans. Niedotlenienie tkanki tłuszczowej jest też następstwem zmniejszonej gęstości kapilar z powodu upośledzonej angiogenezy oraz nasilonej wazokonstrykcji, wtórnej do wzmożonej aktywności angiotensyny II.

Hipoksja tkanki tłuszczowej prowadzi do dysfunkcji mitochondriów oraz rozwoju stresu w siateczce śródplazmatycznej. W przerośniętych adipocytach liczba mitochondriów jest zredukowana [22], a ich dysfunkcja prowadzi do obniżonej sekrecji adiponektyny [23]. Zaburzeniom ulegają wewnątrzkomórkowe szlaki przekaźnictwa sygnałów. Jednocześnie dochodzi do przekształcania komórek prekursorowych adipocytów w komórki zapalne, między innymi w makrofagi. Główną cytokiną produkowaną przez adipocyty i makrofagi jest TNF-α, który inicjuje procesy zapalne oraz insulinooporność. Redukuje aktywność kinazy tyrozynowej receptora insulinowego, hamuje ekspresję GLUT-4 oraz IRS-1, a także blokuje przekaźnictwo sygnałów insuliny [24]. Pod wpływem TNF-α wzrasta ekspresja NF-kB, i nasila się aktywność NF-kB. TNF-α zwiększa też ekspresję molekuł adhezyjnych na powierzchni komórek endotelialnych i mięśni gładkich naczyń oraz redukuje ekspresję PPARγ i adiponektyny.

Przeładowanie komórek tłuszczowych lipidami prowadzi do rozerwania błony komórkowej i uwolnienia do mikrośrodowiska zawartości wewnątrzkomórkowej (lipidów, cytokin, kwasów nukleinowych, ATP). Stanowi to sygnał aktywujący makrofagi i monocyty, ich rekrutację i lokalną proliferację. Czynnikiem przyciągającym komórki żerne jest przede wszystkim MCP-1, produkowany przez przerośnięte adipo-

cyty [25]. Zwiększona produkcja MCP-1 pobudza lokalną infiltrację tkanki tłuszczowej przez makrofagi, nasilając dalszą produkcję cytokin zapalnych oraz rekrutację komórek odpowiedzi immunologicznej. W badaniach *in vitro* zahamowanie wytwarzania MCP-1 skutkowało zahamowaniem procesów zapalnych [26]. Wzmożoną sekrecję MCP-1 stwierdzono w raku płuca, piersi, jajnika, szyjki macicy i czerniaku [27].

Makrofagi otaczają adipocyty tworząc struktury w kształcie korony (CLS – C*rown Like Structures)*. Pojawienie się struktur CLS aktywuje w obrębie układu immunologicznego mechanizmy nieswoiste, czyli wrodzone oraz swoiste, czyli nabyte. Liczba komórek zapalnych w CLS wzrasta wraz ze średnicą adipocytów i stopniem otyłości [28]. Część z nich zmienia się w olbrzymie komórki wielojądrzaste lub komórki piankowate. Odkąd uznano tworzenie struktur CLS jako wykładnika stanu zapalnego w tkance tłuszczowej, wzrosło zainteresowanie wykorzystania tych zmian jako biomarkera onkogenezy [29]. W badaniach histologicznych raka piersi i prostaty struktury CLS stwierdza się w 50% przypadków. Dodatnie wyniki badań w kierunku CLS wiązały się z wyższym BMI i zespołem metabolicznym oraz większą nawrotowością i śmiertelnością. W raku piersi obecność CLS jest powiązana z wyższą aktywnością aromatazy, która stymuluje tumorogenezę i inicjuje kancerogenezę [30].

W procesach zapalnych w otyłości rolę odgrywają też neutrofile, których liczba w tkance tłuszczowej trzewnej istotnie wzrasta w trakcie stosowania diety bogatołuszczowej. Neutrofile produkują elastazę, która rozkłada substrat receptora insuliny IRS-1, przez co rozwija się insulinooporność [31].

Uważa się, że przewlekły stan zapalny dotyczy różnych depozytów tkanki tłuszczowej, a miejscowy stan zapalny prowadzi do gromadzenia we krwi zwiększonych poziomów cytokin i innych białek prozapalnych. U osób otyłych stężenia IL-6 we krwi są podwyższone [32].

Ustalono, że wśród 1822 uczestników badania REGARDS, których obserwowano 8 lat, wyższe stężenia IL-6 były predyktorem zgonu z powodu choroby nowotworowej (HR 5,3 dla górnego tercyla *vs.* dolnego tercyla). W analizie wieloczynnikowej niezależnymi czynnikami ryzyka były zarówno stężenie IL-6, jak i obecność nadwagi lub otyłości [33]. Z kolei Bachelot i wsp. wykazali, że podwyższone stężenia IL-6 korelowały ze stężeniami VEGF i wiązały się z gorszym rokowaniem u kobiet z rakiem piersi [34].

W otyłości wzrasta we krwi stężenie czynników prozapalnych, ale też maleje przeciwzapalnych takich jak IL-10, adiponektyna. IL-10 posiada działanie plejotropowe, uczestniczy w regulacji procesów zapalnych. Początkowo opisywano ją jako czynnik hamujący syntezę cytokin (CSIF – *Cytokine Synthesis Inhibitory Factor*). Niektóre nowotwory mają zdolność samodzielnej sekrecji IL-10, wykorzystując jej immunosupresyjne działanie na organizm. Obecnie pojawia się coraz więcej badań na temat immunostymulującego działania. Podanie IL-10 po wcześniejszej immunizacji szczepionką przeciwnowotworową znacznie poprawia jej skuteczność działania – wzrasta aktywność limfocytów T cytotoksycznych oraz stymulacja komórek NK [35].

INSULINOOPORNOŚĆ

Ogniwem łączącym tkankę tłuszczową w otyłości i choroby nowotworowe jest insulinooporność. W otyłości insulinooporność rozwija się jako konsekwencja zaburzeń lipogenezy / lipolizy z hipertrofią adipocytów podskórnych brzusznych i nasiloną lipolizą w adipocytach trzewnych oraz ektopowego odkładanie tłuszczu w wyniku ograniczonej zdolności adipocytów do ich gromadzenia. Kolejne mechanizmy patofizjologiczne prowadzące do insulinoporności u otyłych osób to upośledzone utlenianie tłuszczów, oporność na leptynę, defekt proliferacji i różnicowania adipocytów, zmieniona produkcja adipocytokin, przewlekły proces zapalny. Na poziomie molekularnym oporność jest wywołana dysregulacją kaskady pobudzeń wywołanych przez insulinę oraz zmienioną ekspresją czynników transkrypcyjnych. Najczęstszym mechanizmem jest hiperfosforylacja reszt seryny i treoniny w białkach IRS, co prowadzi do zmniejszenia fosforylacji w resztach tyrozyny i zmniejszenia interakcji IRS z PI3-K. Insulinooporność może prowadzić do rozwoju onkogenezy w mechanizmie kompensacyjnej hiperinsulinemii. Insulina jest znanym czynnikiem wzrostowym i mitogennym, pobudzającym wzrost komórek. W insulinooporności wzrasta też stężenie proinsuliny i peptydu C. Wykazano, że u pacjentów z wysokim stężeniem peptydu C ryzyko zachorowania na raka jelita grubego wzrasta trzykrotnie [36]. Również sama hiperglikemia spoczynkowa obserwowana często w otyłości i insulinooporności jest czynnikiem ryzyka raka [37].

Hiperglikemia sprzyja kancerogenezie w mechanizmie stymulacji wytwarzania wolnych rodników tlenowych, późnych produktów glikacji oraz aktywacji czynnika transkrypcyjnego NF-kB [38].

Obniżona wrażliwość tkanek na insulinę to również zwiększona produkcja IGF-1 oraz IGF-2 (insulinopodobnych czynników wzrostu typ 1 i 2). Czynniki te aktywują receptor IRS-1, stymulują szlak sygnałowy MAPK (kinaza białkowa zależna od mitogenu) oraz PKB i mTOR. Skutkuje to efektami mitogennymi i antyapoptycznymi oraz pobudza w komórkach nowotworowych nasilone podziały. IGF-1 wpływa też na aktywność genu Ras, która odgrywa dużą rolę w początkowych etapach kancerogenezy [39]. Znaczenie tego mechanizmu zostało udowodnione przede wszystkim w przypadku raka piersi i jelita grubego [40].

WPŁYW TKANKI TŁUSZCZOWEJ NA ROZWÓJ CHORÓB NOWOTWOROWYCH

Tkanka tłuszczowa w otyłości sprzyja rozwojowi chorób nowotoworowych poprzez zmienioną produkcję steroidów płciowych. U kobiet, zwłaszcza po menopauzie, jest ważnym miejscem lokalnej syntezy estrogenów: estradiolu, estriolu i estronu. Wynika to głównie ze zwiększonej aktywności aromatazy. Estrogeny poprzez receptory alfa wpływają na szlaki związane z IRS-1, PI3-K i Akt oraz regulują aktywność GLUT-4. W efekcie przemiany steroidów płciowych skutkują zmienioną sygnalizacją insuliny oraz pobudzają proliferację i wzrost komórek [41].

Estrogeny mają ukierunkowany wpływ na rozwój chorób nowotworowych, przede wszystkim żeńskich

narządów rozrodczych. W wielu pracach wykazano, że oporności insulinowej u otyłych kobiet towarzyszą wysokie stężenia endogennych estrogenów [42], a u chorujących na cukrzycę występuje hiperestrogenemia [43]. Kalish i wsp. wykazali, że wysokie stężenia estradiolu całkowitego i bioaktywnego u kobiet po menopauzie wiążą się z opornością insulinową i z zespołem metabolicznym niezależnie od BMI i WHR [44].

Wysokie poziomy produkowanych lokalnie estrogenów zwiększają ryzyko zachorowania na raka gruczołu piersiowego [45], a także wiążą się z szybkim wzrostem guza [46]. Długotrwała ekspozycja na produkowane lokalnie estrogeny, nieprzeciwstawiona antyproliferacyjnemu działaniu progesteronu, może też sprzyjać rozwojowi raka macicy. Podwyższone poziomy estrogenów we krwi stwierdzono również w raku jajnika. [47].

Czynniki zwiększające liczbę cykli menstruacyjnych (wczesne rozpoczęcie miesiączkowania i późna menopauza) znacznie podnoszą prawdopodobien'stwo rozwinięcia się raka piersi. Zredukowanie liczby owulacji, na przykład poprzez wielorództwo, zdaje się mieć działanie ochronne [48].

U otyłych mężczyzn zazwyczaj występuje hipotestosteronemia [49; 50]. Charakterystycznym dla mężczyzn nowotworem związanym z otyłością jest rak prostaty. Niskie poziomy testosteronu wiążą się z większym ryzykiem zachorowania i gorszym przebiegiem tego nowotworu [51; 52]. Mechanizm wiążący otyłość z rakiem prostaty to przede wszystkim proces zapalny toczący się w tkance tłuszczowej [53] oraz insulinooporność. Sugeruje się też spadek ekspresji białka wiążącego ubikwitynę [54]. W przypadku raka jądra i nowotworu prącia nie stwierdzono powiązań tych nowotworów z otyłością [55; 56].

ZMIENIONA PRODUKCJA ADIPOKIN PRZEZ TKANKĘ TŁUSZCZOWĄ A ROZWÓJ CHORÓB NOWOTWOROWYCH

Zmieniona produkcja adipokin przez tkankę tłuszczową sprzyja rozwojowi chorób nowotworowych.

Adiponektyna odgrywa ważną rolę w metabolizmie glukozy i kwasów tłuszczowych w obrębie wątroby i mięśni szkieletowych. Zwiększa wrażliwość na insulinę, wykazuje działanie przeciwzapalne i przeciwmiażdżycowe. Białko to jest produkowane przez drobne, różnicujące się adipocyty, a ekspresja genu adiponektyny wzrasta podczas dojrzewania preadipocytów nawet 100-krotnie [57]. Korelacja stężenia adiponektyny ze wskaźnikiem BMI jest ujemna, a poziom adiponektyny u kobiet jest nieco wyższy niż u mężczyzn. Obniżone stężenia adiponektyny wiążą się ze zwiększonym ryzykiem wystąpienia cukrzycy typu 2 [58; 59].

Adiponektyna wykazuje efekty biologiczne poprzez 2 receptory błonowe: AdipoR1 i AdipoR2. Obydwa receptory aktywują przekazywanie sygnałów poprzez AMPK (AMP-zależna kinaza) oraz PPARγ receptor alfa aktywowany peroksysomalnym czynnikiem proliferacyjnym), a także poprzez MAPKp38 (kinaza białkowa zależna od mitogenu p38MAPK).

Chociaż dane z piśmiennictwa dotyczące roli adiponektyny w patogenezie kancerogenezy nie są spójne, to większość doniesień sugeruje, że adiponektyna hamuje rozwój i progresję niektórych chorób nowotworowych, głównie piersi, jelita grubego, płuca, tarczycy [60]. W badaniu przeprowadzonym przez Tworoger i wsp. u prawie 1500 kobiet stwierdzono ujemną korelację między stężeniem we krwi adiponektyny i ryzykiem raka sutka. Zależność ta była najsilniejsza wśród kobiet po menopauzie [61]. Wykazano, że u otyłych kobiet uzyskana w wyniku operacji bariatrycznej redukcja masy ciała o 14–25% prowadziła do zwiększenia stężenia adiponektyny i tym samym do zmniejszenia ryzyka rozwoju raka piersi [62].

Kolejną adipokiną istotną w procesie onkogenezy jest leptyna. Białko należy do tej samej rodziny cytokin co IL-6 i tak jak pozostałe cytokiny klasy I oddziaływuje poprzez receptor ObR na ścieżkę sygnałową JAK2-STAT3. Inne szlaki promujące kancerogenezę pod wpływem leptyny to MAPK/ERK, TGFβWnt/βkatenina, PI3-K (kinaza 3-fosfatydyloinozytolu) i AKT czyli PKB (kinaza białkowa B). Aktywna kinaza AKT fosforyluje wiele białek zaangażowanych w podstawowe procesy komórkowe. Głównymi substratami PI3-K oraz AKT są kinaza treoninowo-serynowa mTOR, cyklina D, czynniki transkrypcyjne FOXO. Wymienione ścieżki sygnałowe odgrywają kluczowa rolę w proliferacji, zahamowaniu apoptozy, angiogenezie, migracji i inwazji [63].

Wydaje się, że główny mechanizm oddziaływania leptyny na wzrost komórek nowotworowych to lokalny wpływ na układ immunologiczny i stymulacja procesów zapalnych oraz insulinooporności. Za pośrednictwem receptorów ObR, które są obecne na powierzchni komórek zapalnych, leptyna aktywuje makrofagi M2, odpowiedzialne za procesy nowotworowe oraz wzmaga produkcję cytokin IL-6, IL-8, IL-12, IL-18, TNF-α, przy jednoczesnym zahamowaniu IL-10 i IL-4. Wykazano, że leptyna jest ściśle związana z aktywnością fibroblastów podścieliska guza nowotworowego i poziomem ekspresji metaloproteinaz. [64].

Stwierdzono, że w raku piersi leptyna nasila aktywność aromatazy [65], a wysokie poziomy leptyny mogą sprzyjać przerzutom raka piersi [66; 67]. Badania *in vitro* przeprowadzone na komórkach nowotworowych raka piersi potwierdziły wpływ leptyny na szlaki onkogeniczne poprzez cyklinę D, surwiwinę, integrynę, interleukinę-1, receptor dla IL-1, naczyniowo-śródbłonkowy czynnik wzrostu [68].

W badaniu przeprowadzonym we Włoszech na grupie 216 kobiet z rakiem piersi stężenia leptyny istotnie korelowały z wielkością guza, obecnością przerzutów do węzłów i odległych [69].

Podwyższone stężenia leptyny występują też u kobiet z inwazyjnym, endometrialnym rakiem macicy [70]. W przypadkach raka endometrium z przerzutami do węzłów chłonnych w badaniach immunohistochemicznych obserwowano wyższą ekspresję leptyny i ObR [71].

Podobne obserwacje dotyczą raka jajnika i raka jelita grubego. W przerzutach tych nowotworów stwierdzono wyższą ekspresję receptorów leptynowych niż w guzie pierwotnym [72; 73].

W wielu badaniach prospektywnych wykazano związki pomiędzy hiperleptynemią i zwiększonym ryzykiem raka jelita grubego, raka sutka, przełyku, żołądka, trzustki, prostaty, jajników i płuc [74].

Adipokiną o zupełnie odmiennej, zdawałoby się opiekuńczej, charakterystyce od poprzednio opisanych związków jest chemeryna. Białko to odgrywa znaczącą rolę w procesie chemotaksji i migracji komórek odpornościowych do tkanek objętych procesem zapalnym. Wykazano, że ma właściwości przeciwzapalne, ale może też wywierać wpływ prozapalny.

W badaniach eksperymentalnych wykazano, że wzmożona ekspresja chemeryny wiąże się z nasiloną angiogenezą i migracją komórek nowotworowych [75], a w badaniach klinicznych wyższe stężenia chemeryny wiązały się z większą agresywnością nowotworu [76; 77].

W badaniu przeprowadzonym wśród 221 pacjentów z rakiem jelita grubego i ponad 2300 osobników stanowiących grupę kontrolną stężenia chemeryny z górnego kwartyla w porównaniu ze stężeniami z dolnego kwartyla kojarzyły się ze statystycznie znamiennym większym ryzykiem raka okrężnicy (HR=2.27) [78].

Chemeryna może również zwiększać prawdopodobieństwo wystąpienia raka trzustki poprzez podtrzymywanie stanu przewlekłego zapalenia [79].

Jednakże w raku wątrobowokomórkowym spada poziom chemeryny, co autorzy tłumaczą obniżona gęstością komórek NK [80]. Tak więc rola chemeryny w onkogenezie nie jest do końca jasna i badania nad tą adipokiną na pewno będą kontynuowane w najbliższych latach.

ROZWÓJ CHORÓB NOWOTWOROWYCH W OTYŁOŚCI A STYL ŻYCIA

Rozwojowi chorób nowotworowych w otyłości oprócz nadmiernego gromadzenia tkanki tłuszczowej sprzyja styl życia. Kancerogeneza może być wynikiem nadmiernego gromadzenia tkanki tłuszczowej, ale znaczenie może też mieć nieodpowiednia dieta. Badania epidemiologiczne nie wyjaśniają względnego udziału tych 2 czynników. Dieta bogatotłuszczowa, bogatowęglowodanowa, mięso oraz skłonność do zaparć – zwiększają ryzyko raka jelita grubego. Dieta oparta o wysoki indeks glikemiczny prowadzi do wysokich poziomów glukozy i insuliny, co w konsekwencji sprzyja rozwojowi tego nowotworu [81].

Stwierdzono, iż dieta bogata w nasycone kwasami tłuszczowymi i tłuszcze typu trans promuje przerzuty raka prostaty [82], a metabolity bakterii związanych z otyłością sprzyjają rozwojowi raka wątroby [83].

PODSUMOWANIE

Nie podlega wątpliwości, iż zwiększona obecność tkanki tłuszczowej ma znaczny wpływ na występowanie i progresję wielu chorób nowotworowych. Komórki tkanki tłuszczowej (adipocyty) uwalniają liczne substancje biologicznie czynne (adipokiny), mające wpływ na przemiany metaboliczne w całym organizmie. Niezależnie, czy dotyka to piersi, układu pokarmowego, czy układu rozrodczego, powiązanie wzrostu z nowotworem jest niekorzystne dla ludzi. Zmieniające się poziomy adipokin wydzielanych takich jak adiponektyna, chemeryna, czy leptyna mają znaczy wpływ na zjawiska insulinooporności, stymulacji procesów zapalnych, czy zmian w układzie immunologicznym.

PIŚMIENNICTWO

1. Solinas G, Germano G, Mantovani A et al. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. J Leukoc Biol. 2009; 86(5):1065-73. doi: 10.1189/jlb.0609385.
2. Nakamura T, Matsumoto K, Kiritoshi A et al. Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: in vitro analysis of tumor-stromal interactions. Cancer Res. 1997; 57(15): 3305-13.
3. Jiang W, Hiscox S, Matsumoto K et al. Hepatocyte growth factor/scatter factor, its molecular, cellular and clinical implications in cancer. Crit Rev Oncol Hematol. 1999; 29(3): 209-48.
4. Yang X, Hu Q, Hu LX et al. miR-200b regulates epithelial-mesenchymal transition of chemo-resistant breast cancer cells by targeting FN1. Discov Med. 2017; 24(131): 75-85.
5. Steele CB, Thomas CC, Henley SJ et al. Vital Signs: Trends in Incidence of Cancers Associated with Overweight and Obesity—United States, 2005–2014. MMWR Morbidity Mortality Wkly. Rep. 2017; 66(39):1052–1058. doi: 10.15585/mmwr.mm6639e1
6. Lauby-Secretan B, Scoccianti C, Loomis D et al. Body Fatness and Cancer–Viewpoint of the IARC Working Group. N Engl J Med 2016; 375(8): 794–8.
7. Chen Y, Wang X, Wang J et al. Excess body weight and the risk of primary liver cancer: an updated meta-analysis of prospective studies. Eur J Cancer. 2012; 48(14): 2137-45. doi: 10.1016/j.ejca.2012.02.063.
8. Chen Y, Liu L. Wang X et al. Body mass index and risk of gastrin cancer: a meta-analysis of a population with more than ten milion from 24 prospective studies. Cancer Epidemiol Biomarkers Prev. 2013; 22(8): 1395-408.
9. Pischon T, Lahmann PH, Boeing H et al. Body size and risk of colon and rectal cancer In the European Prospective Investigation Into Cancer and Nutrition (EPIC). J Natl Cancer Inst. 2006; 98(13): 920-31.
10. Parr CL, Batty D, Lam TH et al. Obesity, overweight and cancer mortality In the Asia-Pacific Cohort Studies Collaboration: pooled analyses of 424,519 participants. Lancet Oncol. 2010; 11: 741-52.
11. Montazeri A, Sadighi J, Farzadi F et al. Weight, height, body mass index and risk of breast cancer in postmenopausal women: a case-control study. BMC Cancer. 2008; 8: 278. doi: 10.1186/1471-2407-8-278.
12. Tworoger SS, Eliassen AH, Kelesidis T et al. Plasma adipo- nectin concentrations and risk of incident breast cancer. J Clin Endocrin Metab. 2007; 92: 1510–1516.
13. Himbert C, Delphan M, Scherer D et al. Signals from the adipose microenvironment and the obesity-cancer link - a systematic review. Cancer Prev Res. 2017. ; 494-506.
14. Trayhurn P, Wood S, Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr. 2004; 92(3): 347-55.
15. Li-Man Li, Huan Liu, Xing-Hui Liu et al. Clinical significance of exosomal miRNAs and proteins in three human cancers with high mortality in China. Oncol Lett. 2019; 17(1): 11–22.
16. Lu J, Getz G, Miska EA et al. MicroRNA expression profiles classify human cancers. Nature 2005, 435: 834-8.
17. Hursting SD, Digiovanni J, Dannenberg AJ et al. Obesity, energy balance, and cancer: New opportunities for prevention. Cancer Prev Res (Phila) 2012; 5: 1260–72.

18. Sokolova O, Naumann M. NF-κB Signaling in Gastric Cancer.Toxins (Basel).2017; 28:9 (4). pii: E119. doi: 10.3390/toxins9040119.
19. Zuo T, Zhu M, Xu W et al. Iridoids with Genipin Stem Nucleus Inhibit Lipopolysaccharide-Induced Inflammation and Oxidative Stress by Blocking the NF-κB Pathway in Polycystic Ovary Syndrome. Cell Physiol Biochem. 2017; 43(5):1855-1865. doi: 10.1159/000484074.
20. Poczęta M, Bednarek I,STAT3 – ukryty czynnik transkrypcyjny celem terapii przeciwnowotworowych. Ann. Acad. Med. Siles. 2013; 67, 2, 133–141.
21. Bolinder J, Kerckhoffs DA, Moberg E et al. Rates of skeletal muscle and adipose tissue glycerol release in nonobese and obese subjects. Diabetes. 2000; 49: 797-802.
22. Keijer J, van Schothorst EM. Adipose tissue failure and mitochondria as a possible target for improvement by bioactive food components. Curr Opin Lipidol. 2008; 19: 4-10.
23. Koh EH, Park JY, Park HS et al. Essential role of mitochondrial function in adiponectin synthesis in adipocytes. Diabetes 2007; 56: 2973-81,
24. Hotamisligil GS, Peraldi P, Budavari A et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 1996; 271: 665-8.
25. Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J Clin Endocrinol Metab. 2005; 90: 2282-9.
26. Weisberg SP, McCann D, Desai M et al. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003; 112:1796-808.
27. Anisiewicz A, Okła K, Wawruszak A. Makrofagi towarzyszące nowotoworowi – pochodzenie, charakterystyka oraz znaczenie w raku piersi. Review and Research on Cancer Treatment. 2015; 1.
28. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab. 2007; 92: 1023-33.
29. Quail DF, Dannenberg AJ. The obese adipose tissue microenvironment in cancer development and progression. Nat Rev Endocrinol. 2019; 15: 139–54
30. Carter JM, Hoskin TL, Pena MA, i wsp. Macrophagic "crown-like structures" are associated with an increased risk of breast cancer in benign breast disease. Cancer Prev Res. 2018; 11: 113–9.
31 .Quail DF, Dannenberg AJ. The obese adipose tissue microenvironment in cancer development and progression. Nat Rev Endocrinol. 2019; 15: 139–54.
32. Hursting SD, Digiovanni J, Dannenberg AJ, et al. Obesity, energy balance, and cancer: New opportunities for prevention. Cancer Prev Res (Phila). 2012; 5:1260–1272.
33. Dibaba DT1, Judd SE, Gilchrist SC et al. Association between obesity and biomarkers of inflammation and metabolism with cancer mortality in a prospective cohort study. Metabolism. 2019; 94:69-76. doi: 10.1016/j.metabol.2019.01.007.
34. Bachelot T, Ray-Coquard I, Menetrier-Caux C, i wsp. Prognostic value of serum levels of interleukin 6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients. Br J Cancer. 2003; 88: 1721–6.
35. Kicielińska J, Pajtasz- Piasecka E. Rola IL-10 w modulowaniu odpowiedzi odpornos'ciowej w warunkach prawidłowych oraz w s'rodowisku nowotworu. Postepy Hig Med Dosw. 2014; 68: 879-892.
36. Bardou M, Barkub AN, Martel M. Obesity and colorectal cancer. Gut. 2013; 62: 933-47.
37. Rapp K, Schroeder J, Klenk J. Fasting blood glucose and cancer risk in a cohort of more than 140000 adults in Austria. Diabetologia. 2006; 49: 945-52.
38. Logsdon CD, Fuentes MK, Huang EH i wsp. RAGE and RAGE ligands in cancer. Curr Mol Med. 2007; 8: 777-82.
39. Leitner JW, Kline T, Carel K, et al. Hyperinsulinemia potentiates activation of p21Ras by growth factors. Endocrinology. 1997; 138(5): 2211–2214, doi: 10.1210/ endo.138.5.5240.
40. Fromowitz FB, Viola MV, Chao S et al. ras p21 expression in the progression of breast cancer. Hum Pathol.1987; 18(12):1268-75.
41. Alonso A, González C. Relationship between non-genomic actions of estrogens and insulin resistace. Infect Disord Drug Targets. 2008; 8: 48-51.
42. Livingstone C, Collison M. Sex steroids and insulin resistance. Clin Sci. 2002; 102:151-66.
43. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2006; 295:1288-99.
44. Kalish GM, Barrett-Connor E, Laughlin GA, Gulanski BI. Postmenopausal Estrogen/Progestin Intervention Trial. Association of endogenous sex hormones and insulin resistance among postmenopausal women: results from the Postmenopausal Estrogen/Progestin Intervention Trial. J Clin Endocrinol Metab. 2003; 88: 1646-52.
45. Key TJ, Appleby PN, Reeves GK et al. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst. 2003; 95: 1218-26.
46. Noruzinia M, Coupier I, Pujol P. Is BRCA1/BRCA2-related breast carcinogenesis estrogen dependent? Cancer. 2005;104(8):1567-74.
47. Biela A, Pacholska-Bogalska J. Nowotwory hormonozależne u kobiet. Nowa Medycyna 4/2012, s. 76-81.
48. Travis RC, Key TJ. Oestrogen exposure and breast cancer risk. Breast Cancer Res. 2003; 5(5): 239–247.
49. Fui MN, Dupuis P, Grossmann M. Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J Androl. 2014;16(2):223-31. doi: 10.4103/1008-682X.122365.
50. Kelly DM, Jones TH. Testosterone and obesity. Obes Rev. 2015; 16(7):581-606. doi: 10.1111/obr.12282.
51. Kaplan AL, Hu JC, Morgentaler A. Testosterone Therapy in Men With Prostate Cancer. Eur Urol. 2016; 69(5):894-903. doi: 10.1016/j.eururo.2015.12.005.
52. Wang Y, Dai B, Ye DW. Serum testosterone level predicts the effective time of androgen deprivation therapy in metastatic prostate cancer patients. Asian J Androl. 2017; 19(2):178-183. doi: 10.4103/1008-682X.174856.
53. Cao Y, Giovannucci E. Obesity and Prostate Cancer. Recent Results Cancer Res. 2016; 208:137-153.
54. Zoubeidi A, Ettinger S, Beraldi E et al. Clusterin facilitates COMMD1 and I-kappaB degradation to enhance NF-kappaB activity in prostatecancer cells. Mol Cancer Res. 2010; 8(1):119-30. doi: 10.1158/1541-7786.MCR-09-0277.
55. Abufaraj M, Mari A, Mansy K, Obesity and its implications on oncological urological surgery.Curr Opin Urol. 2017 ;27(5):446-455. doi: 10.1097/MOU.0000000000000429.
56. Hashibe M, Abdelaziz S, Al-Temimi M3 et al. Long-term health effects among testicular cancer survivors. J Cancer Surviv. 2016; 10(6): 1051-1057..
57. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev. 2005; 6:13-21.
58. Liu ZQ , Zhang MX, Wang J. Analysis of correlation between the mild cognitive impairment (MCI) and level of adiponectin in elderly patients with type 2 diabetes mellitus (T2DM). Eur Rev Med Pharmacol Sci. 2017; 21(23):5471-5477. doi: 10.26355/eurrev_201712_13937.

59. Banerjee A, Khemka VK, Roy D et al. Role of Serum Adiponectin and Vitamin D in Prediabetes and Diabetes Mellitus. Can J Diabetes. 2017; 41(3):259-265.
60. Di Zazzo E, Polito R, Bartollino S et al. Adiponectin as link factor between adipose tissue and cancer. Int J Mol Sci. 2019; 20. pii: E839. doi: 10.3390/ijms20040839.
61. Tworoger SS, Eliassen AH, Kelesidis T et al. Plasma adiponectin concentrations and risk of incident breast cancer. J Clin Endocrinol Metab. 2007; 92: 1510-6.
62. Sjostrom L, Gummesson A, Sjostrom Cdet al. Effects of bariatric burgery on cancer incidence In obese patietns in Sweden (Swedish Obese Subjects Study): a prospective, controlled intervention trial. Lancet Oncol. 2009; 10: 653-2.
63. Ray A, Cleary MP. The potential role of leptin in tumor invasion and metastasis. Cytokine Growth Factor Rev. 2017; 38: 80-97.
64. Ahn JH, Choi YS, Choi JH. Leptin promotes human endometriotic cell migration and invasion by up-regulating MMP-2 trough the JAK2/STAT3 signaling pathway. Mol Hum Reprod. 2015; 21: 792-802.
65. Liu E, Samad F, Mueller BM. Local adipocytes enable estrogen-dependent breast cancer growth: Role of leptin and aromatase. Adipocyte. 2013; 2: 165-9.
66. Choi J , Cha YJ, Koo JS. Adipocyte biology in breast cancer: From silent bystander to active facilitator. Prog Lipid Res. 2018; 69:11-20. doi: 10.1016/j.plipres.2017.11.002.
67. Li K, Wei L, Huang Y et al. Leptin promotes breast cancer cell migration and invasion via IL-18 expression and secretion.Int J Oncol. 2016; 48(6):2479-87. doi: 10.3892/ijo.2016.3483. Epub 2016..
68. Ahlin C, Lundgren C, Embretsén-Varro E. High expression of cyclin D1 is associated to high proliferation rate and increased risk of mortality in women with ER-positive but not in ER-negative breast cancers. Breast Cancer Res Treat. 2017; 164(3):667-678. doi: 10.1007/s10549-017-4294-5.
69. Madeddu C, Gramignano G, Floris C et al. Role of inflammation and oxidative stress in post-menopausal oestrogen-dependent breast cancer. J Cell Mol Med. 2014; 18(12): 2519–2529. doi: 10.1111/jcmm.12413.
70. Ma Y1, Liu Z, Zhang Y et al. Serum leptin, adiponectin and endometrial cancer risk in Chinese women. J Gynecol Oncol. 2013; 24(4):336-41. doi: 10.3802/jgo.2013.24.4.336.
71. Zhang Y, Liu L, Li C et al. Correlation analysis between the expressions of leptin and its receptor (ObR) and clinicopathology in endometrial cancer. Cancer Biomark. 2014; 14(5):353-9. doi: 10.3233/CBM-140415.
72. Kato S, Abarzua-Catalan L, Trigo C et al. Leptin stimulates migration and invasion and maintains cancer stem-like properties in ovarian cancer cells: an explanation for poor outcomes in obese women. Oncotarget. 2015; 6(25):21100-19.
73. Erkasap N, Ozkurt M, Erkasap S et al. Leptin receptor (Ob-R) mRNA expression and serum leptin concentration in patients with colorectal and metastatic colorectal cancer. Braz J Med Biol Res. 2013; 46(3): 306–310. doi: 10.1590/1414-431X20122559.
74. Byers T, Sedjo RL. Body fatness as a cause of cancer: epidemiologic clues to biologic mechanisms.Endocr Relat Cancer. 2015; 22(3):R125-34. doi: 10.1530/ERC-14-0580.
75. Wang N, Wang QJ, Feng YY et al. Overexpression of chemerin was associated with tumor angiogenesis and poor clinical outcome in squamous cell carcinoma of the oral tongue. Clin Oral Investig. 2014; 18(3):997-1004. doi: 10.1007/s00784-013-1046-8.
76. Wang C, Wu WK, Liu X, To KF, Chen GG, Ng EK. Increased serum chemerin level promotes cellular invasiveness in gastric cancer: a clinical and experimental study. Peptides. 2014; 51: 131-8.
77. Siemińska L, Borowski A, Marek B et al. Serum concentrations of adipokines in men with prostate cancer and benign prostate hyperplasia. Endokrynol Pol. 2018; 69(2):120-127.
78. Eichelmann F, Schulze MB, Wittenbecher C et al. Association of Chemerin Plasma Concentration With Risk of Colorectal Cancer. JAMA Netw Open. 2019; 2(3):e190896. doi: 10.1001/jamanetworkopen.2019.0896.
79. Kiczmer P, Szydło B, Seńkowska A et al. Serum omentin-1 and chemerin concentrations in pancreatic cancer and chronic pancreatitis. Folia Med Cracov. 2018; 58(2):77-87.
80. Li JJ, Yin HK, Guan DX et al. Chemerin suppresses hepatocellular carcinoma metastasis through CMKLR1-PTEN-Akt axis.Br J Cancer. 2018; 118(10):1337-1348. doi: 10.1038/s41416-018-0077-y.
81. Sieri S, Krogh V, Agnoli C et al. Dietary glycemic index and glycemic load and risk of colorectal cancer: results from the EPIC-Italy study. Int J Cancer. 2015; 136(12):2923-31. doi: 10.1002/ijc.29341.
82. Allott EH, Arab L, Su LJ et al. Saturated fat intake and prostate cancer aggressiveness: results from the population-based North Carolina-Louisiana Prostate Cancer Project. Prostate Cancer Prostatic Dis. 2017; 20(1):48-54. doi: 10.1038/pcan.2016.39.
83. Loo TM, Kamachi F, Watanabe Y et al. Gut Microbiota Promotes Obesity-Associated Liver Cancer through PGE2-Mediated Suppression of Antitumor Immunity. Cancer Discov. 2017;7(5):522-538. doi: 10.1158/2159-8290.CD-16-0932.

Konflikt interesów

Autorzy deklarują brak konfliktu interesów.

AUTOR KORESPONDUJĄCY
Jakub Warakomski
ul. Grottgera 10/1, 41-800 Zabrze, Polska
tel. 509549907
e-mail: kuba.warakomski@gmail.com

Nadesłano: 30.05.2019
Zaakceptowano: 18.07.2019

PRACA POGLĄDOWA
REVIEW ARTICLE

PILONIDAL DISEASE: CHANGES IN UNDERSTANDING OF ETIOLOGY, PATHOGENESIS AND APPROACH TO TREATMENT

Viktor Konoplitskyi, Ruslan Shavliuk, Dmytro Dmytriiev, Kostiantyn Dmytriiev, Oleksii Kyrychenko, Bohdan Zaletskyi, Oleksandr Olkhomiak
NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY , VINNYTSIA, UKRAINE

ABSTRACT
Data from Web of Science, SCOPUS, Pub Med, Medline, E-library, and other sources was used in writing this article. The main focus was directed towards literature written in English. The selection of literature was based on such concepts as: etiopathogenesis, historical principles of treatment, methods of surgical and non-surgical intervention. Data from metanalysis publications and randomized clinical trials pertaining to the treatment of the pilonidal sinus at various stages of its formation was used, as well.

KEY WORDS: pilonidal disease, analysis, literary sources

Wiad Lek 2019, 72, 8, 1559-1565

INTRODUCTION

Pilonidal disease (PD), or the epithelial coccygeal passage (ECP) is defined as a thin epithelial tube, located in the subcutaneous tissue of the intergluteal cleft, at the level of the coccyx, or less frequently at the level of the sacrum. The disease is characterized by recurrent, or chronic inflammation. Often ECP is 2-5 cm in length and ends blindly in the soft tissue at the top of coccyx. On the skin it can present as a single, or less frequently as multiple spot-like, round openings in the intergluteal cleft. The openings are frequently located within 0.3 – 1 cm from one another. The primary openings often have tufts of hair growing from them. Additionally, PD can manifest as secondary, abscess-like fistulas.

The first description of PD appears in the works of A. W. Anderson in a letter to Boston Medical and Surgical Journal, titled "Hair Extracted from an Ulcer". Anderson described a fistula that contained hair, which he found near the coccyx of his patient [1]. Later, in 1854, J. M. Warren added to the works of Anderson, by describing PD in greater detail, providing guidelines for treatment, as well as proposing the first hypothesis for the development of PD. Warren thought that the etiology involved atypical hair growth (from superficial to deep, instead of the normal deep to superficial) in the intergluteal cleft [2]. Later yet, in 1880, R. M. Hodges described PD as a separate disease, naming it for the Latin words "pillus" – hair and "nidus" – nest [3].

Since then, there has been a wide variety of medical terms, which describe the same pathological process: epithelial coccygeal passage, pilonidal cyst, pilonidal sinus, extra dermal sacro-coccygeal sinus, sacral epithelial indentation, epithelial coccygeal fistula, epithelial coccygeal cyst, sacral funnel, "posterior umbilicus", sequestral dermoid, dermoid cyst, coccygeal fistula, Sinus of Grayje, Buie Pilonidal Disease, Bredow's Suture Fistula, mucous cyst, cystic hygroma, pilonidal dimple, pilonidal fistula, coccygeal pits, fovea coccygea, Jeep Disease, sacrococcygeal fistula, ectodermal cyst, sacrococcygeal ectodermal sinus, postanal sinus, congenital postnatal dermal fistulae, and Barber's Disease. The sole multitude of terms used to describe PD throughout history reflects the amount of research put into studying the disease, at the same time, however, introducing confusion, and making apparent the difficulty in understanding the pathology of the disease, difficulty in finding conclusive literary sources, and difficulty in evaluating the said sources.

REVIEW AND DISCUSSION

PREVALENCE

In the general population, PD is seen with frequency of 26 per every 100,000 individuals, among whom the majority are males (2:1) [4,5]. The majority of cases present in individuals between 15 ad 30 years of age [6]. The average age patients at the time of diagnosis is 21 in males, and 19 in females [6]. PD is most often seen in individuals of European descent and those of Arabic and Caucasus Mountain Region descent. The disease is seen much more rarely in individuals of African descent [7]. Researches have noted a correlation between the prevalence of PD and certain morphological factors, such as: density of hair growth, obesity, and depth of the intergluteal fold. Certain lifestyle factors, such as: time spent sitting, and time spent walking have been found to play a role in the development of PD. Certain authors cite genetic factors that may predispose the individual to PD [8].

ETIOLOGY AND PATHOGENESIS

Often, PD is asymptomatic for long periods of time, as it doesn't cause, the child or his/her parents any discomfort. Still some parents choose to consult a surgeon with concerns over apparent openings near their child's coccyx, or sacrum. PD is also often discovered during routine health maintenance procedures. According to some authors, PD should not be considered a disease due to its asymptomatic nature. Rather, according to these authors, PD should be considered a disease from the time of its first clinical manifestations, which present in the form of an abscess in the sacrococcygeal region.

Discussions over the etiology pathogenesis of PD continue since its first description. It's worth noting that researchers residing in post-Soviet countries exhibit cardinally different views of PD than their Western counterparts. This tendency is reflected in the different approaches to treatment of patients with PD.

The existing theories of PD etiology and pathogenesis can be divided into several categories: I – historical theories, II – the neurogenic theory, III – the ectodermal theory, IV – the theory of acquired PD.

The first category has strictly historical significance, as it's based on the propositions of physicians living in 18th – early 20th century, which were predominantly not based on clinical findings. For example, J. M. Warren in 1854 explained the appearance of fistulas in the coccygeal region by pathological behavior of hair follicles in the same region.

The neurogenic theory has found its reflections in the works of F. G. Mallory (1892), who found neural tube remnants lined with cylindrical and cuboidal epithelium in embryos during 3-6 months of gestation. M. Cage (1939) has found in his research a 2-chambered cyst in the coccygeal region. The cysts were connected by a passage, which he proposed exhibited neurogenic etiology. In 1887 F. Tourneax and G.J Hermann have proposed that the epithelial coccygeal passage develops from rudimentary spinal cord remnants. During embryogenesis the cranial end of the spinal cord (which forms the brain), and the caudal end (which attaches to the skin in the intergluteal fold) becomes fixed. During the 3rd month of gestation, uneven growth of the spine and the spinal cord is noted, as a result of which, the latter eventually becomes thinner. During further development, normally, the thinned region of the spinal cord (filum terminale) partially involutes, and partially remains in the form of a trough lined by cylindrical epithelium. This trough will normally disappear by the end of the 6th month of gestation. In the case of failure of this trough to involute PD may arise.

Various theories of congenital PD are often grouped together under "ectodermal origin". The beginnings of the theories of ectodermal origin can be traced back to the works of O. Lannelongue (1882), who proposed that PD arises as a result of ectodermal invagination into the intergluteal fold. This invagination, Lannelongue thought, occurs due to disproportional development of subcutaneous fat tissue in the coccygeal region, as a result of abnormal skin attachment within the region. Independently, C. Gussenbauer (1893) and E. Crone (1917) have discovered that pilonidal cysts exhibit tissues of dermal origin, complete with hair follicles and sweat glands.

H.B. Stone (1931) connected the development of PD to changes observed in puberty. Stone made his observations in the uropygial glands, which are secondary sex organs in avian species [9, 10].

The findings of L. Tail (1924) are considered to be the most well founded, and well-studied. Tail proposed that the invagination of ectoderm towards the floor of the intergluteal cleft is connected to the reduction of coccygeal vertebrae (theory of tail`s ligament). This theory is based on the particular pattern of development seen in the caudal end of the spinal column. During the 5th-6th week of gestation the coccyx is composed of 9 vertebrae, which during further development will reduce to only 5 vertebrae. If this reduction does not occur, pilonidal cysts may form.

In modern literature, the theory of acquired PD has gained popularity. This theory encompasses several different proposed mechanisms of PD development. R. M Hodges (1880) is considered to be the first to propose the acquired etiology of PD. Hodges stated that the PD arises as a result of entrapment of hair follicles within the coccygeal passage. He further noted that these trapped follicles originate from neighboring skin regions, rather than from within the coccygeal passage itself. R. M. Hodges has also stated that the presence of asymptomatic coccygeal passages in itself should be considered a pathology, rather the entrapment of hair follicles within them, and subsequent inflammation of the passages is what defines Pilonidal Disease. In the later years, studies of the anatomical and physiological features of the sacrococcygeal region have been conducted, in an effort to explain how these features may effect the development of PD. In 1934 M. Burgdorf has turned his attention towards connective tissue adhesions between the sacral bone and the skin. He also noted that excess development of subcutaneous fat in this region leads to the development of traction diverticula, in which inflammation may arise as a result of accumulation of the various metabolic products of the skin.

In 1952 W.P Kleitsch and L. Cherry connected PD with such morphological features as a narrow pelvis, and a deep intergluteal fold [11]. These factors, according to the authors, promote friction, excess sweating and maceration within the intergluteal fold, which lead to the formation of a "weak spot", into which microbes and hair may penetrate.

In 1954 O.N. Davage proposed that PD is of inflammatory origin, stating that hair may be a secondary factor in the pathogenesis, while inflammation within the intergluteal fold should be considered the primary factor, due to which the epithelial coccygeal passage forms [12]. O.N. Davage considered mechanical injury of the epithelium, namely by use of coarse toilet paper to be one of the key factors in the formation of pilonidal cysts. This notion was later supported by R.M. Hardawa (1958), who found small pieces of toilet paper inside a pilonidal cyst [13].

In 1959 R.A. Raffman connected the development of PD to the penetration of hair into the macerated skin,

however, he proposed that the subsequent epithelization of these macerated regions may be due to the epithelial cells, which have been introduced into the region together with the hairs [14].

A year earlier, D.H. Patey and R.W Scarff (1958) demonstrated that the presence of hairs within the epithelial coccygeal passages isn't simply due to chance, but rather due to the presence of negative pressure within the lumen of the epithelial passage, which in turn can be explained by rhythmic contraction and relaxation of gluteal muscles [15]. According to the researches, this pump-like mechanism may explain the high incidence of PD among American troops (predominantly those serving in the mechanized infantry platoons) during the Second World War. The disease was seen especially often in Jeep drivers. This gave PD one of its many names – "the Jeep Disease" [16].

In 1980 J. Bascom proposed the follicular retention theory of acquired PD, which is widely accepted today among most researchers. The differentiating characteristic of this theory is in that the author was the first to propose a direct connection between the formation of pilonidal passages and inflammation of hair follicles, located in the midline of the intergluteal fold floor. The Author describes in detail a "trichogenic-pump" mechanism of secondary ulcer formation. Bascom further proposes 6 stages of PD development, characterizing each stage by morphological findings:

Stage I (a dilated hair follicle) – excess accumulation of keratin takes place, often as a result of some precipitating factor (increased production of keratin, as a result of hormonal imbalances during puberty, decreased outflow of keratin as a result of the tight closure of the intergluteal cleft in the sitting position, inflammation as a result of poor personal hygiene, an inflammation and swelling due to injury, or bunt force trauma of sacrococcygeal region)

Stage II (an infected hair follicle, or acute pyogenic folliculitis) – an infection of the follicle occurs, with subsequent spread of the infection to the neighboring subcutaneous fat tissue.

Stage III (an acute pilonidal abscess) – the hair follicle ruptures at its weakest point, more often at its base, due to excess pressure during sitting, or due to multiple force vectors in the standing position.

Stage IV (formation of a primary fistula, or the development of a chronic pilonidal abscess) –the inflammatory exudate drains, while the acute process becomes chronic, preventing the fistula from healing.

Stage V (formation of a primary epithelial passage) – the epithelial passage develops from cells of the basal cell layer beneath the ruptured follicle.

Stage VI (formation of secondary epithelial passages) –propagated by the "trichogenic pump", which first leads to the accumulation, and subsequently to the penetration of hair from neighboring skin regions into the primary epithelial passage. This process occurs via the "fish hook" mechanism (cutaneous protrusions allow the movement of hairs in only one direction). Concurrently, there is inflammation in the underlying subcutaneous fat tissue, which leads to the formation of a secondary acute abscess, which in turn drains, forming a secondary fistula. This secondary fistula becomes chronically inflamed (secondary chronic pilonidal abscess). In the absence of adequate treatment, eventually the walls of the secondary fistula will undergo epithelization with cells from the surface of the skin [17,18,19,20].

J. Bascom's theory was supported and further developed by G.E. Karyadaki, who in 1992 published his own research based on the observation and treatment of 6000 patients. He noted that cut hairs, imbedded in a normal tissue can trigger a foreign body response. Furthermore, Karyadaki formulated a theory of PD pathogenesis based on 3 variables: shaved hairs (H), which is acted on by some force (F), which is the result of some secondary factors, such as depth of the intergluteal fold, width of the gluteal fold, and the amount of friction within it. This force is what causes the penetration of shaved hairs into the fistula. The third factor is some sustained trauma of the skin and the soft tissues (V) [24]. Therefore, the formula reads as:

$PD = H*F*V^2$

A.V. Kulyapin (1989) found by investigating the hair follicles and the sebaceous glands under the microscope, the latter were without changes. Conversely, he has found notable changes in the observed sweat glands, from the same region. Based on these findings, Kulyapin proposed that acute Hidradenitis Suppurativa, which arises secondary to blockage of sweat gland outflow channels by hairs and various other debris may be the cause of PD. It is worth noting that sweat glands in the intergluteal folds are apocrine, and so they begin to function at the beginning of puberty.

METHODS OF TREATMENT

Radical surgical treatment is currently the universally accepted method of treatment of PD. However, even today there isn't a single universal approach which satisfies both; the physician, and the patient. Therefore, specialists within the surgical and coloproctological fields continue developing new surgical approaches. In these developments, the researchers seek to decrease the recurrence of disease, especially during periods of remote observation. Researchers seek to minimize the amount of postoperative pain, and the frequency of postoperative infection. Finally, they aim to decrease the amount of time for which the patient must remain in the hospital following the operation, likewise, they seek to minimize the recovery time period [21,22].

Overall, all existing surgical treatments of PD can be divided into 4 categories: conservative surgical intervention, open surgical intervention, closed surgical intervention, and minimally invasive surgical intervention.

Acetic acid, Ethanol and Chloroform, as well as other so-called sclerosing agents are used to treat PD non-surgically. Electrocoagulation, and cauterization with acetic acid or nitrous oxide have remained common options until the end of the 20th century [24]. In 1956 A.A. Klaus only used strict measures of hygiene, as well as shaving in the treatment of his PD patients. According to Klaus, he saw

recovery in 89.3% of all cases [25].

B. Mauirece and R. Greenwood were known components of conservative treatment methods. In 1964, they performed injections of 40% and 80% phenol into their patients' coccygeal passages. [26]. Subsequent randomized trials, which compared the phenol injection method to surgical intervention, have shown that recurrence of disease was higher in the case of the former. However, conservatively treated patients noted less pain, and were able to return to carrying out their normal daily activities sooner, compared to those who were treated surgically [27,28]. Later D. Ahmet [2012] treated 83 PD patients with 80% phenol, and found that on average, the patients were able to return to their normal daily activities in 2-3 days, while there was a 13.3% rate of recurrence [29].

Open surgical approaches to the treatment of PD imply excision of the pilonidal cyst, achievement of hemostasis, and treatment of the cavity with antiseptic agents. The wound remains open, which leads to healing by secondary intention. This technique has been reported to show a low rate of PD recurrence, as well a low rate of infectious complications. This is attributed to the unobstructed drainage of fluid from the wound's surface. Another advantage of open surgical approaches is the relative simplicity of the techniques, which causes these techniques to be favored in many countries around the world. One notable disadvantage of the open surgical approaches is the relatively long period of time the patient must remain in the hospital, due to the long time required for complete healing of the wound (several months, or more). At the same time, the patients tend to dislike the visual appearance of the scar (which often undergoes keloidal transformation, and ulceration). Patients also tend to experience itching, reduced mobility in the region, and discomfort in the sitting and squatting positions [30].

In order to reduce the duration of patients' stay in the hospital, and the over-all healing time, a wide variety of modified open approaches have been proposed. These modified techniques imply the suturing of the wound's edges to its floor, thus reducing the wound's area, and reducing the duration of time, in which the wound heals by secondary intention. These techniques have been demonstrated to only marginally reduce the time spent in hospital, meanwhile no notable improvements along other parameters have been noted at all. This category of surgical approaches includes the so-called "marsupialization of the pilonidal cyst", and its many variants, proposed by Obeid, McFee, and others [31].

In 2014 I. Varnalidis et al published their research, in which they compared 3 methods of surgical treatment of PD: the open approach, the marsupialization approach, and the closed approach with complete suturing of the wound along the midline. The researchers have determined that the average amount of time the patients took to recover after the marsupialization method was 27.3 days, with a maximum of 40 days. The closed approach with tight suturing along the midline showed a recovery time of 11.7 days, with a maximum of 15 days. Finally, the patients recovered in 46.4 days, on average (with a maximum of 90 days) after the open surgical approach with healing by secondary intention. The researches also noted the rates of PD recurrence, which for the marsupialization method, and the open approach were observed to be 6.35% and 3.45%, respectively. Meanwhile the rate of PD recurrence after the closed approach with tight suturing was a much higher 57.8% [32].

The closed, midline suturing approach falls under the third category of surgical PD treatment. The technique is also relatively wide-spread around the world, mainly because of its simplicity. After the cyst is excised, the wound is sutured with a simple surgical knot along the midline. This approach, and all other closed approaches encourage healing of the wound by primary intention, thus decreasing post-operative recovery time, and decreasing the size of the scar. One notable disadvantage of this technique is a high rate of PD recurrence, and a high rate of infection. This can be attributed to obstructed draining, increased tension along the suture line, and increased friction between tissues in the area [33].

In 2015 Doll D. et al published their analysis based on findings made in the treatment of patients with PD over 20 years. In their analysis, they compared the outcomes of open and closed surgical approached. According to the data, the recurrence rate after 20 years of observation post-surgery was higher in the closed approached with tight suturing, than in the open approach, with rates of 44% and 28%, respectively. Also, according to the gathered data, the researchers have concluded that the though the separation of wound edges during the healing process lengthens recovery time, it has no statistically significant influence on the rate of PD recurrence, at the 5 and 10 years of observation [34].

A Al-Khamis et al (2010) have composed a meta-analysis of PD patient treatment with various open and closed surgical approaches, in which the authors have found that when comparing the two, neither showed any significant advantage over the other. As with other similar studies, when assessing the duration to complete healing, closed approaches have been found to take on average 15 days to heal, while the open approaches took on average 41 days to heal. Conversely, open techniques have shown a much lower rate of PD recurrence (12.5% against 24% observed in closed techniques). No statistically significant difference in rate of post-operative infection was noted between the two. However, a significant advantage of the "lateralization technique", which is based on suturing laterally from midline, was shown over the tight midline suture method. The lateralization technique demonstrated healing times reduced by 5.4 days, compared to the tight midline suture technique. Frequency of infection was also noted to be reduced by 3.72%, while frequency of recurrence was observed to be reduced by 4.54% [35].

Proponents of the acquired origin theory have proposed a new view on the current methods of surgical intervention, stating the importance of forming the postoperative wound on the buttock, instead of at the floor of the intergluteal

cleft. They argue for this approach based on the assumption that a surgical wound formed at the floor of the cleft is under the influence of many unfavorable forces. Instead, these researchers propose moving the wound by 3-4cm laterally, thus somewhat flattening the intergluteal cleft, and according to them, reducing the possibility of hairs entering the wound and formation of a "residual cavity". The latter can be explained by better alignment of wound edges and the decrease in tension between tissues [42,43].

G.E. Karydakis (1973) first proposed the use of the aforementioned "eccentric" suturing technique, with mobilization of two "flabs" of skin after the pilonidal cyst has been excised. This method has gained popularity for its reported high efficacy. Later, in 1992, the author has provided a convincing base of evidence, grounded on the treatment of over 6500 patients, according to which, the rate of PD recurrence was 1%, rate of complications – 8% and the amount of time spent in hospital was on average 3 days, with a home recovery time of 15 days on average [36].

Subsequently, other researchers have attained date similar to that of G.E Karyadakis. Can et al [37], in their research have found frequency of PD recurrence to be 4.6%, while post-operative complications were noted in 8.69% of all cases [37]. According to the data gatered by S. Sozen et al (2010), the rate of recurrence was noted to be at 1.5%, while the rate of complications was noted to be 6% [38]. M. Sakr et al (2003) have reported a 2.4% rate of recurrence, while complications were noted in 10% of patients during their in hospital stay (3 days). Additionally, the Karydakis method was shown to be highly effective in treating obese patietns (BMI > 26.5 kg/m^2) [39].

The "rhomboidal plastic method" proposed by A. Limberg in 1946 is currently another widespread surgical technique for the treatment of PD. This method is characterized by resecting the pilonidal cyst rhomboidally and covering the surface of the wound with an accordingly shaped piece cutaneous-subcutaneous tissue, resected from the buttock. This placement of 2 adjacent triangular pieces of tissue over the wound provides an adequate redistribution of tension. The main advantage of this technique is in the low rate of recurrence and postoperative complications [23].

In 1929 F. Lahey was first to successfully cover the surgical wound after resecting a pilonidal cyst with a cutaneous-subcutaneous pedunculated graft. However, this approach saw a large number of recurrences, and was a fairly complicated procedure over-all, and therefore never gained widespread popularity [60]. R.H Fishbein (1979) performed a closing of the defect with a cutaneous-subcutaneous-fascial graft, which allowed for a decrease in recurrence to 2.5% over 36 months of postoperative observation [56]. R.S Monroe and F.T. McDermott (1965) used a so-called "Z-like plastic method", the results of which were inconclusive: despite the low rate of recurrence (up to 8.9%), major postoperative complications, such as necrosis of the graft (2.5%), formation of keloids (up to 14%), diminished sensitivity of the soft tissues, and unsightly scars (up to 50%) were noted [57,58,59,60]. R.F Roth et al (1977) proposed a "W-like plastic method", which also never gained popularity [61].

In 2015 I. Bali et al conducted a comparative analysis between the Karydakis and Limberg techniques, in which the researches have noted the Limberg technique to be advantageous according to all parameters: the duration of hospital stay – 1,4 against 3 days, early regaining of mobility by the patient – 1 against 2 days post-surgery, return to normal activities – 8 against 17 days, duration of overall healing time – 22 against 24 days. Finally, it was found that the Limberg technique resulted in higher overall patient satisfaction [40]. However, M. Ates et al (2011), in their research have noted a lower rate of recurrence in patients treated with the Karydakis method, as opposed to the Limberg method [41].

According to many modern authors, surgical techniques completed by lateralization of the wound show better results compared to other surgical approaches, in which lateralization is not used [41].

As to the closed surgical techniques and their modifications, researchers list the inactivation, of the trichogenic pump, due to the reduction in depth, or the complete flattening of the intergluteal cleft. This results in the reduction of skin debris present, normally present in the cleft, and reduces the possibility of hairs infiltrating the wound. The residual cavity is also less likely to form [45].

J. Bascom, as a proponent of the acquired theory of PD pathogenesis, proposed a number of surgical techniques in the 1980ies. One such technique is called Bascom I, and it is based on subdermal removal of the pilonidal cyst, beginning at the primary opening of the pilonidal fistula, which is later sutured, and finishing at the secondary opening, through which drainage later occurs. These measures reduced pressure within the postoperative wound, and allowed Bascom to achieve an average postoperative healing time of 39 days. 68% of the wounds were noted to heal by primary intention, with recurrence of 7.3% [23].

Some time later, J. Bascom developed a new surgical approach, called Bascom II (cleft lift), according to which, after the subdermal excision of the cyst from the intergluteal cleft, a thick skin flap is formed, which is place eccentrically, thus closing the residual openings within the cleft. At the end of the procedure, the intergluteal fold becomes "smoothed out", in other words maximally flattened. The Bascom II procedure has been demonstrated to be especially effective during repeated surgeries [20,23].

According to the majority of modern researchers, shaving, or laser removal of hairs surrounding the postoperative wound until the time of its full healing is crucial to the success of surgical intervention aimed at treating PD. Researchers also agree that maintenance of through personal hygiene around the postoperative area is very important [48,49,50,51].

Another important aspect of surgical intervention is a through and precise alignment of the postoperative wound edges. If the edges are not well aligned, a so-called "false recurrence" of PD may occur, characterized by uneven healing, and formation of a residual cavity underneath the scar, with subsequent penetration of hair inside the said cavity [52,53].

C. Soll (2001) proposed a minimally invasive method of PD treatment, characterized by subdermal resection of the primary and secondary fistulas, followed by the formation of a tunnel in the subcutaneous fat tissue, which connects the proximal and distal openings. According to the author, notable advantages of this method are in the formation of a relatively small residual cavity, a low likelihood of deformation of the sacrococcygeal region, a low rate of recurrence (7%), minimal postoperative pain, and a short healing time (under 10 days) [62].

P. Meinero (2013) proposed a technique of endoscopic pilonidal sinus treatment (EPSiT) characterized by the destruction of coccygeal passages by a monopolar coagulator, with assistance of a fistuloscope, inserted through the secondary opening. This technique allows for the removal of hair, hair follicles, and various other debris from within the fistula [63]. A. Mortiz et al (1997) proposed treating PD with a carboxide laser [59] .

I.M. Nordon et al (2009) conducted a study, comparing the outcomes of Bascom I and Bascom II techniques. According to the results, the average healing time following Bascom I procedure was 4-4.5 weeks, while the average healing time after Bascom II procedure was 10-13 days [46].

M.H Abo-Ryia et al (2017) published the results of treatment according to a modified Karydakis procedure. During the surgery, the authors mobilized a fascial-dermal-subcutaneous graft, which was subsequently attached to sacral fascia, resulting in a satisfactory outcome in 96.7% of the patients [47].

In 2018 a team of researchers submitted a metanalysis, in the form of a general evaluation of data , in which they gathered results from a total of 101313 studies by various authors, published from 1833 to 2017. From the gathered data, the team or researchers concluded that Karydakies, Bascom, Limberg and Dufourmentel procedures, as well as their modified variants show the least amount of recurrence [54].

CONCLUSIONS

In summary, it can be concluded that PD is an important socio-economic issue, as the patients are most often work-able adults (16-25 years of age). However, existing methods of treatment don't entirely satisfy neither the physicians, nor their patients, due to a high incidence of less than favorable outcomes. Furthermore, despite the extensive research, there still doesn't exist a universal consensus as to the etiology of PD and its definitive pathogenesis.

REFERENCES

1. Anderson A.W. Hair extracted from an ulcer Boston Med. and Surg. J.1847;36:74.
2. Warren J.M. Abscess containing hair on the nates. Am. J. Med. Sci.1854;28:113-117.
3. Horwood J., Hanratty D., Chandran P., Billings P. Primary closure or rhomboid excision and Limberg flap for the management of primary sacrococcygeal pilonidal disease. A meta-analysis of randomized controlled trials. Colorectal Dis. 2012 Feb;14(2):143-51
4. Anil Sunkara, Wagh D. D, Sameer Harode. Intermammary Pilonidal Sinus. Int. J Trichology. 2010; 2:116-118.
5. Marco Gallinella Muzi, M.D., Giovanni Milito, M.D., Federica Cadeddu, M.D., Casimiro Nigro, M.D., Federica Andreoli, M.D., Dalia Amabile, M.D., Attilio Maria Farinon, F.A.C.S.? Randomized comparison of Limberg flap versus modified primary closure for the treatment of pilonidal disease, The American Journal of Surgery. 2010; 200:9–14.
6. Khanna A., John L., Rombeau M. Pilonidal Disease. Clin Colon Rectal Surg. 2011;1:46–53.
7. Fansler W.A., Anderson J.K. Case of pilonidal sinus a negro. Minnesota Med. 1934;17:146-147.
8. Doll D., Matevossian E., Wietelmann K., Evers T. Family history of pilonidal sinus predisposes to earlier onset of disease and a 50% long-term recurrence rate. Dis Colon Rectum. – 2009; 9:5-11.
9. Stone H.B. Pilonidal sinus. Ann Surg. 1924;79(3):410-414.
10. Stone H.B. The origin of pilonidal sinus (coccygeal fistula). Ann Surg. 1931;94:317–320.
11. Kleitsch W.P., Cherry L.D. Pilonidal sinus; a rational scheme of treatment. Mil Surg. 1952;111(2): 96-106
12. Davage O.N. The origin of sacrococcygeal pilonidal sinuses. American jour. pathol. 1954;30(6):1191-1205
13. Hardaway R.M. Pilonidal cyst: neither pilonidal nor cyst. AMA Arch Surg.1958;76(1):143-147.
14. Raffman R.A. A re-evaluation of the pathogenesis of pilonidal Sinus. Ann Surg. 1959;150(5):895-903.
15. Patey D.H., Scarff R.W. The hair of the pilonidal sinus. Lancet. 1958;9(268):772-773.
16. Buie L.A. Jeep disease. South Med Journal. 1944;37:103-109.
17. Bascom J. Pilonidal disease: origin from follicles of hairs and results of follicle removal as treatment. Surgery. 1980; 87(5):567-572.
18. Bascom J. Pilonidal disease: origin from follicles of hairs and results of follicle removal as treatment. Surgery. 1980:87(5):567-572.
19. Bascom J., Bascom T. Failed pilonidal surgery: new paradigm and new operation leading to cures. Arch Surg. 2002;137(10):1146-1151.
20. Bascom J., Bascom T. Utility of the cleft lift procedure in refractory pilonidal disease. Am. J. Surg. 2007;193(5):606-609.
21. Kose E., Hasbahceci M., Tonyali H., Karagulle M. Comparative analysis of the same technique-the same surgeon approach in the surgical treatment of pilonidal sinus disease: a retrospective cohort study. Ann Surg Treat Res. 2017;93(2):82-87.
22. Tavassoli A., Noorshafiee S., Nazarzadeh R. Comparison of excision with primary repair versus Limberg flap. Int J Surg. 2011;9:343-346.
23. Khanna A., Rombeau J.L. Pilonidal Disease. Clin Colon Rectal Surg. 2011; 24(1):46-53.
24. Rignoult D. Traitement non operatoire des fistules pilonidal. Gastroent. clin. Biol. 1977;11:941-944.
25. Klass A.A. The so called pilonidal sinus. Canadian Medical Association Journal. 1956;75:53-57.
26. Maurice B.A., Greenwood R.K., A conservative treatment of pilonidal sinus. Br J Surg. 1964;4:45-48.
27. Gulpinar K., Pampal A., Ozis S., Kuzu M. Non-operative therapy for pilonidal sinus in adoles-cence: crystallised phenol application, 'report of a case'. BMJ Case Rep. 2013;5:88-91.
28. Olmez A., Kayaalp C., Aydin C. Treatment of pilonidal disease by combination of pit excision and phenol application. Tech Coloproctol. 2013;2:201-216.
29. Ahmet D., Tahsin C. Ozgur T. Phenol procedure for pilonidal sinus disease and risk factors for treatment failure. Surgery. 2012;151:113-117.

30. Karydakis G E. Easy and successful treatment of pilonidal sinus after explanation of its causative process. Aust N Z J Surg. 1992;62(5):385–389.
31. Khanna A., Rombeau J.L. Pilonidal Disease. Clin Colon Rectal Surg. 2011;24(1):46-53.
32. Varnalidis I., Ioannidis O., Paraskevas G., Papapostolou D., Malakozis S., Gatzos S., Tsigkriki L., Ntoumpara M., Papadopoulou A., Makrantonakis A., Makrantonakis N. Pilonidal sinus: a comparative study of treatment methods. J. Med. Life. 2014;7(1): 27-30.
33. Khanna A., Rombeau J.L. Pilonidal Disease. Clin Colon Rectal Surg. 2011;24(1):46-53.
34. Doll D., Matevossian E., Luedi M.M., Schneider R., Zypen D., Novotnyc A. Does full wound rupture following median pilonidal closure alter long-term recurrence rate? Med Princ Pract. 2015;24(6):571-577.
35. Al-Khamis A., McCallum I., King P.M., Bruce J. Healing by primary versus secondary intention after surgical treatment for pilonidal sinus. Cochrane Database Syst Rev. 2010 Jan 20. Pub. 3.
36. Karydakis G.E. Easy and successful treatment of pilonidal sinus after explanation of its causative process. Aust. J Surg. 1992;62(5):385-389.
37. Can M.F., Sevinc M.M., Yilmaz M. Comparison of Karydakis flap reconstruction versus primary midline closure in sacrococcygeal pilonidal disease: results of 200 military service members. Surg. Today. 2009;39(7):580-586.
38. Sozen S., Topuz O., Donder Y., Emir S., Ozdemir C.S., Keceli M. et al. Comparison between Karydakis flap repair and Limberg flap for surgical treatment of sacrococcygeal pilonidal sinus (short term results). Turk J Surg. 2010;26(3):153-156.
39. Sakr M., El-Hammadi H., Moussa M., Arafa S., Rasheed M. The effect of obesity on the results of Karydakis technique for the management of chronic pilondal sinus. Int J Colorectal Dis. 2003;18(1):36-39.
40. Bali I., Aziret M., Sozen S., Emir S., Erdem H., Çetinkunar S., Irkorucu O. Effectiveness of Limberg and Karydakis flap in recurrent pilonidal sinus disease. Clinics (Sao Paulo). 2015;70(5):350-355.
41. Ates M., Dirican A., Sarac M., Aslan A., Colak C. Short and long-term results of the Karydakis flap versus the Limberg flap for treating pilonidal sinus disease: a prospective randomized study. Am J Surg. 2011;202(5):568-573.
42. Mentes B.B., Leventoglu S., Cihan A., Tatlicioglu E., Akin M., Oguz M. Modified Limberg transposition flap for sacrococcygeal pilonidal sinus. Surg Today. 2004;34(5):419-423.
43. Kose E., Hasbahceci M., Tonyali H., Karagulle M. Comparative analysis of the same technique-the same surgeon approach in the surgical treatment of pilonidal sinus disease: a retrospective cohort study. Ann Surg Treat Res. 2017;93(2):82-87.
44. Eryilmaz R., Sahin M., Alimoglu O., Dasiran F. Surgical treatment of sacrococcygeal pilonidal sinus with the Limberg transposition flap. Surgery. 2003;134:745-749.
45. Bascom J., Bascom T. Failed pilonidal surgery: new paradigm and new operation leading to cures. Arch Surg. 2002;137(10):1146-1151
46. Nordon I. M., Senapati A., Neil P.J. A prospective randomized controlled trial of simple Bascom's technique versus Bascom's cleft closure for the treatment of chronic pilonidal disease. Am. J. Surg. 2009;197(2):189-192.
47. Abo-Ryia M.H., Abd-Allah H.S., Al-Shareef M.M., Abdulrazek M.M. Fascio-adipo-cutaneous lateral advancement flap for treatment of pilonidal sinus: A modification of the Karydakis operation-cohort study. World J Surg. 2017;21:93-105.
48. Bascom J. Pilonidal disease: origin from follicles of hairs and results of follicle removal as treatment. Surgery. 1980;5:567-572.
49. Demircan F., Akbulut S., Yavuz R., Agtas H. The effect of laser epilation on recurrence and sat-isfaction in patients with sacrococcygeal pilonidal disease: a prospective randomized controlled trial. Int. J. Clin. Exp. Med. 2015;2:2929-2933.
50. Lukish R. Laser epilation is a safe and effective therapy for teenagers with pilonidal disease. Journal of Pediatric Surgery. 2009;1:282-285.
51. Oram Y. Evaluation of 60 patients with pilonidal sinus treated with laser epilation after surgery. Medical Laser Application. 2010;19:155-159.
52. Marza L. Reducing the recurrence of pilonidal sinus disease. Nursing Times. 2013;25(109):22-24.
53. Mentes B., Leventoglu S., Cihan A. Modified Limberg transposition flap for sacrococcygeal pi-lonidal sinus. Surg. Today. 2004;34:419-423.
54. Stauffer V.K., Luedi M.M., Kauf P., Schmid M., Diekmann M., Wieferich K., Schnüriger B., Doll D. Common surgical procedures in pilonidal sinus disease: A meta-analysis, merged data analysis, and comprehensive study on recurrence. Sci Rep. 2018;8:3058.
55. Lahey F.H. An operation for pilonidal sinus. Surg. Gynec. Obst. 1929;48:109-111.
56. Fishbein R.H. A method for primary reconstruction follo-wing radical exision of sacrococcygeal pilonidal disease. Ann. Surg. 1979;2:231-235.
57. Middletone M.D. Treatment of pilonidal sinus by Z-plasty. Br. J.Surg. 1968 4:67-70.
58. 100. Mouly R., Martin F.F. Plase de la chirurgie plastigue dans le traitement chirurgical du sinus pi-lonidale. Gastroenterol. Clin. Biol. 1977;2:33-39.
59. 101. Monroe R.S., Mc Dermott F.T. The elimination of causal factors in pilonidal sinus treated by Z-plasty. Brit.иJ. Surg. 1965;52:98-101.
60. Tschudi J., Ris H. Morbidity of Z- Plasty in the treatment of pilonidal sinus. Chirurg. 1988;59:486-490.
61. Roth R.F., Moorman W.L. Treatment of pilonidal sinus and cyst by conservative exision and W-plasty closure. Plastic and Reconstructive Surgery. 1977;3:412-415.
62. Soll C., Dindo D., Steinemann D. Sinusectomy for primary pilonidal sinus: less is more. Surge-ru. 2011;5:996-1001.
63. Meinero P., Mori L., Gasloli G. Endoscopic pilonidal sinus treatment (E.P.Si.T.). Tech. Colo-proctol. 2014; 4:389-392.
64. Moritz, A., Gutknecht, N., Doertbudak, O.Goharkhay, K., Schoop, U., Schauer, P. and Sperr, W.Bacterial reduction in periodontal pockets through irradiation with diode laser, a pilot study. J. Clin. Laser Med. Surg. 1997;15:33-37.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Bohdan Zaletskyi
National Pirogov Memorial Medical University
Vinnytsia, Ukraine
tel: +380965363996
e-mail: zaletskyi.bohdan@gmail.com

Received: 17.02.2019
Accepted: 15.07.2019

 © Wydawnictwo Aluna

PRACA POGLĄDOWA
REVIEW ARTICLE

THE ROLE OF 1,25(OH)$_2$D$_3$ AND ITS ANALOGS IN PROLIFERATION AND DIFFERENTIATION OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK – LITERATURE REVIEW

WPŁYW 1,25(OH)$_2$D$_3$ I JEGO ANALOGÓW NA WZROST I RÓŻNICOWANIE RAKA PŁASKONABŁONKOWEGO GŁOWY I SZYI – PRZEGLĄD PIŚMIENNICTWA

Mateusz Mizgalski, Krzysztof Zub, Karolina Dorobisz, Tomasz Zatoński
DEPARTMENT OF OTOLARYNGOLOGY - HEAD AND NECK SURGERY, MEDICAL UNIVERSITY OF WROCLAW, WROCLAW, POLAND

ABSTRACT

Epidemiological studies correlate sun or UV light exposure with a lower incidence of a variety of malignancies, including breast, colon and prostate cancer. The biologically active 1,25(OH)$_2$D$_3$ and its analogs have been shown to have antiproliferative and differentiating effects in a variety of malignant and non-malignant cells. The effects of 1,25(OH)$_2$D$_3$ are mediated by the binding of calcitriol to a specific intracellular receptor, vitamin D receptor [VDR]. **The aim** of this study is to review the literature concerning the role of 1,25(OH)$_2$D$_3$ and its analogs in squamous carcinoma cell lines of the head and neck (SCCHN).

KEY WORDS: 1,25(OH)$_2$D$_3$, vitamin D$_3$, squamous cell carcinoma of the head and neck

STRESZCZENIE

Badania epidemiologiczne wskazują na związek pomiędzy ekspozycją na światło ultrafioletowe a mniejszą częstością zapadania na nowotwory złośliwe w różnych lokalizacjach – sutka, okrężnicy czy gruczołu krokowego. Wykazane zostało antyproliferacyjne działanie czynnego biologicznie 1,25(OH)$_2$D$_3$ i jego analogów na komórki guzów złośliwych i łagodnych. Wpływ 1,25(OH)$_2$D$_3$ jest regulowany poprzez powinowactwo kalcytriolu do specyficznego wewnątrzkomórkowego receptora witaminy D. Autorzy pracy dokonali przeglądu literatury dotyczącej wpływu 1,25(OH)$_2$D$_3$ i jego analogów na komórki raka płaskonabłonkowego głowy i szyi.

SŁOWA KLUCZOWE: 1,25(OH)$_2$D$_3$, witamina D$_3$, rak płaskonabłonkowy głowy i szyi

Wiad Lek 2019, 72, 8, 1566-1570

INTRODUCTION

It is well established that the metabolically active form of vitamin D, 1α,25-dihydroxycholecalciferol [1,25(OH)$_2$D$_3$] plays a key role as a regulator in calcium and phosphate homeostasis. By definition, a vitamin is an essential nutritional factor. Therefore the term "vitamin D3" is misleading and should be referred to as a hormone. The steroid hormone 1,25(OH)$_2$D$_3$ is synthesized in the skin from 7-dehydrocholesterol in reaction catalyzed by UV light. Thereafter vitamin D is transported to the liver where it is 25-hydroxylated to 25(OH)-D$_3$ and then 1-hydroxylated to 1,25(OH)$_2$D$_3$ in kidneys. Apart from its classical role, 1,25(OH)$_2$D$_3$ regulates gene expression in a variety of target cells and has been shown to regulate cell proliferation and differentiation [1–4]. These effects are mediated through binding to a specific, intracellular receptor protein, the vitamin D receptor (VDR) [5]. VDRs are expressed in many types of cancer cells, including cells derived from tumors of the breast, prostate, pancreas, colon, urinary bladder, cervix, thyroid, pituitary, melanoma, glioma, neuroblastoma, leukemia and lymphoma cells[6, 7]. When human myeloid leukemia cells are treated with 1,25(OH)$_2$D$_3$, they differentiate into functional monocytes and macrophages. Similarly, human histiocytic lymphoma cells differentiate in the presence of 1,25(OH)$_2$D$_3$ [8]. Lappe at al [9] demonstrated that higher doses (1,100 IU) of vitamin D plus calcium significantly reduce cancer incidence.

Epidemiological studies have correlated sun or UV light exposure with a lower incidence of a variety of malignancies, including breast, colon and prostate cancer [10, 11] . Colston et al [12] have shown that patients with 1,25(OH)$_2$D$_3$ receptor-positive tumors have a significantly longer disease-free survival than those without this receptor. Shabahang et al [13] have shown that the level of VDR correlates with the degree of differentiation in human colon cancer cell lines. The more well-differentiated the tumor, the higher the level of VDR and conversely, less-differentiated tumor have lower VDR receptor levels. These investigations suggest that the

loss of the VDR receptor may be one of the factors that either promote the progression of normal epithelium to a malignant phenotype or that cells lose their VDR expression as a result of epithelial dedifferentiation[4]. Heist et al [14] have shown also that the T allele of VDR Fok1 polymorphism and the G-T-C (Cdx2-Fok1-Bsm1) haplotype are associated with lower survival of patient with advanced non-small-cell lung cancer. That report demonstrated a relationship between VDR polymorphism and prognosis of patients with cancer.

REVIEW AND DISCUSSION

CYTOLOGICAL EFFECTS OF 1,25(OH)$_2$D$_3$ IN CANCER CELLS

Squamous carcinoma cell lines of the head and neck [HNSCC] respond to 1,25(OH)$_2$D$_3$ by inhibition of their proliferation and stimulation of differentiation in time- and dose-dependent manner. These effects are due to a blockage in the transition of cells from the G0 to S phases in the cell cycle, and occur through the nuclear VDR and interaction with vitamin D response elements [VDREs] in the regulatory region of the vitamin D target genes [15].

The antiproliferative activity of 1,25(OH)$_2$D$_3$ is caused by expression of the cell cycle inhibitory proteins p21 and p27. These proteins inhibit the activity of cyclin-dependent kinases in the G0/G1 cell-cycle phase, resulting in dephosphorylation of the retinoblastoma protein and cell-cycle arrest [16]. Different results have been published concerning the interaction of p21 and vitamin D$_3$ cell-cycle regulation. It was found in several studies that vitamin D$_3$ either had no effect on p21 protein expression [17] or was accompanied by downmodulation of p21 [18]. Other studies [19, 20] linked the antiproliferative effect of 1,25(OH)$_2$D$_3$ to overexpression of this important cell-cycle regulatory protein. Further investigations must be conducted to clarify this process.

Immune regulatory activities of vitamin D is also very important, because HNSCC can inhibit immune defenses. HNSCC patients have an accumulation of immune inhibitory CD34+ progenitor cells and a defect in dendritic cell differentiation [21, 22]. Furthermore, T-cells from about one-third of HNSCC patients are unresponsive to stimulation through the CD3/T-cell receptor [23]. The immune depression is caused also by suppressive mediators produced by the HNSCC cells and by the immune suppressive cells that they induce[24]. Many studies have shown that treatment with active metabolite of vitamin D-1,23(OH)$_2$D$_3$ delayed carcinogenesis in the hamster buccal pouch tumor model [25]. In mouse models 1,25(OH)$_2$D$_3$ therapy can reduce the extent of metastatic disease [26]. Vitamin D$_3$ can activate the immune system in cancer patients and stimulate intratumoral immune infiltration [27].

Many studies showing that the inflammation is protumorogenic. Vitamin D can reduce levels of the pro-inflammatory cytokines-IL-6, IL-17 [28], TNF-alfa [29]it was the aim of the study to establish the role of 1α,25-dihydroxyvitamin D$_3$ (vit. D and COX-2 [30].

THE VITAMIN D ANALOGUES – A WAY OF SIDE-EFFECTS PREVENTION

The main barrier to the clinical use of 1,25(OH)$_2$D$_3$ has been its hypercalcemic effect. More than 800 analogues were developed in an attempt to maintain the inhibitory effect on tumor cell proliferation while reducing hypercalcemia [31,32]. One such analogue is EB1089. It is 60 times more potent than 1,25(OH)$_2$D$_3$ in inhibiting the growth of MCF-7 breast cancer cells in vitro and 100 times more potent than 1,25(OH)$_2$D$_3$ in inhibiting tumor growth in animal models of breast cancer, with only half the hypercalcemic activity [33]. Lianjun Lu et al have shown in the in vitro study that EB1089 inhibits the growth of human laryngeal SCCs. They suggested that EB1089 inhibits human laryngeal SCC via protein p57. p57 is a potent inhibitor of several G 1 cyclin/CDK complexes and its overexpression leads to cell cycle arrest in G1 [34] whereas Cdk inhibitors (CKIs. p57 also controls cell cycle exit and the differentiation of lens fiber cells, placental trophoblasts, chondrocytes, and human skeletal myoblasts [35–37]. Although p57 appears to be a critical terminal effector of signal transduction pathways that control cell differentiation and proliferation, its precise involvement in differentiation and proliferation in response to cytotoxic drugs or during tumorigenesis is unknown. Interestingly, p57 gene expression is also reduced in human laryngeal cancers [38], reinforcing a possible function of this protein in tumorigenesis.

In an in vitro model Chiang et al. have shown that the new generation of vitamin-D analog MART-10 has higher VDR transactivation activity than D-1,23(OH)$_2$D$_3$. MART-10 was also approximately 100-fold more potent in repressing cells growth through cell cycle arrest at the G0/G1 phase. They have revealed that the telomerase expression in the experimental cell line was significantly reduced after MART-10 and D-1,23(OH)$_2$D$_3$ treatment. In the in vivo study they have shown higher anti-cancer effectiveness of MART-10 in comparison to D-1,23(OH)$_2$D$_3$ [39].

Yang et al. have proved that MART-10 and D-1,23(OH)$_2$D$_3$ can effectively inhibit a metastatic process of HNSCC. This aim may be achieved by repressing Snail and Twist expressions. Both molecules play a role in EMT process (epithelial-mesenchymal transmission), which is an important step for cancer cells to become invasive [40]. In addition EMT process is partially responsible for the resistance to chemotherapy and radiotherapy [41–43]. Zhao at al. have demonstrated the increased level of Snail in the laryngeal squamous cell carcinoma (LSCC) cells and proved that knockdown of Snail can significantly inhibit the ability of adhesion, migration and invasion of the cancer cells. In the same study they have investigated the level of parameters, which are crucial for the EMT process such as Vimentin, E-cadherin, N-cadherin, Integrin, β-catenin. Increased level of all of the factors was detected in LSCC cells. Moreover they have proved that Snail silencing can lead to remarkable inhibition of expression of all of them.

Yang et al. have noticed that the inhibition of metastatic process was implemented by reversing cadherin switch

(upregulation of E-cadherin and downregulation of N-cadherin). Low E-cadherin and high N-cadherin expression was previously linked with increased invasiveness of cancer cells [44]throughout adult life and in some pathological conditions. Cadherins, and more specifically the neural cell adhesion molecule N-cadherin, play an important role in migration. In embryogenesis, N-cadherin is the key molecule during gastrulation and neural crest development. N-cadherin mediated contacts activate several pathways like Rho GTPases and function in tyrosine kinase signalling (for example via the fibroblast growth factor receptor. Authors have also confirmed that MART-10 and D-1,23$(OH)_2D_3$ inhibit the expression of collagenases MMP -9 and MMP-2 in HNSCC cells which is another factor responsible for invasive potential of cancer cells.

THE CORRELEATION BETWEEN VITAMIN D SERUM LEVEL AND CLINICAL DATA IN HNSCC PATIENTS

Bochen at al. have compared vitamin D serum level in 231 HNSCC patients with 232 healthy controls matched for age and sex. The results have shown that vitamin D serum level in HNSCC patients was notably lower. The authors have excluded influence of general malnutrition status on that result. In addition they have revealed a significant correlation between low vitamin D serum level and a positive lymph node status. Follow up time has manifested an influence of Vitamin D on overall survival (OS), which was significantly shorter in HNSCC patients with low vitamin D.

Bochen at al. have also investigated the influence of Vitamin D serum level on the anti-tumor activity of the immune system. Significantly higher infiltration of CD3+ T cells, helper T cells, cytotoxic T cells, NK cells, CD 86+ macrophages and M1 macrophages was observed in the tumor tissues of vitamin D high patients. On the other hand the infiltration of M2 macrophage (CD 163+) was notably lower in the tumor tissue of the Vit D high HNSCC patients [45]it is known that vitamin D can stimulate the patients' antitumor immunity. However, valid epidemiological data for head and neck squamous cell carcinoma (HNSCC. It is well known that all of the immune cells play an important role in the anti-tumor activity of the immune system[46–48]. In addition Bochen at al. have shown that the infiltration of CD3+ T cells, helper T cells, cytotoxic T cells, NK cells, CD 86+ macrophages and M1 macrophages is associated with longer overall survival, whereas the infiltration of the of M2 macrophage (CD 163+) is correlated with shorter OS.

INDIRECT EFFECTS OF 1,25$(OH)_2D_3$ IN THE ANTI-TUMOR THERAPY

As mentioned above one of the potential actions of vitamin D in the therapy of HNSCC is its role in the augmentation of chemotherapy and radiotherapy. Latest studies have shown that this effect may be achieved also in case of Erlotinib therapy, which is currently undergoing clinical evaluation for its chemopreventive potential in head and neck cancer patients [49–51]. Activation of epidermal growth factor (EGFR) is an early stage of the neoplastic process in HNSCC [52the epidermal growth factor receptor (EGFR, 53]. Erlotinib acts through inhibiting EGFR and due to this fact has a significant potential in chemoprevention [54]. In mouse model D-1,23$(OH)_2D_3$ has been shown to augment the inhibition of the EGFR activation in a combination treatment including erlotinib and D-1,23$(OH)_2D_3$. The mechanism of this action depends on inhibition of phosphorylation of Erk and Akt, which is one of the stages in EGFR activation cascade [49].

CONCLUSIONS

Vitamin D due to its pleyomorphic effect can play an important role in many different processes. An influence on all of the mentioned prooncogenic processes gives a possibility to use Vitamin D in the additional therapy of HNSCC. A supplementation of Vitamin D may improve a therapy primarily in those patients, whose natural production of D-1,23$(OH)_2D_3$ is low. It is important to take into account, that high doses of Vit D may lead to clinically relevant side effects. The correlation between serum level of Vit D and the aggressiveness of HNSCC is not precisely defined. More research is required to assess an impact of Vit D on HNSCC patients who are currently undergoing a standard therapy.

REFERENCES

1. Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. Cancer Res. 1994;54:805–10.
2. Kornfehl J, Formanek M, Temmel A, Knerer B, Willheim M. Antiproliferative effects of the biologically active metabolite of vitamin D3 (1,25 [OH]2 D3) on head and neck squamous cell carcinoma cell lines. Eur Arch Otorhinolaryngol. 1996;253:341–4.
3. Vink-van Wijngaarden T, Pols HA, Buurman CJ, Birkenhäger JC, van Leeuwen JP. Inhibition of insulin- and insulin-like growth factor-I-stimulated growth of human breast cancer cells by 1,25-dihydroxyvitamin D3 and the vitamin D3 analogue EB1089. Eur. J. Cancer 1996;32A:842–8.
4. McElwain MC, Modzelewski RA, Yu WD, Russell DM, Johnson CS. Vitamin D: an antiproliferative agent with potential for therapy of squamous cell carcinoma. Am J Otolaryngol. 1997;18:293–8.
5. Hager G, Formanek M, Gedlicka C, Thurnher D, Knerer B, Kornfehl J. 1,25(OH)2 vitamin D3 induces elevated expression of the cell cycle-regulating genes P21 and P27 in squamous carcinoma cell lines of the head and neck. Acta Otolaryngol. 2001;121:103–9.
6. Bouillon R, Okamura WH, Norman AW. Structure-Function Relationships in the Vitamin D Endocrine System. Endocr Rev. 1995;16:200–257. DOI: 10.1210/edrv-16-2-200.
7. Haussler MR, Whitfield GK, Haussler CA et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res. 1998;13:325–49. DOI: 10.1359/jbmr.1998.13.3.325.
8. Olsson I, Gullberg U, Ivhed I, Nilsson K. Induction of differentiation of the human histiocytic lymphoma cell line U-937 by 1 alpha,25-dihydroxycholecalciferol. Cancer Res. 1983;43:5862–7.

9. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr. 2007i; 85:1586–1591. DOI: 10.1093/ajcn/85.6.1586.
10. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol. 1980;9:227–31.
11. Schwartz GG, Hulka BS. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). Anticancer Res. 1990;10:1307–11.
12. Colston KW, Chander SK, Mackay AG, Coombes RC. Effects of synthetic vitamin D analogues on breast cancer cell proliferation in vivo and in vitro. Biochem Pharmacol. 1992;44:693–702.
13. Shabahang M, Buras RR, Davoodi F, Schumaker LM, Nauta RJ, Evans SR. 1,25-Dihydroxyvitamin D3 receptor as a marker of human colon carcinoma cell line differentiation and growth inhibition. Cancer Res. 1993;53:3712–8.
14. Heist RS, Zhou W, Wang Z et al. Circulating 25-Hydroxyvitamin D, VDR Polymorphisms, and Survival in Advanced Non–Small-Cell Lung Cancer. J Clin Oncol. 2008;26:5596–5602. DOI: 10.1200/JCO.2008.18.0406.
15. Hager G, Kornfehl J, Knerer B, Weigel G, Formanek M. Molecular analysis of p21 promoter activity isolated from squamous carcinoma cell lines of the head and neck under the influence of 1,25(OH)2 vitamin D3 and its analogs. Acta Otolaryngol. 2004;124:90–6.
16. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. 1999;13:1501–12.
17. Akutsu N, Lin R, Bastien Y, Bestawros A, Enepekides DJ, Black MJ, White JH. Regulation of gene Expression by 1alpha,25-dihydroxyvitamin D3 and Its analog EB1089 under growth-inhibitory conditions in squamous carcinoma cells. Mol Endocrinol. 2001;15:1127–39. DOI: 10.1210/mend.15.7.0655.
18. Hershberger PA, Modzelewski RA, Shurin ZR, Rueger RM, Trump DL, Johnson CS. 1,25-Dihydroxycholecalciferol (1,25-D3) inhibits the growth of squamous cell carcinoma and down-modulates p21(Waf1/Cip1) in vitro and in vivo. Cancer Res. 1999;59:2644–9.
19. Muto A, Kizaki M, Yamato K et al. 1,25-Dihydroxyvitamin D3 induces differentiation of a retinoic acid-resistant acute promyelocytic leukemia cell line (UF-1) associated with expression of p21(WAF1/CIP1) and p27(KIP1). Blood 1999;93:2225–33.
20. Liu M, Lee MH, Cohen M, Bommakanti M, Freedman LP. Transcriptional activation of the Cdk inhibitor p21 by vitamin D3 leads to the induced differentiation of the myelomonocytic cell line U937. Genes Dev. 1996;10:142–53.
21. Lathers DM, Achille N, Kolesiak K, Hulett K, Sparano A, Petruzzelli GJ, Young MR. Increased levels of immune inhibitory CD34+ progenitor cells in the peripheral blood of patients with node positive head and neck squamous cell carcinomas and the ability of these CD34+ cells to differentiate into immune stimulatory dendritic cells. Otolaryngol. Head Neck Surg. 2001; 125:205–12. DOI: 10.1067/mhn.2001.117871.
22. Almand B, Clark JI, Nikitina E, van Beynen J, English NR, Knight SC, Carbone DP, Gabrilovich DI. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol. 2001; 166:678–89.
23. Shibuya TY, Wei WZ, Zormeier M et al. Anti-CD3/anti-CD28 bead stimulation overcomes CD3 unresponsiveness in patients with head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg. 2000;126:473–9.
24. Young M, Day T. Immune Regulatory Activity of Vitamin D3 in Head and Neck Cancer. Cancers (Basel). 2013;5:1072–1085. DOI: 10.3390/cancers5031072.
25. Meier JD, Enepekides DJ, Poirier B, Bradley CA, Albala JS, Farwell DG. Treatment with 1-alpha,25-dihydroxyvitamin D3 (vitamin D3) to inhibit carcinogenesis in the hamster buccal pouch model. Arch Otolaryngol Head Neck Surg. 2007;133:1149–52. DOI: 10.1001/archotol.133.11.1149.
26. Wiers KM, Lathers DM, Wright MA, Young MR. Vitamin D3 treatment to diminish the levels of immune suppressive CD34+ cells increases the effectiveness of adoptive immunotherapy. J Immunother. 2000;23:115–24.
27. Kulbersh JS, Day TA, Gillespie MB, Young MRI. 1α,25-Dihydroxyvitamin D 3 to skew intratumoral levels of immune inhibitory CD34 + progenitor cells into dendritic cells. Otolaryngol Neck Surg. 2009;140:235–240. DOI: 10.1016/j.otohns.2008.11.011.
28. Correale J, Ysrraelit MC, Gaitán MI. Vitamin D-mediated immune regulation in Multiple Sclerosis. J Neurol Sci. 2011;311:23–31. DOI: 10.1016/j.jns.2011.06.027.
29. Bessler H, Djaldetti M. 1α,25-Dihydroxyvitamin D3 modulates the interaction between immune and colon cancer cells. Biomed Pharmacother. 2012;66:428–32. DOI: 10.1016/j.biopha.2012.06.005.
30. Moreno J, Krishnan A V, Peehl DM, Feldman D. Mechanisms of vitamin D-mediated growth inhibition in prostate cancer cells: inhibition of the prostaglandin pathway. Anticancer Res. 2006;26:2525–30.
31. Hansen CM, Hamberg KJ, Binderup E, Binderup L. Seocalcitol (EB 1089): a vitamin D analogue of anti-cancer potential. Background, design, synthesis, pre-clinical and clinical evaluation. Curr Pharm Des. 2000;6:803–28.
32. Hansen CM, Mäenpää PH. EB 1089, a novel vitamin D analog with strong antiproliferative and differentiation-inducing effects on target cells. Biochem Pharmacol. 1997; 54:1173–9.
33. VanWeelden K, FIanagan L, Binderup L, Tenniswood M, Welsh J. Apoptotic regression of MCF-7 xenografts in nude mice treated with the vitamin D3 analog, EB1089. Endocrinology 1998;139:2102–10. DOI: 10.1210/endo.139.4.5892.
34. Matsuoka S, Edwards MC, Bai C et al. p57KIP2, a structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. Genes Dev. 1995;9:650–62.
35. Zhang Z, Adappa ND, Lautenbach E et al. The effect of diabetes mellitus on chronic rhinosinusitis and sinus surgery outcome. Int Forum Allergy Rhinol. 2014;4:315–320. DOI: 10.1002/alr.21269.
36. MacLean HE, Guo J, Knight MC, Zhang P, Cobrinik D, Kronenberg HM. The cyclin-dependent kinase inhibitor p57(Kip2) mediates proliferative actions of PTHrP in chondrocytes. J Clin Invest. 2004;113:1334–43. DOI: 10.1172/JCI21252.
37. Endesfelder S, Bucher S, Kliche A, Reszka R, Speer A. Transfection of normal primary human skeletal myoblasts with p21 and p57 antisense oligonucleotides to improve their proliferation: a first step towards an alternative molecular therapy approach of Duchenne muscular dystrophy. J Mol Med. (Berl). 2003;81:355–62. DOI: 10.1007/s00109-003-0439-6.
38. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65:55–63.
39. Chiang KC, Yeh CN, Hsu J Te et al. MART-10, a novel vitamin D analog, inhibits head and neck squamous carcinoma cells growth through cell cycle arrest at G0/G1with upregulation of p21 and p27 and downregulation of telomerase. J. Steroid Biochem Mol Biol. 2013;138:427–434. DOI: 10.1016/j.jsbmb.2013.09.002.
40. Yang SW, Tsai CY, Pan YC et al. MART-10, a newly synthesized vitamin D analog, represses metastatic potential of head and neck squamous carcinoma cells. Drug Des Devel Ther. 2016; 10:1995–2002. DOI: 10.2147/DDDT.S107256.

41. Hollier BG, Evans K, Mani SA. The epithelial-to-mesenchymal transition and cancer stem cells: a coalition against cancer therapies. J Mammary Gland Biol Neoplasia 2009; 14:29–43. DOI: 10.1007/s10911-009-9110-3.
42. Kurrey NK, Jalgaonkar SP, Joglekar A V, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA. Snail and slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. Stem Cells 2009;27:2059–68. DOI: 10.1002/stem.154.
43. Arumugam T, Ramachandran V, Fournier KF et al.Epithelial to Mesenchymal Transition Contributes to Drug Resistance in Pancreatic Cancer. Cancer Res. 2009;69:5820–5828. DOI: 10.1158/0008-5472.CAN-08-2819.
44. Derycke LDM, Bracke ME. N-cadherin in the spotlight of cell-cell adhesion, differentiation, embryogenesis, invasion and signalling. Int J Dev Biol. 2004;48:463–76. DOI: 10.1387/ijdb.041793ld.
45. Bochen F, Balensiefer B, Körner S et al. Vitamin D deficiency in head and neck cancer patients–prevalence, prognostic value and impact on immune function. Oncoimmunology 2018;7:1–10. DOI: 10.1080/2162402X.2018.1476817.
46. Chen Y, Zhang S, Wang Q, Zhang X. Tumor-recruited M2 macrophages promote gastric and breast cancer metastasis via M2 macrophage-secreted CHI3L1 protein. J Hematol Oncol. 2017; 10:36. DOI: 10.1186/s13045-017-0408-0.
47. Rivera Vargas T, Humblin E, Végran F, Ghiringhelli F, Apetoh L. TH9 cells in anti-tumor immunity. Semin Immunopathol. 2017;39:39–46. DOI: 10.1007/s00281-016-0599-4.
48. Janssen LME, Ramsay EE, Logsdon CD, Overwijk WW. The immune system in cancer metastasis: friend or foe? J Immunother Cancer 2017;5:79. DOI: 10.1186/s40425-017-0283-9.
49. Bothwell KD, Shaurova T, Merzianu M et al. HHS Public Access. 2016;8:765–776. DOI: 10.1158/1940-6207.CAPR-14-0454.Impact.
50. Saba NF, Hurwitz SJ, Kono SA et al.Chemoprevention of head and neck cancer with celecoxib and erlotinib: results of a phase ib and pharmacokinetic study. Cancer Prev Res. (Phila). 2014;7:283–91. DOI: 10.1158/1940-6207.CAPR-13-0215.
51. Rosenthal EL, Chung TK, Carroll WR, Clemons L, Desmond R, Nabell L. Assessment of erlotinib as adjuvant chemoprevention in high-risk head and neck cancer patients. Ann Surg Oncol. 2014;21:4263–9. DOI: 10.1245/s10434-014-3878-0.
52. Grandis JR, Tweardy DJ. Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res. 1993;53:3579–84.
53. Taoudi Benchekroun M, Saintigny P, Thomas SM et al.Epidermal Growth Factor Receptor Expression and Gene Copy Number in the Risk of Oral Cancer. Cancer Prev Res. 2010;3:800–809. DOI: 10.1158/1940-6207.CAPR-09-0163.
54. Gross ND, Bauman JE, Gooding WE et al. Erlotinib, erlotinib-sulindac versus placebo: a randomized, double-blind, placebo-controlled window trial in operable head and neck cancer. Clin Cancer Res. 2014; 20:3289–98. DOI: 10.1158/1078-0432.CCR-13-3360.

Confilct of interest

All authors declare no conflict of interest

CORRESPONDING AUTHOR
Mateusz Mizgalski
St. Grota Roweckiego 119/29, 52-232 Wroclaw, Poland
tel.: 608570038
e-mail: mat.mizgalski@gmail.com

Received: 21.01.2019
Accepted: 17.07.2019

PRACA POGLĄDOWA
REVIEW ARTICLE

SAFE NATURAL ENVIRONMENT AS GUARANTEE OF EXERCISING THE RIGHT TO HEALTH

Antonina H. Bobkova[1], Maryna V. Trotska[2]
[1]LAW FACULTY, DONETSK NATIONAL UNIVERSITY, VINNYTSIA, UKRAINE
[2]DEPARTMENT OF CONSTITUTIONAL, ADMINISTRATIVE, ENVIRONMENTAL AND LABOUR LAW, POLTAVA LAW INSTITUTE OF YAROSLAV THE WISE NATIONAL LAW UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

Introduction: The right to health is one of the most important human rights and its proper exercising allows for the exercise of other rights. One of the guarantees of this right is the safe natural environment, which, on the one hand, allows to maintain the health at the proper level without worsening it, and, on the other hand, to improve and prevent negative consequences concerning it. The proper state of the natural environment is reflected through the relevant qualitative characteristics of each of the natural resources that are included in it, and in aggregate, reflected in the corresponding natural interactions between them.

The aim: The study is aimed at inquiring into the right to health and importance of the safe natural environment in order to provide it.

Materials and methods: Statutory regulation and scientific positions of scholars in the field of the above-mentioned issue are studied in this article. The study analyzes generalized information from scientific journals by means of scientific methods from a medical and legal point of view. This article is based on dialectical, comparative, analytic, synthetic and comprehensive research methods. Within the framework of the systematic approach, as well as analysis and synthesis, the concept of the right to health and its place in the provision of the safe natural environment is researched.

Review: The right to health is regarded as a person's awareness of the existence of appropriate ways, means and conditions that enable them to take care of their physical and psychological state by carrying out appropriate actions or refraining from doing so, thereby preventing or eliminating negative consequences that may threaten or do threaten their proper state of health. Along with others, the safe natural environment is a prerequisite that allows their full enjoyment of the right to health.

Conclusions: The guarantee of the right to health must be based on an integrated approach in understanding the nature of the factors, their interconnection and impact on each other in providing it. The safe natural environment is a prime element when implementing the outlined opportunity. Failure to comply with its proper condition, both directly and indirectly, affects its other components while implementing it. The level of its safety is determined by the proper qualitative state of natural resources, their interconnection and impact on human health. Ensuring the safety of the natural environment, both directly and indirectly, means creating requisite conditions for exercising the right to health.

KEY WORDS: health, right to health, natural environment, safe natural environment, environmental risks

Wiad Lek 2019, 72, 8, 1571-1575

INTRODUCTION

Man is both creature and moulder of his environment, which gives him physical sustenance and affords him the opportunity for intellectual, moral, social and spiritual growth... Both aspects of man's environment, the natural and the man-made, are essential to his well-being and to the enjoyment of basic human rights even the right to life itself [1].

Principle 1 of the Stockholm Declaration established a foundation for linking human rights, health, and environmental protection, declaring that man has the fundamental right to freedom, equality and adequate conditions of life, in an environment of a quality that permits a life of dignity and well-being [2]. In fact, one cannot but agree with this principle, since only in the presence of the safe environment one can speak of exercising human rights which allows developing properly, having an appropriate level of living standards in the existence of which there is the fullest possible exercise of the right to health.

In accordance with the Preamble of the Constitution of the World Health Organization [3], the enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being without distinction of race, religion, political belief, economic or social condition. The importance given to the "underlying determinants of health", that is, the factors and conditions which protect and promote the right to health beyond health services, goods and facilities, shows that the right to health is dependent on, and contributes to, the realization of many other human rights [4, p. 6]. The idea that all human rights are interdependent is consistent with the public health notion of the social determinants of health, which links to numerous rights – the rights to water, housing, food, health, education, a clean environment, etc. [5]. The right to health is a fundamental part of our human rights and of our understanding of a life in dignity [4, p. 1].

In other words, regardless of any given aspect, the right to health is an opportunity to attain an appropriate state of its highest level, which cannot depend on various factors, on the one hand, but on the other hand, it is interrelated

with other rights that are connected with it both directly or indirectly. In particular, the right to health is one of a set of internationally agreed human rights standards and is inseparable or 'indivisible' from these other rights. This means achieving the right to health is both central to, and dependent upon, the realization of other human rights, to food, housing, work, education, information, and participation [6].

THE AIM

The study is aimed at inquiring into the right to health and importance of the safe natural environment in order to provide it.

MATERIALS AND METHODS

Understanding health as a human right creates a legal obligation on states to ensure access to timely, acceptable, and affordable health care of appropriate quality as well as to providing for the underlying determinants of health, such as safe and potable water, sanitation, food, housing, health-related information and education, and gender equality [6].

Along with others, one of these determinants is the proper condition of the natural environment which may be different in terms of its qualitative characteristics, starting with the minimum that meets the safety criteria, which in turn will allow saving the possibility of realizing the vital functions of the human body, and on the other hand, it is such a state of it that does not only preserve the vital functions of the human body but also has a positive effect on it, which allows it to recover and heal. For instance, under section 8 of paragraph 1 of the Additional Provisions of the Law of Health [7], one of the components by which promotion of health is provided is an environmental one, namely, a safe condition of the natural environment. Environmental degradation plays a decisive role in many conflict situations; it can contribute to the outbreak of a conflict and result in the infringement of fundamental human rights, such as the right to life and the right to health [8]. The goal to study of certain aspects of the influence of the relevant factors on human health, which concerns exercising the right to health in one way or other, has been pursued by various authors [9-13].

The human right to health means that everyone has the right to the highest attainable standard of physical and mental health, which includes access to all medical services, sanitation, adequate food, decent housing, healthy working conditions, and a clean environment [14, p. 155]. The right to health is not a right to be healthy, but rather a right to a health system – including health care and the underlying determinants of health – that provides the opportunity for people to attain the highest possible standard of health (paragraph 8 Committee on Economic, Social and Cultural Rights General Comment 14 [15]) [5].

Analyzing this definition, we can conclude that safety of the environment, in general, is one of the components of the complex conception of factors having a direct or indirect effect on the exercise of this right.

The human rights principles of universality, equality and nondiscrimination, participation, accountability and interdependency, together with the right to health framework of availability, accessibility, acceptability and quality, provide a human rights framework for assessment of policy, program and project proposals [5]. One cannot but agree that each of the above components cannot be implemented as effectively as possible if the natural environment does not meet the necessary quality requirements. Measuring the health of the country's natural environment is vital in understanding the role that it can play in protecting a community and reducing the impacts from hazard events [16].

REVIEW AND DISCUSSION

The human right to health is provided for in part 1 of Art. 25 of the Universal Declaration of Human Right [17] which stipulates that everyone has the right to a standard of living adequate for the health and well-being of himself and of his family, including food, clothing, housing and medical care and necessary social services, and the right to security in the event of unemployment, sickness, disability, widowhood, old age or other lack of livelihood in circumstances beyond his control. Pursuant to part 1 of Art. 12 of the International Covenant on Economic, Social and Cultural Rights [18], the States Parties to the present Covenant recognize the right of everyone to the enjoyment of the highest attainable standard of physical and mental health. In accordance with part 1 of Art. 24 of the Convention on the Rights of the Child [19], States Parties recognize the right of the child to the enjoyment of the highest attainable standard of health and to facilities for the treatment of illness and rehabilitation of health. States Parties shall strive to ensure that no child is deprived of his or her right of access to such health care services and others. When analyzing these provisions with respect to the consideration of the safe environment, in general, and the natural one as an important component in exercising the right to health, in particular, it can be noted that each of them directly or indirectly refers to any given characteristics through which one can assert its significance. In a point of fact, the wording of the availability of a respective living standard consists of certain characteristics (part 1 of Art. 25 of the Universal Declaration of Human Right), the realization of which is not possible in the natural environment, which is a threat. For instance, according to part 2 of Art. 12 of the International Covenant on Economic, Social and Cultural Rights, the measures ensuring the right of every person to the highest achievable level of physical and mental health include (b) the improvement of all aspects of environmental and industrial hygiene, which also confirms the special attention paid to the proper condition of the environment, in general, and the natural one, in particular. In addition, according to part 2 of Art. 24 of the Convention on the Rights of the Child, in order to exercise the right of the child to the enjoyment of the highest attainable standard

of health and to facilities for the treatment of illness and rehabilitation of health, the following measures are specified: (c) to combat disease and malnutrition, including within the framework of primary health care, through, inter alia, the application of readily available technology and through the provision of adequate nutritious foods and clean drinking-water, taking into consideration the dangers and risks of environmental pollution; (e) to ensure that all segments of society, in particular parents and children, are informed, have access to education and are supported in the use of basic knowledge of child health and nutrition, the advantages of breastfeeding, hygiene and environmental sanitation and the prevention of accidents. These provisions constitute grounds for arguing that the proper state of the natural environment is both directly and indirectly reflected in exercising the right to health.

Excessive concentration of industrial facilities leads to extremely anthropogenic pressure on the environment and consequently adversely affects public health [20, p. 26].

Before we decide on the conception of what kind of the environment is a safe one, let us mention that the natural environment is the complex of atmospheric, geological and biological characteristics found in an area in the absence of artifacts or influences of a well-developed technological, human culture [21]. The natural environment is the thin layer of life and life supports, called the biosphere, that contains the earth's air, soil, water, and living organisms [22]. Natural environment is the sum-total of natural factors which directly or indirectly affects human life and economic activity (the Preamble of the Law on Environmental Protection [23]). The "environment" is everything that creates natural conditions of the existence of organisms including Man and it is a precondition of their further development. Its components are mainly the air, water, minerals, soil, organisms (Art. 2 of the Environmental Protection Act [24]). "Environment" means the whole of mutually related elements functioning in nature (the earth's surface and underground, air, water, soil, flora, fauna, organic and inorganic material, anthropogenic components), as well as the natural and anthropogenic systems uniting them (Art. 1 of the Environmental Protection Law [25]). In other words, one of the components of the environment is its natural element and the exercise of the right to health will be considered through the prism of its influence.

The health and vitality of the natural environment are critical when measuring vulnerability to each identified hazard. A healthy and productive natural environment can provide excellent protection from a variety of hazards, while a damaged and unhealthy natural environment can reduce protection from specific hazards and, in some cases, increase a hazard's impact [16].

Environmental risks to health are defined as "all the physical, chemical and biological factors external to a person, and all related behaviors, but excluding those natural environments that cannot reasonably be modified". These factors include as follows: - pollution of air (including from second-hand tobacco smoke), water or soil with chemical or biological agents; - ultraviolet (in particular, protection from) and ionizing radiation; - noise, electromagnetic fields; - occupational risks, including physical, chemical, biological and psychosocial risks, and working conditions; - built environments, including housing, workplaces, land-use patterns, roads; - agricultural methods; - man-made climate and ecosystem change; - behavior related to environmental factors, e.g. the availability of safe water for washing hands, physical activity fostered through improved urban design [26]. Pursuant to part 4 of paragraph 23 of the General Part of the Environmental Code Act [27], the non-compliance of the environment with the health and well-being needs is presumed if the limit value of the quality of the environment has been exceeded. That is to say that the safe natural environment is the one that does not endanger human lives and health as well as does not contain any risks of different nature of origin that would not allow the proper exercise of the right to health.

When exercising the right to health to confirm the importance of the proper state of the natural environment it would be appropriate to refer to the decisions of the European Court on Human Rights (hereinafter, the ECHR) concerning the outlined issue. Before doing that, we should note that in cases tried in the context of violation of the requirements concerning protection of the natural environment and, as a result, impossibility of proper exercise of the right to health, reasoning is justified with reference to violations of Art. 8 "Right to respect for private and family life" of the Convention for the Protection of Human Rights and Fundamental Freedoms (hereinafter, the Convention) [28]: everyone has the right to respect for his private and family life, his home and his correspondence (part. 1). There shall be no interference by a public authority with the exercise of this right except such as is in accordance with the law and is necessary in a democratic society in the interests of national security, public safety or the economic well-being of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others (part. 2).

In the decision of the ECHR (the case of López Ostra v. Spain) there is a complaint alleging the Spanish State's failure to protect the home, private and family life of one of its citizens from the pollution caused by a waste treatment facility. It specifies the violation of the right to respect for home, private and family life; relationship between the right to a healthy environment and the right to respect for private life, home and family life; state accountability for actions by private companies in its jurisdiction. This case is significant because it shows the interdependence between civil and political rights on the one hand, and economic, social and cultural rights on the other hand. In many cases, protecting civil rights, such as the right to private and family life, and to respect for the home, involves also protecting economic, social and cultural rights, such as the right to a healthy environment and the right to health. The European Court ruled that "severe environmental pollution may affect individuals' well-being and prevent them from enjoying their homes in such a way as to affect their private

and family life adversely" [29]. In other words, the relevant state of the environment, in general, and the natural one directly or indirectly affects not only the proper exercise of the right to health but also other rights.

In the case of Guerra and Others v. Italy, the applicants made two complaints one of which concerned with the fact that authorities had not taken appropriate action to reduce the risk of pollution by the chemical factory and to avoid the risk of major accidents; that situation, they asserted, infringed their right to life and physical integrity as guaranteed by Article 2 of the Convention for the Protection of Human Rights and Fundamental Freedoms. In this case, the question was whether national authorities had taken the necessary steps to ensure effective protection of the applicants' right to respect for their private and family life. Severe environmental pollution could affect the individuals' well-being and prevent them from enjoying their homes in such a way as to affect their private and family life adversely. It concluded that the respondent State had not fulfilled its obligation to secure the applicants' right to respect for their private and family life [30].

Under the factual background of the next case, the ECHR also concluded that under Art. 8 of the Convention the human right was violated. To the Court's opinion, "... the fact remains that for several years her right to respect for her home was seriously impaired by the dangerous activities carried out at the plant built thirty meters away from her house. Having regard to the foregoing, and notwithstanding the margin of appreciation left to the respondent State, the Court considers that the State did not succeed in striking a fair balance between the interest of the community in having a plant for the treatment of toxic industrial waste and the applicant's effective enjoyment of her right to respect for her home and her private and family life" (the case of Giacomelli v. Italy) [31].

Consequently, analyzing the provisions worded in the above decisions, it can be noted that ensuring the possibility of exercising the right to health depends on the proper qualitative state of the natural environment. Moreover, safety is expressed in the proper condition of both respective natural resources, which are its components, and its state in general.

CONCLUSIONS

Exercising the right to health, a person has to approach such an opportunity comprehensively taking general care of various factors that directly or indirectly allow it to realize the indicated opportunity as fully as possible. The safe environment, in general is a primary element with the proper existence of which it becomes possible to create other conditions that are important in the process of exercising the right to health.

Effectiveness of administering liability for violating human rights in case of pollution of the natural environment depends on the selection of types of liability which fully ensure the restoration of rights of the aggrieved.

REFERENCES

1. Stockholm Declaration of the United Nations Conference on the Human Environment, 16 Jun 1972, U.N. Doc. A/.CONF.48/14/Rev.1 at 3 (1973).
2. Human Rights, Health and Environmental Protection: linkages in law and practice. Available at: https://www.who.int/hhr/information/Human_Rights_Health_and_Environmental_Protection.pdf
3. Constitution of the World Health Organization. Available at: https://www.who.int/governance/eb/who_constitution_en.pdf.
4. The Right to Health. Fact Sheet No. 31. Available at: https://www.ohchr.org/Documents/Publications/Factsheet31.pdf.
5. Gillian Mac Naughton and Lisa Forman. The Value of Mainstreaming Human Rights into Health Impact Assessment. International Journal of Environmental Research and Public Health, 2014;11(10):10076-10090. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4210968/.
6. Human Rights and Health. Available at: https://www.who.int/news-room/fact-sheets/detail/human-rights-and-health/.
7. LAW OF HEALTH, Prom. SG. 70/10, Aug 2004, amend. SG. 46/3 Jun 2005, amend. SG. 76/20 Sep 2005. Available at: https://www.ecolex.org/details/legislation/law-of-health-lex-faoc061743/?type=legislation&q=medical+rehabilitation&page=3.
8. Factsheet on Human Rights and the Environment, UNEP, Nairobi, Kenya, 2015, 4p.
9. Vitalii Pashkov, Olena Batyhina, Maryna Trotska. Legal Restraints of Pesticide Effect on Human Organism and Environment under International Legislation. Wiad Lek, 2017;2:366-372.
10. Vitalii Pashkov, Olena Batyhina, Maryna Trotska. Concept of Waste and Its Impact on Human Health. Wiad Lek, 2017;5:964-969.
11. Vitalii Pashkov, Olena Batyhina, Maryna Trotska. International Legal Regulation of Impact of Occupational Injuries and Diseases on Agricultural Workers' Health. Wiad Lek, 2017;5:953-958.
12. Gutorova N., Batyhina O., Trotska M. Legal Protection of Public Health through Control over Genetically Modified Food. Wiad Lek, 2018;2:366-370.
13. Pashkov V., Trotska M. Natural Environment as Component of Public Health: Some Aspects of its Legal Regulation. Wiad Lek, 2019;2:261-267.
14. Yuwa Wei. Issues Decisive for China's Rise or Fall: An International Law Perspective. Springer, 2019.
15. Committee on Economic, Social and Cultural Rights General Comment 14: The Right to the Highest Attainable Standard of Health (11 August 2000) Document E/C.12/200/4. [(accessed on 29 August 2014)]. Available online: http://www.un.org/en/ga/search/view_doc.asp?symbol=E/C.12/2000/4.
16. Coppola Damon P. Risk and Vulnerability, Introduction to International Disaster Management (Third Edition), 2015. Available at: https://www.sciencedirect.com/topics/social-sciences/natural-environment.
17. Universal Declaration of Human Rights of 10.12.1948. Available at: https://www.un.org/en/universal-declaration-human-rights/.
18. International Covenant on Economic, Social and Cultural Rights of 16.12.1966. Available at: https://www.ohchr.org/en/professionalinterest/pages/cescr.aspx.
19. Convention on the Rights of the Child of 20.11.1989. Available at: https://www.ohchr.org/en/professionalinterest/pages/crc.aspx.
20. Paszkov V.M. Gospodarsko-pravova polityka u sferi ochorony zdorovia: problem vitalnoi bezpeky. Kyiv: Morion, 2017.152s.
21. Natural environment. Glossary European Environment Agency. Available at: https://www.eea.europa.eu/help/glossary#c4=10&c0=all&b_start=30&c2=natural+environment+

22. Health and the Environment in the Southeastern United States: Roundtable on Environmental Health Sciences, Research, and Medicine; Editors: Howard Frumkin, Richard J. Jackson, and Christine M. Coussens. Institute of Medicine (US). Available at: https://www.ncbi.nlm.nih.gov/books/NBK221127/.
23. Law on Environmental Protection, 1991 (Latvia). Available at: https://www.ecolex.org/details/legislation/law-on-environmental-protection-lex-faoc037834/?xregion=Europe&q=the+right+to+health&type=legislation&page=41.
24. Environmental Protection Act No.17/1992 of 1991 (Slovakia). Available at: https://www.ecolex.org/details/legislation/environmental-protection-act-no171992-of-1991-lex-faoc045130/?xregion=Europe&q=the+right+to+health&type=legislation&page=95.
25. Environmental Protection Law No.I-2223 of 1992 (Lithuania). Available at: https://www.ecolex.org/details/legislation/environmental-protection-law-noi-2223-of-1992-lex-faoc038897/?xregion=Europe&q=the+right+to+health&type=legislation&page=96.
26. World Health Organization. Preventing Disease through Healthy Environments: Towards an Estimate of the Environmental Burden of Disease. Available at: https://apps.who.int/iris/bitstream/handle/10665/204585/9789241565196_eng.pdf?sequence=1.
27. General Part of the Environmental Code Act passed 16.02.2011 (Estonia). Available at: http://extwprlegs1.fao.org/docs/pdf/est178392.pdf.
28. Convention for the Protection of Human Rights and Fundamental Freedoms. Rome, 4.XI.1950. Available at: https://www.echr.coe.int/Documents/Convention_ENG.pdf.
29. López Ostra v. Spain (Application no. 16798/90), Dec 9 1994. Available at: https://www.escr-net.org/caselaw/2008/lopez-ostra-vs-spain-application-no-1679890.
30. Guerra and Others v. Italy, Feb 19, 1998. Available at: https://www.ecolex.org/details/court-decision/case-of-guerra-and-others-v-italy-a1113ab0-f99e-47d6-8076-1a51e18e353e/.
31. Giacomelli v. Italy, Mar 26, 2007. Available at: https://www.ecolex.org/details/court-decision/giacomelli-v-italy-a1362da3-5f8c-47ef-a40a-a542c6f6ed8b/.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Maryna V. Trotska
Department of Constitutional, Administrative,
Environmental and Labour Law,
Poltava Law Institute of
Yaroslav the Wise National Law University
Poltava, Ukraine
tel: +380954909415
e-mail: marinatrockaa@ukr.net

Received: 04.04.2019
Accepted: 18.07.2019

 © Wydawnictwo Aluna

PRACA POGLĄDOWA
REVIEW ARTICLE

USING THE SAMPLES OF HUMAN BIOLOGICAL MATERIALS IN THE CRIMINAL PROCEDURE: THE PRACTICE OF THE EUROPEAN COURT OF HUMAN RIGHTS

Oksana V. Kaplina[1], Olha H. Shylo[1], Ivan A. Titko[2]
[1]YAROSLAV MUDRYI NATIONAL LAW UNIVERSITY, KHARKIV, UKRAINE
[2]POLTAVA LAW INSTITUTE OF YAROSLAV MUDRYI NATIONAL LAW UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

Introduction: The use of modern advances in medicine to investigate crimes has caused a number of problems that require scientific reflection. In particular, today there are quite acute questions: medical intervention without the person's consent; forced sampling of human biological materials; clinical methods, the use of which in the biological samples taking will not be regarded as violation of international standards of human rights protection; the correlation of the need for the formation of DNA profile databases and the right of the person to non-disclosure of medical information.

The aim: The aim of this work is to identify and analyze the key points of the European Court of Human Rights (hereinafter referred to as the ECHR) regarding the peculiarities of retention and use of human biological material samples in the investigation of crimes, and the retention of such materials after the completion of the investigation and trial.

Materials and methods: In the preparation of the article, scientific works, the provisions of international normative acts regulating the use of human biological materials as well as the practice of the ECHR concerning the use of human biological materials in the investigation of crimes were used (8 decisions were analyzed in which the ECHR concerned the use of biological samples or related issues). In the research process to achieve the goal, a complex of general scientific and special methods of cognition was used, in particular, the comparative legal method, the system and structural method, the method of generalization, the method of analysis and synthesis, etc.

Review: The positions of the ECHR concerning the following were distinguished and generalized: a) the criteria for the permissibility of compulsory medical intervention for taking of human biological material within the framework of the crime investigation; b) the possibilities of spreading the right not to incriminate oneself on the compulsory taking of human biological materials samples; c) the retention features of cell samples and DNA information in the context of respect for the right to non-interference in the person's private life.

Conclusions: Obtaining and using the human material for the investigation of crimes are not a violation of the European Convention on Human Rights (hereinafter – the Convention), subject to the requirements stated in the practice of the ECHR.

KEY WORDS: human biological materials, taking of biological samples, criminal procedure, practice of the ECHR, human rights, investigation of crimes

Wiad Lek 2019, 72, 8, 1576-1581

INTRODUCTION

Nowadays the use of samples of human biological materials, obtaining of which, as a rule, is often carried out with the use of medical procedures, is quite common in the criminal procedure. At the same time, procedures for such samples taking, their use and retention are often bordered with violations of human rights guaranteed by the Convention. Therefore, there may be a violation of the prohibition of torture (Article 3 of the Convention); violation of the right to a fair trial, in particular in the context of the right not to incriminate oneself (Article 6 of the Convention)[1]; as well as the violation of the right to respect for private life (Article 8 of the Convention). Therefore, today there are quite acute questions: medical intervention without the person's consent; compulsory takiing of human biological materials; clinical methods, the use of which in the biological samples taking will not be regarded as violation of international standards of human rights protection; the correlation of the need for the formation of DNA profile databases and the right of the person to non-disclosure of medical information.

THE AIM

The aim of this work is to identify and analyze the key points of the ECHR regarding the peculiarities of retention and use of human biological material samples in the investigation of crimes, and the retention of such materials after the completion of the investigation and trial.

MATERIALS AND METHODS

In the preparation of the article, scientific works, the provisions of international normative acts regulating the use

[1] The right to freedom from self-closure is not directly specified in aArt. 6 of the Convention, however, is an element of the presumption of innocence (Funke v. France (25 February 1993) and John Murray v. The United Kingdom (8 February 1996).

of human biological materials as well as the practice of the ECHR concerning the use of human biological materials in the investigation of crimes were used (8 decisions were analyzed in which the ECHR concerned the use of biological samples or related issues). In the research process to achieve the goal, a complex of general scientific and special methods of cognition was used, in particular, the comparative legal method, the system and structural method, the method of generalization, the method of analysis and synthesis, etc.

REVIEW

Obtaining samples of biological materials without the person's consent and the prohibition of torture (Art. 3 of the Convention). In this context, first of all it should be pointed out that in practice, the ECHR clearly distinguishes between the need for compulsory medical procedures that are caused by therapeutic indications and compulsory medical procedures that are not conditioned by therapeutic indications and aimed at obtaining evidence in the investigation of a crime. At the same time, the use of compulsory procedures for the taking of biological samples for the purpose of investigating a crime by itself does not indicate a violation of the rights of a person provided for in Art. 3 and Art. 8 of the Convention. Accordingly, in Case of Jalloh v. Germany, the ECHR indicated the following, "Even where it is not motivated by reasons of medical necessity, Articles 3 and 8 of the Convention do not as such prohibit recourse to a medical procedure in defiance of the will of a suspect in order to obtain from him evidence of his involvement in the commission of a criminal offence. Thus, the Convention institutions have found on several occasions that the taking of blood or saliva samples against a suspect's will in order to investigate an offence did not breach these Articles in the circumstances of the cases examined by them" [1]. However, in view of the aforementioned delimitation of medical intervention (with a therapeutic aim and for obtaining evidence to investigate a crime), the ECHR uses slightly different approaches in assessing the admissibility of such medical intervention limits. In particular, the ECHR permits a greater degree of coercion in situations that are conditioned precisely by medical indications. With regard to the same medical intervention in order to obtain evidence, it will not be violated by the Convention solely on condition that such a procedure would correspond to the key principles formulated by the ECHR. The ECHR position in Case of Salikhov v. Russia is quite indicative in this key, where the ECHR stated the following, "It is observed at the outset that the clipping of the applicant's fingernails and the attempt to take his blood were not required by medical reasons, that is, they were not needed to protect the applicant's health. Rather, those procedures were aimed at securing evidence of a rape. This finding does not of itself warrant the conclusion that the intervention contravened Article 3, as the Convention does not, in principle, prohibit recourse to a forcible medical intervention that will assist in the investigation of an offence. Nevertheless, any interference with a person's physical integrity carried out with the aim of obtaining evidence must be the subject of rigorous scrutiny, with the following factors being of particular importance: the extent to which forcible medical intervention was necessary to obtain the evidence, the health risks for the suspect, the manner in which the procedure was carried out and the physical pain and mental suffering it caused, the degree of medical supervision available and the effects on the suspect's health. In the light of all the circumstances of the individual case, the intervention must not attain the minimum level of severity that would bring it within the scope of Article 3. The Court will now examine each of these elements in turn" [2].

Obtaining samples of biological materials without the person's consent and the right not to incriminate oneself (Art. 6 of the Convention). It is also worth pointing out that the ECHR practice raised the question of whether the compulsory taking of biological samples violated the right not to incriminate oneself. It should be recalled that according to the legislation of most European states, as well as in accordance with the provisions of the Convention, a person has the right not to testify against himself, and the forced obtaining of such testimony is considered illegal. Taking into account the above rule, there were cases where the applicants, applying to the ECHR, extended the right not to incriminate oneself to situations of compulsory taking of biological samples. At the same time, the ECHR in this key follows a clear position that the right to freedom from self-restraint extends solely to verbal information (testimony) and does not relate to the acquisition of human biological samples. In particular, the position of the ECHR was expressed in Case of Saunders v. United Kingdom, where the following was stated, "The right not to incriminate oneself is primarily concerned, however, with respecting the will of an accused person to remain silent. As commonly understood in the legal systems of the Contracting Parties to the Convention and elsewhere, it does not extend to the use in criminal proceedings of material which may be obtained from the accused through the use of compulsory powers but which has an existence independent of the will of the suspect such as, inter alia, documents acquired pursuant to a warrant, breath, blood and urine samples and bodily tissue for the purpose of DNA testing" [3].

However, it is interesting to note, that in another judgment (Case of P.G. and J.H. v. The United Kingdom), a similar approach not only found complete support, but was also extended to human voice samples. In particular, as the ECHR pointed out, "In so far as the applicants complained of the underhand way in which the voice samples for comparison were obtained and that this infringed their privilege against self-incrimination, the Court considers that the voice samples, which did not include any incriminating statements, may be regarded as akin to blood, hair or other physical or objective specimens used in forensic analysis and to which privilege against self-incrimination does not apply" [4].

The use of human biological materials samples in the criminal procedure and the right to respect for private life (Art. 8 of the Convention). Today, taking of biological samples (cell samples, DNA information, fingerprints[2]) is quite common for many European countries. In particular, most European countries allow the compulsory taking of fingerprints and cell sampling in the context of criminal proceedings. At least 20 member states of the Council of Europe have provided provisions for DNA information taking and its retention in national databases or in other forms (Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Luxembourg, the Netherlands, Norway, Poland, Spain, Sweden and Switzerland). The number of such countries is gradually increasing [see at: 5]. It is permitted to use DNA material in the investigation of crimes and at the level of international regulations (in particular, the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to Application of Biology and Medicine [6]).

At the same time, an analysis of the ECHR practice provides an opportunity to argue that the main disputes are not because of taking and use of such data within the framework of crime investigation, but the further retention of bio samples while the investigation and trial are already completed. The question of retention of human tissue samples that were taken during the investigation of a crime is very relevant nowadays in the first place from the point of view of ensuring the right to respect for private life. At the same time, not being new, this question has not yet found a clear solution either in science or in practice. In particular, an analysis of the ECHR practice provides an opportunity to state that there is no unified approach. The question is usually solved based on individual circumstances of the case. Nevertheless, an analysis of the existing decisions of the ECHR still gives the opportunity to distinguish some general laws.

However, before turning to the description of these laws, we should note that some of the most general provisions regarding the rules for the use and retention of biological samples of human tissues (in particular, DNA data) in the investigation of crimes have been raised at the level of the Recommendations of the Committee of Ministers of the Council of Europe of 10 February 1992 No. R(92)1 On the use of analysis of deoxyribonucleic acid (DNA) within the framework of the criminal justice system [7]. According to this document, member states are encouraged to adhere to the following principles in relation to the use of bio samples and information derived from them:

- The taking of samples for the purpose of DNA analysis should only be carried out in circumstances determined by the domestic law; it being understood that in some states this may necessitate specific authorisation from a judicial authority;
- Recourse to DNA analysis should be permissible in all appropriate cases, independent of the degree of seriousness of the offence;
- Samples collected for DNA analysis and the information derived from such analysis for the purpose of the investigation and prosecution of criminal offences must not be used for other purposes;
- Samples taken for DNA analysis and the information so derived may be needed for research and statistical purposes. Such uses are acceptable provided the identity of the individual cannot be ascertained. Names or other identifying references must therefore be removed prior to their use for these purposes;
- Samples or other body tissues taken from individuals for DNA analysis should not be kept after the rendering of the final decision in the case for which they were used, unless it is necessary for purposes directly linked to those for which they were collected. Measures should be taken to ensure that the results of DNA analysis and the information so derived is deleted when it is no longer necessary to keep it for the purposes for which it was used. The results of DNA analysis and the information so derived may, however, be retained where the individual concerned has been convicted of serious offences against the life, integrity or security of persons. In such cases strict retention periods should be defined by domestic law. Samples and other body tissues, or the information derived from them, may be stored for longer periods: when the person concerned so requests; or when the sample cannot be attributed to an individual, for example when it is found at the scene of an offence. Where the security of the state is involved, the domestic law of the member state may permit retention of the samples, the results of DNA analysis and the information so derived even though the individual concerned has not been charged or convicted of an offence. In such cases strict retention periods should be defined by domestic law [7].

It is clear that in its decisions the ECHR cannot ignore the Committee of Ministers of the Council of Europe's general recommendations; therefore starting positions are drawn from them. In particular, when considering complaints related to the retention of bio samples, the ECHR not only addresses the question of whether there is interference with the sphere of privacy and whether such interference with public interests is commensurate, but also explores aspects such as:

- Differentiation of the interference degree with privacy in the use of bio samples, depending on the specificity of such bio samples (or information received from them): fingerprints, cells, DNA profiles;
- Severity (qualification) of a crime as a factor influencing the decision on the question of the admissibility of the use and retention of bio samples and the information obtained with their use and the term of such retention;
- The results of the investigation (in which the biological samples were taken) and the trial, in particular, whether the person was convicted or acquitted.

Therefore, summarizing some practice, we will try to distinguish the ECHR's generalized positions according to key aspects.

1. Human tissue samples (including DNA samples) contain personal data, and therefore their taking and retention is

[2] The reference of fingerprints to biomaterials is controversial, but we recall them in the light of the ECHR practice, which follows the principle of delimiting such samples as (a) cells, (b) DNA data and (c) fingerprints.

always an interference with privacy. In general, the position of the ECHR on this issue was as follows. In addition to the person's name, their private and family life may include other means of personal identification and family ties. Data contained in cells and DNA data are definitely a kind of personal data, since it is not only possible to identify a person, but also to observe their kinship ties, to establish ethnicity, etc. Moreover, any obtaining and retention of personal data by public authorities, no matter how they were received, should be considered as having a direct impact on ensuring respect for the privacy of the person concerned, regardless of whether there is any further use of these data [see at: 5; 8];

2. Such interference with private life is still permissible if this is due to the protection of the public interest. In general, in most cases, the ECHR recognizes that the public interest in combating crime fully justifies the objective of biological samples obtaining and using. At the same time, the issue of the further (that is, after the completion of the investigation and trial) retention of bio samples and the information received from them by the ECHR is not so clear. In particular, as stated by the ECHR in the judgment in S. and Marper v. The United Kingdom, the interests of the data subjects (from whom the biosphere was received) and community as a whole in the protection of personal data, including fingerprints and DNA information, may be outweighed by a legitimate interest in the prevention of crime. The court finds it to be beyond dispute that the fight against crime, and in particular against organized crime and terrorism, which is one of the challenges faced by today's European societies, depends greatly on the use of modern scientific techniques of investigation and identification. The Court agrees that the retention of fingerprints and DNA information pursues the legitimate purpose of detection and, therefore, prevention of crime. If the original taking of this information pursues the aim of linking a particular person to the particular crime of which he or she is suspected, its retention pursues the broader purpose of assisting in the identification of future offenders [see at: 5]. Also, the ECHR, determined by the permissibility of bio samples retention, the importance of public interest that can afford such retention, the length of retention, emphasizes the need to clearly distinguish between types of biological samples, in particular: fingerprints, cells (biological tissue samples), and DNA data. This, in its turn, makes it possible to distinguish and formulate the following position, which is followed in the practice of the ECHR.

3. The degree of interference with the right to privacy with the use of bio samples depends on the type of such bio samples (or information obtained from them): fingerprints, samples of biological tissues (cells), or DNA profiles[3]. The positions on this issue are primarily reflected in Van der Velden v Netherlands [9] and S. and Marper v. The United Kingdom [5] and are as follows. First of all, as it was already noted, the ECHR recognizes the retention of any bio samples, regardless of their variety, as the interference with the right to respect for the private life. At the same time, the ECHR indicates that the retention of fingerprints, DNA profiles and biological samples is generally more controversial than obtaining such bioinformatics, and retention of biological samples (cells) poses more ethical problems than digitized DNA profiles and fingerprints, taking into account the difference in levels of information that can be disclosed. The retention of cell samples implies a particularly strong interference with the right to respect for private life, given the extremely high amount of genetic and health-related information contained there. Thus, according to the degree of intervention in private life, the ECHR provides the following gradation of human bio samples (given the nature and amount of information contained in each of them): cellular material (the highest level of intervention); DNA profile (average level of intervention), fingerprints (the lowest level of intervention). This approach is substantiated by the following arguments:

- Human cell samples contain not only identifying information about a person, but also information about his or her health, the presence of diseases, etc., that is, information that goes beyond the scope of the need for a crime investigation. In addition, human cell samples contain a unique genetic code that can be used to form a DNA profile;
- The DNA profile contains a more limited amount of private information than the cellular material; in particular, it contains only identifying information along the DNA code and does not contain information about the health of the person and the presence of diseases. Nevertheless, the DNA code provides the ability to set data that goes beyond the scope of the investigation, in particular, to identify family ties or the ethnic origin of a person;
- Fingerprints, unlike cellular material and DNA profiles, provide an opportunity to identify only the person, but do not make it possible to identify any other personal information.

4. The severity (qualification) of a crime as a factor affecting the issue resolution of the permissibility of bio samples use and retention and the information obtained with their use and the term of such retention. The position on the need to take into account the severity and seriousness of a crime in deciding the use of the fight against criminality of human tissues is laid down in the Recommendation of the Committee of Ministers of the Council of Europe of 10 February 1992, No. R(92)1 [7]. Moreover, as stated in ECHR judgments, the majority of Contracting States allow the taking of cell materials in criminal proceedings only from persons suspected of committing crimes of a certain minimum severity. For example, in Austria, Belgium, Finland, France, Germany, Hungary, Ireland, Italy, Luxembourg, the Netherlands, Norway, Poland, Spain and Sweden, the taking of DNA information in the context of criminal proceedings is limited to serious crimes, in particular those punishable by imprisonment [see at: 5]. In the judgment of W. v. the

[3] A DNA profile (or genetic passport) is data that contains information about the human genetic code, along with identifying data about the person to which this DNA profile belongs. The decisions of the ECHR refer to DNA profiles as digitized information stored electronically in the National DNA Database along with the identity of the person concerned (see at Case of S. and Marper v. The United Kingdom).

Netherlands of 20 January 2009 (Complaint No. 20689/08), the ECHR stated that the length of retention of DNA data after the investigation and trial should be based on the length of the maximum sentence that can be imposed in relation to the offence. And in the judgment of Peruzzo and Martens v. Germany, dated 4 June 2013 (Complaints Nos. 7841/08 and 57900/12), the ECHR found that the remedies provided by the relevant national law were sufficient: only the genetic material of recidivists or persons suspected of serious crimes, or crimes against sexual self-determination [10, p. 112, 116].

5. The results of the investigation, in which the biological samples were taken, and trial (in particular, whether the person was convicted or acquitted), are also compulsory taken into account by the ECHR in deciding on the justification for the retention of bio samples after the completion of the investigation and trial from the standpoint of respect for the right to privacy. The general concept in this issue is as follows: the unlimited duration of biological samples retention and information received from them about persons who were found guilty of a crime at the police databases is justified by the public interest in the fight against crime. Instead, a similar approach to the question of biological samples retention of persons who were not convicted of any crime violates the presumption of innocence. It is significant that in the vast majority of European states there is a clear distinction between the procedures for retention of biological samples of persons, depending on whether they were found guilty or not guilty of a criminal offence. For example, Belgium, Hungary, Ireland, Italy and Sweden require that such information be automatically deleted in order to justify or stop the criminal proceedings. Germany, Luxembourg and the Netherlands allow the retention of such information if there is any suspicion of a person or if further investigation is required in another case; Austria allows its retention, if there is a risk that the suspect will commit a dangerous offence; Norway and Spain permit the retention of profiles if the defendant was acquitted because he was not subject to criminal liability; Finland and Denmark allow retention for 1 and 10 years respectively, if justified, and Switzerland for 1 year if the proceedings were terminated [see at: 5]. At the same time, the provisions of the French legislation on the protection of fingerprints of justifiable persons during the twenty-five years were recognized by the ECHR as that is equivalent to their lifelong retention. Consequently, the ECHR stated that in this case the restriction of the right to private life was not proportional to the accomplishment of public interests in a democratic state [11]. It is significant that the results of the investigation and trial (recognition of a person guilty or innocent) in different ways affect the issues of enhanced protection of the minor's interests. Thus, these results, in some cases, may further enhance the privacy of minors, and in others, to alleviate the difference in approaches to minors and adults. For example, in the judgment of S. and Marper v. The United Kingdom, the ECHR indicated that "the retention of the unconvicted persons' data may be especially harmful in the case of minors, given their special situation and the importance of their development and integration in society"[5]. Instead, in the judgment of W. v. the Netherlands dated 20 January 2009 (Complaint No. 20689/08), the ECHR indicated that, unlike the case S. and Marper v. The United Kingdom, where the applicants were found to be innocent of a crime, that case involved the retention of DNA data of a person who had been prosecuted. Therefore, the applicant's minor age should not influence the decision on the retention of his or her biological samples after the completion of the investigation and trial [10, p. 113].

DISCUSSION

The questions discussed in this article are not completely new. As the issue of ECHR's interpretation of human rights in healthcare [see at: 12], and the question of samples of human biological materials amples taking and using, in particular regarding the application of compulsory medical procedures to the patient, has already been raised by researchers in different contexts. At the same time, the review of works makes it possible to state that today a number of issues remain controversial. The problem of the formation and use of DNA databases in Poland in the context of the adoption of relevant laws was considered by M. Goc, H. Dybrowska [13], where the authors, based on statistical data and positive experience with the use of human biological samples, expressed their belief in the need for further intensifying the use of such samples in the investigation of crimes. In turn, the issue of constitutionality of the DNA samples use, in particular in the context of Polish law, was raised in the work of J. Wójcikiewicz and V. Kwiatkowska-Wójcikiewicz [14]. The discussion on the relationship between the privacy of information containing human DNA and the public interest in the use of such information in the investigation of crimes is illustrated in the work of R. Erbaş [15]. A similar question was raised by K. Dedrickson [16], where the author concludes that the correct approach to the creation of universal DNA bases will guarantee maximum achievement in the fight against crime, with minimal interference with privacy. Controversial issues of the restriction of somatic human rights, the specifics of the use of compulsory medical procedures depending on the procedural status of a person, peculiarities of the application of various medical methods in order to obtain evidence are illustrated in the work of A. Gambaryan [17].

CONCLUSIONS

1. In practice, the ECHR clearly distinguishes between the need for compulsory medical procedures that are caused by therapeutic indications and compulsory medical procedures aimed at obtaining evidence in the investigation of crime. The use of compulsory medical procedures for taking of biological samples for the purpose of investigating a crime by itself does not indicate a violation of the rights of a person provided for in Art. 3 and Art. 8 of the Convention, but when assessing the admissibility of such procedures, the ECHR pays particular attention to the factors: the extent to which forcible medical intervention

was necessary to obtain evidence, the health risks of the suspect, the way in which the procedure is performed, the physical pain and the mental suffering experienced by the person, the degree of medical supervision and the impact on the suspect's health.

2. The ECHR maintains a clear position that the right not to incriminate oneself is applied exclusively to verbal information (evidence) and does not relate to taking of human biological samples, in particular such as samples of blood, saliva, urine, fingernail clippings, and even voice samples.
3. In resolving the issue of the admissibility of restricting the right to respect for private life in the use of human bio samples during the criminal procedure, the ECHR not only decides whether such interference with the public interest is proportional, but also takes into account aspects such as: (a) differentiation of the interference degree with privacy in bio samples using, depending on the specifics of such bio samples (or information received from them): fingerprints, cages, DNA profiles; (b) the severity (qualification) of the crime as a factor affecting the permissibility question resolution of the use and retention of bio samples and the information obtained with their use and the term of such retention; (c) the results of the investigation, in which the biological samples were taken, and the trial: in particular, whether the person was convicted or acquitted.

REFERENCES

1. Case of Jalloh v. Germany, application no. 54810/00, 11 July 2006. See at: http://hudoc.echr.coe.int/eng?i=001-76307
2. Case of Salikhov v. Russia, application no. 23880/05, 3 May 2012. See at: http://hudoc.echr.coe.int/eng?i=001-110718
3. Case of Saunders v. United Kingdom, application no. 19187/91, 17 December 1996. See at: http://hudoc.echr.coe.int/eng?i=001-58009
4. Case of P.G. and J.H. v. The United Kingdom, application no. 44787/98, 25 September 2001. See at: http://hudoc.echr.coe.int/eng?i=001-59665
5. Case of S. and Marper v. The United Kingdom, applications nos. 30562/04 and 30566/04, 04 December 2008. See at: http://hudoc.echr.coe.int/rus?i=001-90051
6. Konventsiya o zaschite prav i dostoinstva cheloveka v svyazi s primeneniem dostizheniy biologii i meditsinyi: Konventsiya o pravah cheloveka i biomeditsine (Oviedo, 4 April 1997) [The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to Application of Biology and Medicine: Convention on Human Rights and Biomedicine]. See at: https://rm.coe.int/168007d004 (in Russian).
7. Recommendation No. R(92)1 of the Committee of Ministers on the use of analysis of deoxyribonucleic acid (DNA) within the framework of the criminal justice system (Adopted by the Committee of Ministers on 10 February 1992 at the 470th meeting of the Ministers' Deputies). See at: https://rm.coe.int/09000016804e54f7
8. Case of Amann v. Switzerland, application no. 27798/95, 16 February 2000. See at: http://hudoc.echr.coe.int/eng?i=001-58497
9. Case of Van der Velden v. The Netherlands, application no. 29514/05, 07 December 2006. See at: http://echr.ketse.com/doc/29514.05-en-20061207/view/
10. Problemy bioetiki v svete sudebnoy praktiki Evropeyskogo Suda po pravam cheloveka [Problems of bioethics in the light of the jurisprudence of the European Court of Human Rights]. See at: https://rm.coe.int/case-law-2017/1680736451 (in Russian).
11. Case of M.K. v. France, application no. 19522/09, 18 April 2013. See at: http://hudoc.echr.coe.int/eng?i=001-119075
12. Tatsiy V., Gutorova N., Pashkov V. Legal aspects of cancer deseases prophylactics: patients rights context. Wiad Lek. 2017;71(I):1108–1113.
13. Goc M., Dybrowska H. The present and future of DNA database in Poland. Z Zagadnień Nauk Sadowych, z. L. 2002;150–154. See at: http://www.forensicscience.pl/pfs/50_goc.pdf
14. Wójcikiewicz J., Kwiatkowska-Wójcikiewicz V. The constitutionality of taking DNA reference samples. doi: http://dx.doi.org/10.12775/CLR.2017.009
15. Erbaş R. The tension between genome privacy and criminal justice in the wake of DNA databases. Journal of Penal Law and Criminology. 2017;5(2):163–178. See at: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3081456
16. Dedrickson K. Universal DNA databases: a way to improve privacy? Journal of Law and the Biosciences. 2017;4(3):637–647. See at: https://doi.org/10.1093/jlb/lsx041
17. Gambaryan A.S. Otkaz ot prava i voprosyi ugolovno-protsessualnogo vmeshatelstva [Waiver and Issues of Criminal Procedure Intervention]. 2018;352. (in Russian).

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Ivan A. Titko
Poltava Law Institute of Yaroslav Mudryi National Law University
Pershotravnevyi prospekt, 5, 36000, Poltava, Ukraine
tel: +380975150748
e-mail: titko.iv@gmail.com

Received: 24.03.2019
Accepted: 10.07.2019

 © Wydawnictwo Aluna

PRACA POGLĄDOWA
REVIEW ARTICLE

INTERNATIONAL LEGAL STANDARDS FOR CONDUCTING BIOMEDICAL EXPERIMENTS ON ANIMALS: STATUS AND PROSPECTS OF DEVELOPMENT

Mykhailo A. Anishchenko[1], Olexandr G. Aleksieiev[1], Inna A. Hamburh[2]
[1]DEPARTMENT OF MANAGEMENT AND ECONOMICS OF PHARMACY, MEDICAL AND PHARMACEUTICAL LAW OF ZAPORIZHZHIA STATE MEDICAL UNIVERSITY, ZAPORIZHZHIA, UKRAINE
[2]DEPARTMENT OF CIVIL, ADMINISTRATIVE AND FINANCIAL LAW OF PRIVATE CLASSIC UNIVERSITY, ZAPORIZHZHIA, UKRAINE

Cruelty to animals can exist neither where people are truly educated, nor where true scholarship reigns.
Alexander Humbolt

ABSTRACT

Introduction: The article highlights the basic principles of the use of animals in biomedical research raised the main problems of vivisection. It also suggests specific steps to enhance the humanization of this process. The conclusions are the following: it is very important to prevent cruelty to animals in the context of resolving bioethical and legal problems associated with the implementation of the principle of humanism and the protection of animal rights. Modern science and the practice of preclinical and clinical tests should be involved into serious challenge in order to improve and universalize the process of medical and biological research technology in vitro.

The aim:: The purpose of the article is to study the main international legal principles of using animals in biomedical experiments, as well as to determine the main problems on this issue and to outline the ways to resolve them.

Materials and methods: The research material is a modern international regulatory framework that establishes the basic principles for the animals treatment. The methods of information retrieval, analysis, systematization, and generalization were used in this article.

Review: As it is well-known, human treatment of people begins with a human attitude towards animals. Nowadays the international community has adopted enough legal acts regulating the proper behavior with animals, including those specially cultivated for such studies (laboratory) when conducting biomedical experiments. Recently, both, in the national legislation of European countries and international legislation, there have been trends in the protection of both the general rights of animals as well as intensification of their protection in the field of biomedical research.

Conclusions: it is imperative, both at the international and national levels, to adopt programs for development and support of scientific study of the in vitro biosystems introduction into the practice of toxicological studies.

At the national level, it is necessary to criminalize the violation of international standards for conducting biomedical research using animals, strengthen other types of responsibility, and create a special system of state bodies responsible for the policy of using animals for biomedical purposes and exercising control in this area. Both, international and national legislation should impose a direct ban on the use of in vivo technologies in case of the possibility of using in vitro technologies.

KEY WORDS: vivisection, biomedical experiment, animals, legislation

Wiad Lek 2019, 72, 8, 1582-1585

INTRODUCTION

Tests related to the use of animals as experimental material in biomedical research have always caused and continue to cause heated debates. Vivisection (from lat. vivus – living, and sectio a cutting) always had both, supporters and ardent opponents, because vivisection was vivo surgical manipulation with the purpose of studying the properties of pharmaceutical products, development of new methods of surgical, therapeutic, psychological treatment of various diseases., The conflict between them goes beyond the scientific debate very often. In addition, it moves into an active phase. The society becomes a witness against the trials and convictions in respect of both supporters and opponents of vivisection.

THE AIM

The purpose of the article is to study the main international legal principles of using animals in biomedical experiments, as well as to determine the main problems on this issue and to outline the ways to resolve them.

MATERIALS AND METHODS

The research material is a modern international regulatory framework that establishes the basic principles for the treatment of animals. The methods of information retrieval, analysis, systematization, and generalization were used in this research.

REVIEW AND DISCUSSION

There is no treatment for around half of all diseases in the world. It can take many years of painstaking work using a wide range of research techniques to understand how the body works, and how diseases are progressing, and to find cures, vaccines or treatments. There is overwhelming scientific consensus worldwide, where some researches, using animals is still essential for medical progress.[9]. Therefore, the need for regulation of biomedical research the procedure is undoubtful. Firstly, it is necessary for both, for creating new methods of treatment and creating new dosage forms. For example, it is impossible to create new dosage forms with their subsequent registration [10] without research. The issue of preventing the negative effects of individual biochemical compounds on human health and the environment is very important. [11] These are just some of the reasons for the necessity for researches on animals. As it is well-known, human treatment of people begins with a human attitude towards animals. Nowadays the international community has adopted enough legal acts regulating the proper behavior of humans with animals, including those specially cultivated for such studies (laboratory) when conducting biomedical experiments. The Regulation on the Use of Animals in Biomedical Research, adopted by the 41st World Medical Assembly in September 1989, occupies a rather important special place among them. The basic tenets of the provisions of this document of this Regulation determine the following principles for the use of animals when conducting biomedical research are prescribed: conducting training activities with the staff of research institutions on the proper handling, transportation and care of animals; provision of veterinary support for biomedical experiments; all research institutions must comply with the rules of humane treatment of laboratory animals [1].

The International Recommendations (Ethical Code) for conducting biomedical research and using animals, which were developed and published in 1985 by the Council of International Scientific Organizations, is an important document. The main ethical and legal provisions, enshrined in the recommendations, are as follows: in order to develop more advanced model of healthcare there should be used mathematical models, machine modeling and biological systems in vitro; experiments on animals should be carried out only after assessing their value for human health or the animals and exclusively for the development of biological knowledge, for experiments it is necessary to use only floor amount of healthy individuals; researchers should treat animals in such a manner as to minimize inconvenience, suffering and pain, including the use of proper sedatives, analgesic or narcotic drugs in accordance with the standards adopted in veterinary practice (if it is necessary, there can be a diversion protocol, the decisions are made the relevant competent committee); in challenging cases, animals that cannot be treated, are to be mortified in a painless way; laboratory animals should be provided with optimal living conditions and veterinary care; training on animal welfare for personnel, conducting research [2].

As it can be seen from the abovementioned, both documents imply the vital need for biomedical research with use of the animals. According to the first document, namely the Regulation on the use of animals in biomedical research, there is no alternatives to the research involving animals. The second document, namely the International Recommendations (Code of Ethics) for conducting biomedical research using animals, contains an alternative to experiments based on laboratory animals, which includes the use of mathematical models, machine modeling, and biological systems in vitro. Although, according to the above-mentioned International recommendations, such an alternative should be implemented in cases "where necessary" instead of "where possible". That is, according to the logic of the authors of this document, the alternative research methods with animals should be used only if a certain type of research cannot show the proper quality of the results without using alternative methods, and not in an exactly opposite situation, when applying the alternative methods ensures the proper quality of experiments. The latter method of application of the alternative methods could sharply reduce the need for laboratory animals' usage.

The European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes, adopted by the member states of the Council of Europe on 18th of March, in Strasbourg in 1986, establishes the following principles: animals which used for scientific purposes should be provided with proper care, veterinary support and conditions that allow them to meet their physiological and ethological needs; scientific experiments with animals should not be carried out only in case if there is a justified and feasible possibility of using another acceptable scientific method without using animals and states should encourage the use of such methods; if it is necessary to conduct scientific experiments with animals, there should be a careful approach to the selection of specific animals with the desired justification of such a choice by the competent state authority, there is a need for selection of the safest and least traumatic methods with the smallest number of animals tested and the most satisfactory results; widespread usage of methods of general or local anesthesia or methods of analgesia, if possible and appropriate; the most painful and traumatic research activities must be conducted only with the consent of the competent public authority; upon completion of any scientific procedure, the person responsible for this procedure or a veterinarian makes a decision on supporting or sustaining life of the animal or its mercy killing using the euthanasia method in case the animal constantly experiences pain or anxiety; no animal, after participating in an experiment that is grievous for its health, can take part in an experiment for the second time, except conducting procedures under anesthesia and subsequent killing, or if it implies minimal intervention; according to the decision of the competent state bodies, the animal may be released after experiments conducted on it, if the care provided by the researchers allows the animal to be in a normal physiological state, the release of the animal to freedom to achieve educational or training

purposes is not allowed; creation of a special permit system of state bodies that could issue the appropriate permissions to competent persons to conduct experiments with the participation of animals; breeding ground and supplying institutions, as well as user institutions must be registered with the appropriate state authority; animal welfare conditions must comply with international standards, including with appropriate labeling, registry maintenance and proper veterinary care; stray domestic animals cannot be used in research, animals originating only from breeding establishments or supplying institutions must be used for these purposes, if there is not any general or special exemptions; proper training of personnel, who conduct research using animals; conducting research with the use of animals for educational purposes for professional training of specialists may only take place under the supervision of a competent responsible person and only in cases where the purpose of such classes cannot be achieved by other methods (for example, using audiovisual media, etc.); states maintain special statistics on research, the data of which are transmitted to the Secretary General of the Council of Europe; states maintain special statistics on research, the data received is transmitted to the Secretary General of the Council of Europe; in order to avoid unnecessary repetition of research using animals, states recognize the results of such research conducted in other countries [3].

Recently, both, in the national legislation of European countries and international legislation, there have been trends in the protection of both the general rights of animals as well as intensification of their protection in the field of biomedical research.

For instance, in the countries of European Union there is an outright ban on testing of cosmetics on animals as well as it is prohibited to crop the tail, the ears and conduct onychectomy [4].

For the first time in the European region, at the constitutional level, Switzerland has secured the obligation to ensure animal welfare. In 1994, in Switzerland, there was a referendum, which changed the status of animals from "things" to "sentient" creatures. In Switzerland, the Law of 1978 regulated in detail the issues of ensuring the well-being and protection against ill-treatment of domestic animals as well as laboratory and agricultural ones. The law contains requirements for keeping animals, training personnel for working with laboratory animals, conditions for obtaining permits for keeping wild and domestic animals, animal trade, trade transportation. Special attention was paid to obtaining permission to conduct experiments, as well as the need for anesthetization of the animal during experiments.

In 2002, the Federal Republic of Germany completed the provisions of the Constitution on the protection of animals (Article 20a). Active work of public organizations (Tierschutzverein) - Animal Protection Society has led to concrete measures for the protection of animals. The Law on the protection of animals establishes penalties in case of violation of the rules for the treatment of animals, as well as a special taxation [5, p. 373]. In Germany, euthanasia of healthy animals is completely prohibited [4].

Spain is the world's first country that equalized human and animal rights. In 2008, it recognized the right of every great ape to life, freedom, and protection from cruelty. Nowadays in Spain, monkeys cannot be regarded as objects of private property, experiments on monkeys are prohibited, and their keeping in circus and television programs and living conditions in zoos have been significantly improved.

In April 2016, New Zealand officially recognized that all animals are intelligent creatures. Those people, who mistreated animals, are persecuted. Research and experiments on animals are prohibited.

India became the first country in the world, which has acknowledged the rights of dolphins. Dolphins should be understood as "personalities of non-human nature" with the right to be considered individuals. There is a prohibition against dolphinariums and dolphins' captive breeding stations. Furthermore, India became the first country in South Asia that issued a ban on testing cosmetics and its ingredients on animals [4].

The adoption of such humane laws was preceded by a series of scandals, the subject of which were cases of cruel treatment of animals during the conduct of biomedical experiments.

One of the most egregious cases of cruelty to animals took place at the Institute for the Behavioral Research in Silver Spring, Maryland, the USA in 1981.

A group of scientists led by Edward Taub conducted research on monkeys of the Philippines, the purpose of which was to study neuroplasticity. Its aim was to study the possibility of primate brain to change under the influence of experiments. Specifically, it was manifested in the form of removal of the spinal ganglion, and subsequent attempts to control the limbs of monkeys with an electric shock. The animal researcher Taub was charged with 17 sentences, because of the first trial in the history of the United States of America [6].

In Great Britain in 1997, the story of the bullying of the staff of the animal testing laboratory Huntingdon Life Sciences has a wide public response. The public was presented with a video in which the laboratories were puppies, imitated sexual intercourse with them during blood sampling for tests [7].

In 2002, the British Union for the Abolition of Vivisection published videotapes showing incidents of ill-treatment of experimental monkeys at the University of Cambridge.

In particular, images with animals were shown, in which cranial boxes were opened for the subsequent introduction of various toxins into the brain. Animals deliberately caused a stroke and left for a long time without any help. An interesting fact is that despite the enormous public response, the inspection of the university laboratory carried out by government representatives did not reveal any irregularities [8].

It should be noted that the authors of the article understand the need for appropriate biomedical experiments on animals. In some kind of researches there's simply no alternative options to exclude the use of animals in this matter. For example, such research can include the devel-

opment of new products aimed at the treatment of arthritis, which in turn implies a model that completely simulates the physiological characteristics of the human body. At the same time, searching for the ways to minimize the pain and physical suffering of animals must be continued and intensified.

CONCLUSIONS

As the results of the newest biomedical research show, the effectiveness of toxicological studies in biological systems in vitro is in most cases not inferior to the effectiveness of studies using experimental animals (in vivo). The serious task for improving and widespread introduction of technology into the process of biomedical research in vitro aiming to reduce the number of in vivo experiments in order to resolve bioethical and legal problems, associated with the implementation of the principle of humanism and the protection of animal's rights, preventing ill-treatment of them should be given. Modern science and the practice of preclinical and clinical researches must reduce the number of them to a possible minimum with the prospect of completely abandoning of researches using experimental animals, as well as legal regulation of the abovementioned processes in international law and national legislation of the countries of the world should be adopted immediately.

Nowadays, international acts with all their progressiveness and humanism, regulating animal rights in the process of carrying out biomedical research, must focus on the protection of the rights of laboratory animals. A number of shortcomings characterize them, so they must resolve the following provisions: they cannot be legally binding, as they are not accepted by states, but by international organizations (for example, as the Regulation on the Use of Animals in Biomedical Research, International Recommendations for Conducting Biomedical Research with the Use of Animals); there is no international legal act that would apply to countries all over the world, at the UN level with novative achievements of medical and biological science; there is no international competent authority whose task would be to control the use of animals in biomedical experiments; there is no Unified register of biomedical research with the participation of animals in order to avoid inappropriate repetitions.

In addition, it is imperative, both at the international and national levels, to adopt programs for the development and support of scientific researches to study the introduction of in vitro biosystems into the practice of toxicological studies.

At the national level, it is necessary to criminalize the violation of international standards for conducting biomedical research using animals, strengthen other types of liability, and create a special system of state bodies responsible for the policy of using animals for biomedical purposes and exercising control in this area. Both, international and national legislation should impose a direct ban on the use of in vivo technologies in case of the possibility of using in vitro technologies.

REFERENCES

1. Regulation on the use of animals in biomedical research. Adopted by the 41st World Medical Assembly.Hong Kong: 1989; See at URL: http://zakon2.rada.gov.ua/laws/show/ru/990_037.
2. International recommendations (Ethical Code) for conducting biomedical research using animals. See at URL: https://nsau.edu.ru/file/89911/
3. The European Convention for the Protection of Vertebrate Animals used for experiments or for other scientific purposes, adopted by the member states of the Council of Europe on March 18. Strasbourg: 1986; See at URL: https://nsau.edu.ru/file/89911/..
4. Suntsova N. About the rights of animals in different countries. See at URL: http://catsafety.ru/uxodi-i-zabota/o-pravax-zhivotnyx-v-raznyx-stranax/.
5. Korotny T.R., Zubchenko N.I., "Ensuring the welfare of animals and their protection from cruel treatment: from ethical standards to international legal regulation"// International law and international organizations: 2014;3:355-377. doi: 10.7256 / 2226-6305.2014.3.12522.
6. Doidge, Norman., "The Brain That Changes Itself: Stories of Personal Triumph from the Frontiers of Brain Science." 1. Viking Adult. 2007;141. ISBN 067003830X.
7. "It's a Dog's Life", "Countryside Undercover", Channel Four Television, UK: March 26, 1997; also see «It's a dog's life», Archive Copy: 8 March, 2012 Wayback Machine, Small World Productions 2005. 2010.
8. Laville, Sandra. "Lab monkeys scream with fear in tests", The Guardian: 2005.
9. "Research using animals: an overview. University of Oxford". See at URL: http://www.ox.ac.uk/news-and-events/animal-research/research-using-animals-an-overview
10. Vitaliy M. Pashkov, Irina A. Golovanova, Andrii A. Olefir. "The impact of the legal regime of intellectual property protection in the pharmaceutical market". Wiad Lek. 2016;3:582 – 586 .
11. Pashkov V, Batyhina O, Trotska M. "Legal restraints of pesticide effect on human organism and environment under international legislation". Wiad Lek. 2017;2:366 – 371.

Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mykhailo A. Anishchenko
Department of Management and Economics of Pharmacy,
Medical and Pharmaceutical Law of Zaporizhzhia State Medical University
Zaporizhzhia, Ukraine
tel: +380999451534
e-mail: anishchenko9085@gmail.com

Received: 27.04.2019
Accepted: 01.07.2019

OPIS PRZYPADKU
CASE REPORT

LEWOSIMENDAN – CENNY GRACZ W LECZENIU CIĘŻKIEJ PRAWOKOMOROWEJ NIEWYDOLNOŚCI KRĄŻENIA

LEVOSIMENDAN – A VALUABLE PLAYER IN THE TREATMENT OF A RIGHT-SIDED HEART FAILURE

Joanna Płonka, Jarosław Bugajski, Piotr Feusette, Agata Duszańska, Marek Gierlotka
KLINIKA I ODDZIAŁ KARDIOLOGII, UNIWERSYTECKI SZPITAL KLINICZNY, INSTYTUT MEDYCYNY, UNIWERSYTET OPOLSKI, OPOLE, POLSKA

STRESZCZENIE
66-letni otyły mężczyzna, obciążony cukrzycą typu 2 i nadciśnieniem tętniczym, przewlekłą chorobą nerek w okresie 4, utrwalonym migotaniem przedsionków z bradykardią, przyjęty został na oddział kardiologii z powodu objawów ciężkiej prawokomorowej niewydolności krążenia. W leczeniu zastosowano standardową terapię, w tym złożone leczenie moczopędne. Z uwagi na jej nieskuteczność rozpoznano oporność na diuretyki i włączono do leczenia lewosimendan, uzyskując obfitą diurezę, znaczną redukcję wagi ciała i istotną poprawę wydolności krążenia, co pozwoliło na dalszą diagnostykę i adekwatne, przyczynowe leczenie zabiegowe. W artykule zarysowano ponadto aktualny stan wiedzy na temat miejsca lewosimendanu w leczeniu ciężkiej prawokomorowej niewydolności krążenia.

SŁOWA KLUCZOWE: prawokomorowa niewydolność krążenia, lewosimendan, oporność na diuretyki

ABSTRACT
A 66 year-old obese man, suffering from type 2 diabetes, high blood pressure, chronic nephropathy in stage 4, permanent atrial fibrillation accompanied by bradycardia was admitted to a cardiology ward with the signs and symptoms of acute right-sided heart failure. A standard therapy was used including combined diuretics therapy. In spite of the applied methods and pharmaceuticals, no significant reduction of the body weight neither improvement in cardiovascular capacity or renal parameters were observed. Due to the ineffectiveness of the standard combined pharmacotherapy applied in the case of the acute circulatory failure, the resistance to diuretics was recognized and as a result of the above, infusion of levosimendan was decided to be applied. This therapy resulted in rich diuresis, significant loss in body weight and considerable improvement in cardiovascular capacity which allowed to continue further diagnostics and appropriate invasive treatment. The article describes current knowledge on the place of levosimendan and its application in the treatment of an right-sided heart failure.

KEY WORDS: right-sided heart failure, levosimendan, resistance to diuretics

Wiad Lek 2019, 72, 8, 1586-1591

WSTĘP

Ogromny postęp technologiczny, jaki obserwujemy w leczeniu schorzeń sercowo-naczyniowych w ostatnich latach, spowodował poprawę przeżycia, lecz równocześnie epidemię XXI wieku, jaką jest niewydolność krążenia. Niewydolność krążenia dotyczy 61,7 mln ludzi na świecie, więcej niż połowa klasyfikowana jest jako ciężka.

W populacji europejskiej występowanie niewydolności serca ocenia się na 0,4–2%, co oznacza, iż problem ten dotyczy około 6,5–10 miliona Europejczyków. W Polsce na niewydolność serca choruje 0,8–1 miliona osób. Etiologia niewydolności serca to głównie choroba wieńcowa i nadciśnienie tętnicze. Rocznie przybywa 220 tys. nowych chorych na niewydolność serca. Szacunkowo, wśród osób po 40 r.ż. co czwarty Polak i co piąta Polka zachoruje na niewydolność serca [1, 2].

Jeśli spojrzymy na wytyczne Europejskiego Towarzystwa Kardiologicznego [3] dotyczące diagnostyki i leczenia niewydolności serca, ostrą niewydolność serca definiuje się jako stan gwałtownie pojawiających się lub nasilających objawów, między innymi duszności, *orthopnoe*, pogorszenia tolerancji wysiłku, retencji płynów pod postacią zastoju nad polami płucnymi oraz obrzęków obwodowych.

Wyróżnia się ostrą niewydolność serca (AHF) *de novo*, jeśli epizod występuje po raz pierwszy oraz ostrą dekompensację przewlekłej niewydolności serca (ADHF), którą obserwuje się u chorych z wcześniej rozpoznawaną niewydolnością krążenia. Dekompensacja przewlekłej niewydolności serca jest dominującą przyczyną hospitalizacji chorych z niewydolnością krążenia i odpowiada za 80% przyjęć do szpitali z powodu niewydolności serca [1, 4-5].

Mimo postępu farmakoterapii ostrej niewydolności serca, od wielu lat są stosowane wciąż te same leki, w odniesieniu do których w większości nie udowodniono wpływu na rokowanie. W standardach zaleca się stosowanie między innymi dożylnych diuretyków pętlowych w celu zmniejsze-

nia przewodnienia, leków wazodilatacyjnych oraz leków inotropowych, aby zwiększyć rzut serca, poprawić perfuzję obwodową i tym samym utrzymać funkcje poszczególnych narządów. Ze względu na poważne rokowanie pacjentów z zaawansowaną ostrą niewydolnością krążenia ciągle poszukuje się nowych możliwości farmakoterapii [6].

OPIS PRZYPADKU

66-letni chory z ciężką otyłością (przy przyjęciu waga 123 kg przy wzroście 170 cm – BMI = 42,6 kg/m^2), od lat leczony z powodu cukrzycy typu 2 lekami doustnymi, z nadciśnieniem tętniczym w klasie III Polskiego Towarzystwa Nadciśnienia Tętniczego, przewlekłą chorobą nerek w stadium 4, hyperurykemią został skierowany na oddział kardiologii ze szpitala rejonowego w celu dalszego leczenia ciężkiej prawokomorowej niewydolności krążenia z powodu wyczerpania możliwości terapeutycznych w tamtejszym ośrodku. Chory w wywiadzie podawał od około 6 miesięcy duszność, spadek tolerancji wysiłku, kaszel oraz uczucie zatykania w klatce piersiowej. Utraty przytomności nie występowały.

Przy przyjęciu pacjent był w stanie średnio-ciężkim, z kaszlem i dusznością po niewielkim wysiłku (NYHA III), bez możliwości leżenia na płasko, noc spędzał w pozycji półsiedzącej z wysoko położonym wezgłowiem łóżka. W badaniu fizykalnym zwracała uwagę duża otyłość pokarmowa z objawami prawokomorowej niewydolności krążenia pod postacią masywnych obrzęków kończyn dolnych, wodobrzusza oraz ściszenia szmeru pęcherzykowego spowodowanego obecnością płynu w opłucnej oraz trzeszczenia nad polami płucnymi. Dodatkowo stwierdzono bradykardię oraz ciche tony serca, ciśnienie tętnicze krwi wynosiło 140/80 mmHg. W badaniach laboratoryjnych z odchyleń od normy przy przyjęciu stwierdzono: niedokrwistość z Hb = 10,8 g%, obniżony poziom żelaza = 31,9 ug/dl (N:53-167ug/dl), podwyższone CRP = 49 mg/l (N: 0,0-5,0), dodatnie przeciwciała *Chlamydia pneumoniae* (IgG), podwyższony poziom kreatyniny do 2,0 mg/dl z eGFR = 35,5 ml/min/1,73m^2, NTproBNP = 3011 ng/l (N-241ng/l) i D-dimery = 1024 ng/ml (N<500).

W zapisie EKG migotanie przedsionków z wolną akcją komór 50/min. oraz niskim woltażem zespołów QRS, najpewniej spowodowanym obecnością płynu w worku osierdziowym (Ryc. 1). Badanie echokardiograficzne wykazało dobrą funkcję skurczową lewej komory serca z frakcją wyrzutową LVEF = 50% i powiększeniem jam lewego serca (wymiar rozkurczowy lewej komory 58 mm, lewy przedsionek 55 mm). Zwracała uwagę powiększona prawa komora (45 mm) i prawy przedsionek (pole 38 cm^2) z cechami ich przeciążenia i zachowanej funkcji skurczowej (TAPSE 22 mm), wysokie prawdopodobieństwo nadciśnienia płucnego (RVSP 76 mmHg) oraz umiarkowana niedomykalność zastawki trójdzielnej. Ponadto uwidoczniono przerost lewej komory serca. IVSd – 1,4 cm i LVPWd – 1,3cm.

W worku osierdziowym obecny był płyn, głównie za prawą komorą w ilości 0,8 cm, ponadto stwierdzono obecność płynu w opłucnej oraz w jamie brzusznej. W badaniu RTG klatki piersiowej stwierdzono cechy niewydolności krążenia, powiększoną sylwetkę serca, szerokie, naczyniowe wnęki płucne oraz zmiany niedodmowo-zapalne (Ryc. 2).

Biorąc pod uwagę stan kliniczny chorego, nieskuteczność wcześniejszego leczenia dożylnego w szpitalu rejonowym, w tym leczenia dożylnymi wlewami furosemidu, podjęto próbę złożonego leczenia moczopędnego. Zastosowano kombinowaną terapię z użyciem furosemidu max.100mg/dobę i.v. i torasemidu max. 40 mg/dobę i.v. oraz hydrochlorotiazydu 12,5 mg p.o. doustnie. Dodatkowo, z uwagi na nadciśnienie tętnicze, stosowano doustnie ca-bloker - lerkanidypinę 20 mg/dobę p.o. i alfa-bloker w dawce 4 mg/dobę p.o. z dobrym efektem.

W profilaktyce powikłań zakrzepowo-zatorowych w przebiegu migotania przedsionków wstępnie kontynuowano warfarynę, po czym, z myślą o dalszej diagnostyce i leczeniu, heparynę drobnocząsteczkową – enoksaparynę w dawce zredukowanej z powodu niewydolności nerek – 160 mg/dobę podskórnie.

Z uwagi na obserwowaną anemię podawano dożylnie preparaty żelaza w dawce dobowej 100 mg przez 6 dni. Ponadto w leczeniu stosowano wlew 20% albuminy przez 6 dni oraz osłonowo, doustnie inhibitor pompy protonowej – pantoprazol 20 mg/ dobę.

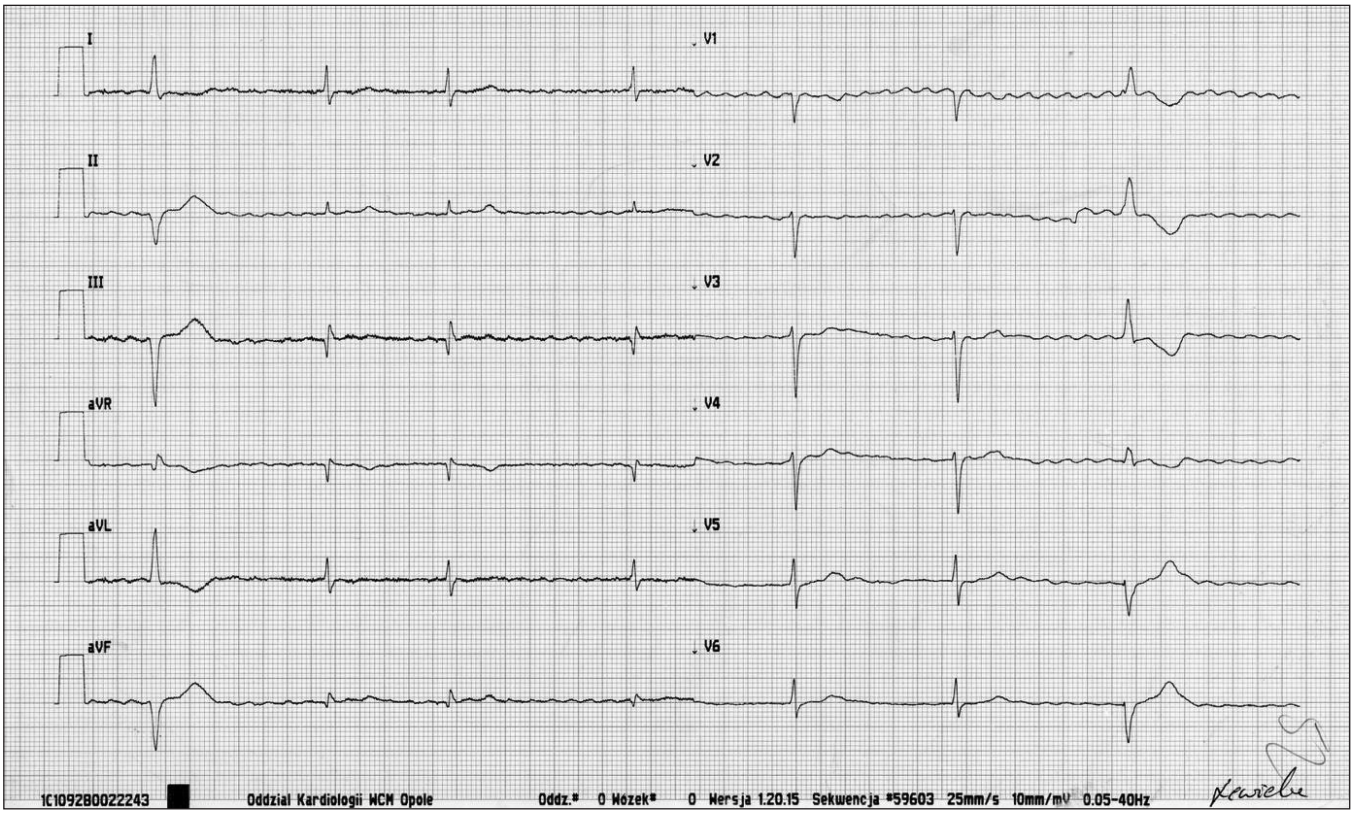
Dodatkowo podjęto decyzję o leczeniu doksycykliną z uwagi na opisywane zmiany zapalne w zdjęciu klatki piersiowej, stany podgorączkowe, kaszel i podwyższone wartości przeciwciał *Chlamydia pneumoniae*.

Diagnostykę poszerzono również o 24-godzinne monitorowanie EKG metodą Holtera, które potwierdziło migotanie przedsionków z okresowymi zwolnieniami rytmu serca do 17/min, średnim rytmem 50/min, licznymi pauzami, najdłuższa o czasie trwania 4 sekund. Ponadto wykonano USG żył kończyn dolnych i angio-TK tętnic płucnych, wykluczając zakrzepicę w łożysku naczyniowym oraz zatorowość płucną.

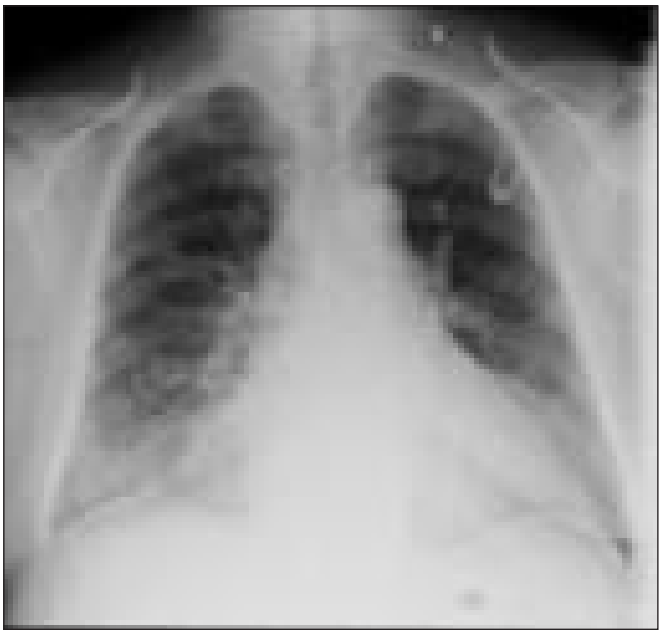
W kolejnych dniach, pomimo stosowanego leczenia, w tym leczenia dożylnego wysokimi dawkami diuretyków nie uzyskano efektywnej diurezy, redukcji obrzęków, spadku wagi ciała ani poprawy klinicznej. W związku z powyższym rozpoznano oporność na leczenie moczopędne. Zdecydowano o zastosowaniu lewosimendanu w 24-godzinnym wlewie dożylnym 0,1 µg/kg/min. Już w dniu rozpoczęcia wlewu uzyskano obfitą diurezę, która utrzymywała się w kolejnych dobach hospitalizacji i pozwoliła na stopniową redukcję dożylnych leków moczopędnych oraz następnie zastosowanie wyłącznie preparatów doustnych. W kolejnych dniach leczenia obserwowano również spadek stężenia kreatyniny (z 2,0 do 1,6 mg/dl), wzrost eGFR (35 do 46 ml/min/1,73m^2) z oraz obniżenie NT-proBNP (3011 do 1077 ng/l). Podczas hospitalizacji waga ciała chorego zmniejszyła się łącznie o 20 kg i uzyskano wyraźną poprawę kliniczną (do klasy NYHA II), ustąpienie wodobrzusza, płynu w osierdziu i opłucnych (Ryc. 3).

W trakcie leczenia wzrosło również ciśnienie tętnicze, co spowodowało konieczność intensyfikacji farmakoterapii i dołączenia inhibitora ACE.

W kontrolnym echo serca obserwowano poprawę funkcji skurczowej lewej komory serca, wyraźny spadek ciśnienia



Ryc. 1. EKG przy przyjęciu. Migotanie przedsionków z akcją komór 50/min oraz niskim woltażem zespołów QRS.



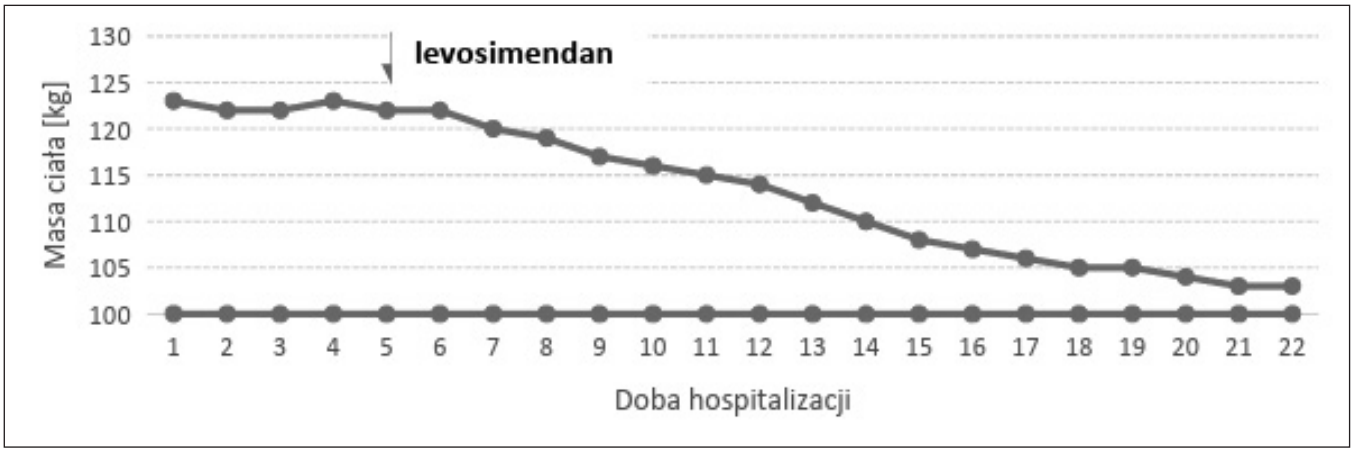
Ryc. 2. RTG klatki piersiowej przy przyjęciu. Radiologiczne objawy niewydolności krążenia, powiększona sylwetka serca, szerokie, naczyniowe wnęki płucne oraz zmiany niedodmowo- zapalne.

w "prawym sercu" (RVSP 34 mmHg) oraz ustąpienie płynu w worku osierdziowym i opłucnej.

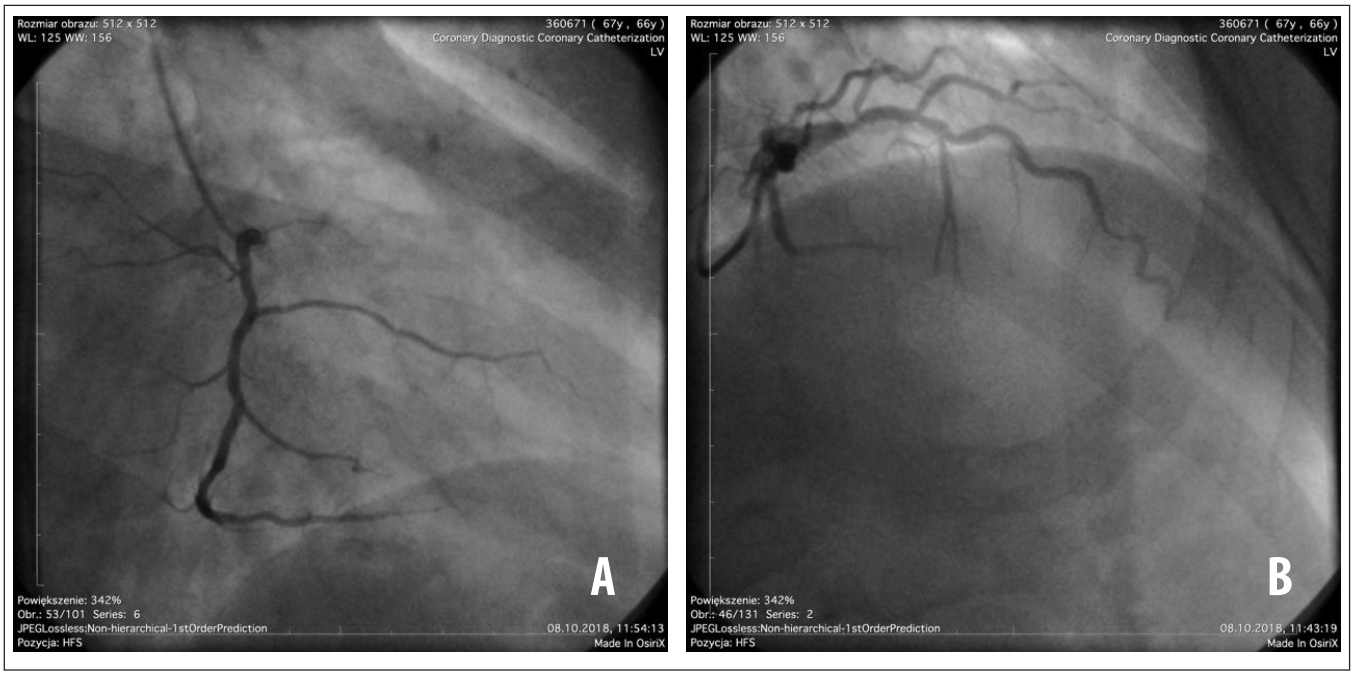
W celu oceny wydolności układu krążenia, chronotropizmu oraz ewentualnego niedokrwienia (z uwagi na liczne czynniki miażdżycy) zdecydowano o wykonaniu testu wysiłkowego na bieżni ruchomej. Badanie przerwano przed uzyskaniem rytmu submaksymalnego z powodu zmęczenia (5 METS). Nie obserwowano zmian ST-T, chory bez stenokardii w trakcie i po wysiłku. Obserwowano słaby przyrost tętna w trakcie badania.

Biorąc pod uwagę całość obrazu klinicznego, obecność czynników ryzyka choroby wieńcowej, zdecydowano o wykonaniu koronarografii z jednoczesnym cewnikowaniem prawego serca oraz pomiarem ciśnień i oporów płucnych. W koronarografii uwidoczniono rozsiane zmiany miażdżycowe z istotnym przewężeniem w zakresie gałęzi przedniej zstępującej (Ryc. 4). W pomiarach ciśnień nie stwierdzono nadciśnienia płucnego (średnie ciśnienie w tętnicy płucnej 19 mmHg, średnie ciśnienie zaklinowania 12 mmHg, naczyniowy opór płucny 1,6 jednostek Wooda). Tym niemniej, z uwagi na brak objawów dławicy piersiowej, chory nie wyraził zgody na rewaskularyzację mięśnia sercowego. Natomiast z uwagi na stwierdzane zaburzenia przewodzenia i upośledzenie choronotropizmu implantowano układ stymulujący serce, na co chory wyraził zgodę.

Ostatecznie, po 22 dniach hospitalizacji, pacjent został wypisany do domu w stanie optymalnej poprawy, w klasie czynnościowej NYHA II, ze zredukowaną o 20 kg masą ciała (masa ciała przy wypisie 103 kg) i 3-krotnie zredukowanym NT-proBNP. Z leków zalecono: apiksaban 10 mg/dobę, bisoprolol 2,5 mg/dobę, perindopril 2,5 mg/dobę, torasemid 20 mg/dobę, furosemid 160 mg/dobę, lerkanidipine 20 mg/dobę, glikwidon 15 mg/dobe, doksazosynę 4 mg/dobę, atorwastatynę 20 mg/dobę, allopurynol 100 mg/dobę, pantoprazol 20 mg/dobę.



Ryc. 3. Pomiary masy ciała w kolejnych dniach hospitalizacji. Strzałką zaznaczono moment zastosowania lewosimendanu.



Ryc. 4. Koronarografia. Po lewej (A) – prawa tętnica wieńcowa bez istotnych zwężeń. Po prawej (B) — lewa tętnica wieńcowa z istotnym zwężeniem w początkowym odcinku tętnicy przedniej zstępującej, potwierdzonym w badaniu czynnościowym cząstkowej rezerwy przepływu (FFR = 0,70).

W obserwacji ambulatoryjnej pacjent po 3 miesiącach od wypisu ze szpitala czuje się dobrze, pozostaje ze stabilną wagą ciała, wartością kreatyniny 1,6 mg/dl i tolerancją wysiłku w klasie NYHA II.

KOMENTARZ

Lewosimendan wprowadzono do leczenia po raz pierwszy w Szwecji w 2000 r. Podstawowym wskazaniem do jego stosowania jest ostra dekompensacja niewydolności krążenia, wymagająca hospitalizacji w sytuacji, gdy standardowa terapia dożylna jest niewystarczająca.

U podstaw działania lewosimendanu leżą trzy mechanizmy:

1. Działanie na komórki mięśnia sercowego poprzez uwrażliwienie na wapń, wybiórcze wiązanie z troponiną C, która łączy się z jonami wapnia i powoduje wzrost kurczliwości miocytów mięśnia sercowego, nie wywiera wpływu na czynność rozkurczową.
2. Poprzez otwarcie ATP-zależnych kanałów potasowych w komórkach mięśni gładkich naczyń krwionośnych lewosimendan powoduje rozszerzenie zarówno tętnic, jak i żył, a także poprawia krążenie w tętnicach wieńcowych.
3. Poprzez otwarcie ATP-zależnych kanałów potasowych w mitochondriach kardiomiocytów wykazuje działanie kardioprotekcyjne w przypadku niedokrwienia mięśnia sercowego.

Jego działaniu nie towarzyszy podwyższone zapotrzebowanie na tlen mięśnia sercowego, niedokrwienie ani rozwój tolerancji. Z obserwacji klinicznych wynika, iż lewosimendan redukuje objawy ostrej niewydolności serca zarówno lewej, jak i prawej komory, obniża poziom neurohormonów (BNP), a jego długotrwałe 7–10-dniowe efekty działania, wynikają z działania samego leku jak i aktywnych metabolitów substancji czynnej (OR-1896

i OR-1855). Powyższe działania widoczne są również podczas równoległego stosowania leku beta-adrenolitycznego. Lewosimendan poprawia parametry hemodynamiczne, tj. powoduje wzrost pojemności minutowej serca, objętości wyrzutowej, obniża ciśnienie zaklinowania w tętnicy płucnej, średnie ciśnienie krwi, średnie ciśnienie w prawym przedsionku oraz całkowity opór obwodowy. Dodatkowo, nie obserwowano wzrostu tolerancji, nawet w przypadku stosowania infuzji 2-dniowej. Zapobiega niedokrwieniu mięśnia sercowego, nie wpływa na funkcję rozkurczową mięśnia sercowego [7].

Pozytywne działanie leku występuje nie tylko w zakresie układu krążenia, ale także w zakresie innych narządów, co pozwala na stwierdzenie, iż lewosimendan posiada działanie plejotropowe. Bezcenny jest wpływ na nerki i wątrobę. Przedstawiony przypadek potwierdza skuteczność lewosimendanu w zespole sercowo-nerkowym. U chorych z niewydolnością krążenia często dochodzi do pogorszenia funkcji nerek, co powoduje niekorzystne efekty leczenia niewydolności krążenia. Mimo stosowania standardowej terapii, w tym terapii dożylnej lekami moczopędnymi w dużych dawkach, często nie obserwuje się efektywnej diurezy, spadku wagi ciała, ustąpienia duszności i wreszcie poprawy klinicznej. Obraz taki daje podstawę do rozpoznania oporności na moczopędną terapię dożylną. U pacjentów leczonych lewosimendanem odnotowano statystycznie znaczącą poprawę GFR i spadek kreatyniny. Mechanizmy odpowiadające za poprawę funkcji nerek pod wpływem lewosimendanu są złożone. Oprócz wpływu na układ krążenia i wymienione powyżej podstawowe parametry hemodynamiczne, lewosimendan wywiera bezpośredni wpływ na krążenia nerkowe. Poprzez bezpośrednie działanie na przedkłębuszkowe naczynia krwionośne, prowadzi do poprawy przepływu krwi przez nerki oraz poprawy filtracji. Przełamuje w ten sposób oporność nerek na standardowe leczenie moczopędne i poprawia diurezę [8–9].

Jak wspomniano, lewosimendan stosuje się zwykle w leczeniu ostrej niewydolności serca, w sytuacji gdy standardowa terapia jest nieskuteczna. Z praktycznego punktu widzenia, ważne jest, aby w takich sytuacjach lek podawać jak najszybciej, nie czekając na całkowitą anurię oraz hypotonię, która wymagać będzie stosowania dużych dawek presorów [10-12].

W ośrodku autorów lek stosuje się w postaci wlewu dożylnego, bez wstępnego bolusa, z prędkością przepływu 0,1 μg/kg/min. Najczęstsze działania niepożądane to hypotonia, tachykardia, zaburzenia rytmu serca (migotanie przedsionków, dodatkowe skurcze komorowe, częstoskurcz komorowy), bóle i zawroty głowy, bezsenność, hypokaliemia, nudności, wymioty, zaparcia, biegunki. Zasadne jest, aby przed podaniem leku wyrównać zaburzenia elektrolitowe w celu uniknięcia złożonej arytmii komorowej. Nie obserwowano zjawiska tachyfilaksji [13].

Liczne badania kliniczne ostatnich lat dotyczące lewosimendanu, a zwłaszcza opublikowana w 2016 roku praca Polleselo i wsp.[14], w której przeanalizowano łącznie 58 publikacji, wykazały poprawę rokowania oraz redukcję hospitalizacji. Niektóre z analizowanych wykazały istotne ograniczenie śmiertelności w porównaniu z dobutaminą [14–15].

Aktualne wytyczne Europejskiego Towarzystwa Kardiologicznego, dotyczące niewydolności krążenia z 2016 roku plasują lewosimendan łącznie z dopaminą, dobutaminą i inhibitorem fosfodiesterazy na jednym poziomie w klasie IIB, poziom wiarygodności danych C. Przedstawiony przypadek kliniczny wskazuje na istotną rolę lewosimendanu w leczeniu opornej na farmakoterapię ostrej niewydolności prawokomorowej. Odpowiednio wczesne jego zastosowanie pozwoliło na kompensację układu krążenia przed wystąpieniem objawów niewydolności wielonarządowej oraz przełamanie oporności na stosowane diuretyki. Coraz liczniejsze dane wskazują na to, iż lek ten winien być stosowany dużo wcześniej, zanim układ krążenia stanie na krawędzi nieodwracalnego uszkodzenia. Pozostaje mieć nadzieję, że nowe standardy leczenia ostrej niewydolności serca uwzględnią dobrą praktykę kliniczną i plejotropowe działanie leku, umieszczając go na lepszej pozycji.

PIŚMIENNICTWO

1. Feusette P, Gierlotka M, Tukiendorf A et al. Niewydolność serca w wojewo'dztwie opolskim – epidemiologia i perspektywy na przyszłość'. Heart failure in Opole voivodeship – epidemiology and future perspectives. Wiad Lek 2019;72(1): 112-119.
2. Cowie MR, Lopatin YM et al. The Optimize Heart Failure Care Program: Initial lesson from global implementation. Int J Cardiol. 2017;236:340-344
3. Ponikowski P, Voors AA, Anker SD et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute a d chronic heart failure of the European Society of Cardiology (ESC). Eur Heart J. 2016;37(27): 2129–2200.
4. Gheorghiade M, Pang PS. Acute heart failure syndr omes. J Am Coll Cardiol. 2009;53(7):557-573. doi: 10.1016/j.jacc.2008.10.041.
5. Nessler J, Kozierkiewicz A, Gackowski A et al. Kompleksowa opieka nad chorymi z niewydolnos'cią serca w Polsce: propozycje rozwiązań' organizacyjnych. Kardiol Pol. 2018;76(2):479-487.
6. Janikowski K, Lelonek M. Skuteczna terapia lewosimendanem u chorego z zaostrzeniem przewlekłej niewydolności serca z obniżoną frakcją wyrzutowa lewej komory. Folia Cardiolog 2017;12(4,):378-385.
7. SIMDAX® monografia produktu. Orion Pharma 2015.
8. Rozentryt P. Zastój narządowy w niewydolności serca. Terapia 2016;9(2).
9. Fedel F, Karson K, Matskeplishvili S. Pharmacological approaches to cardio-renal syndrome:a role for the inodilator levosimendan. Eur Heart J Suppl.2017;19 (Suppl C): C22-C28.
10. Harjola VP, Giannakoulas G, von Lewinski D et al. Use of levosimendan in acute heart failure. European Heart Journal Supplements 2018;20(Suppl I):12-I10.
11. Bouchez.S, Fedele F. Giannakoulas G. Levosimendan in Acute and Advanced Heart Failure: an Expert Perspective on Posology and Therapeutic Application. Cardiovasc Drugs Ther 2018;32:617-624.
12. Polz G. Levosimendan in acute and advanced heart failure: still some chapter to be written. Eur Heart J Suppl. 2018;20(Suppl I)
13. Kivikko M, Lehtonen L. Colucci WS. Sustained hemodynamic effects of intravenous levosimendan. Circulation. 2003;107(1):81-86.
14. Pollesello P, Parissis J, Kivikko M et al. Levosimendan meta- analyses: is there a pattern in the effect on mortality: Int J Cardiol. 2016;209:77-83.

15. Ribeiro RA, Rohde LE, Polanczyk CA. Levosimendan in acute decompensated heart failure: systematic review and meta- analysis. Arq Bras Cardiol. 2010;95(2):230-237.

Konflikt interesów:
Joanna Płonka – wykłady dla firmy Orion i Novartis
Jarosław Bugajski, Marek Gierlotka – wykłady dla firmy Orion
Agata Duszańska, Joanna Płonka, Jarosław Bugajski – grant wyjazdowy ESC.

AUTOR KORESPONDUJĄCY
Joanna Płonka
Klinika i Oddział Kardiologii
Uniwersytecki Szpital Kliniczny w Opolu
al. W. Witosa 26, 45-401 Opole, Polska
Tel.: +48 77 45 20 660
e-mail: jbplonka@wcm.opole.pl

Nadesłano: 12.05.2019
Zaakceptowano: 01.07.2019

 © Wydawnictwo Aluna

LETTER TO THE EDITOR OF "WIADOMOSCI LEKARSKIE"
LIST DO REDAKTORA "WIADOMOŚCI LEKARSKICH"

Szanowna Redakcjo,

z zainteresowaniem zapoznałem się z *Listem do Redaktora*, zamieszczonym w czerwcowym numerze „Wiadomości Lekarskich", współautorstwa Pana Profesora Zbigniewa K. Wszołka „Programy edukacyjne dla Polaków chcących studiować/podnosić kwalifikacje za granicą". W imieniu zespołu NAWA dziękuję za słowa uznania pod adresem nowej, funkcjonującej od zaledwie 20 miesięcy Narodowej Agencji Wymiany Akademickiej (NAWA). Zapewniam, że jesteśmy jako zespół świadomi pokładanych w naszej pracy nadziei na wzmocnienie umiędzynarodowienia polskiego szkolnictwa wyższego i nauki, a także współpracy akademickiej pomiędzy polskimi i zagranicznymi naukowcami.

Cieszą nas pozytywne komentarze dotyczące programów stypendialnych dla doktorantów, pozwalających im na – z jednej strony – krótkie wizyty w zagranicznych instytucjach (wspominany w *Liście* program międzynarodowej wymiany stypendialnej doktorantów i kadry akademickiej – PROM), jak i na odbycie dłuższych, nawet dwunastomiesięcznych staży w ramach Programu im. Wilhelminy Iwanowskiej. Co więcej, bardzo cieszą pozytywne opinie o naszych stypendystach, ich kompetencjach i zaangażowaniu w prace badawcze. Wspierani przez szereg ekspertów, dokładamy starań, by nasi stypendyści prezentowali wysoką jakość i mogli w zagranicznych jednostkach stanowić wizytówkę nowoczesnej polskiej nauki.

Aby czytelnicy „Wiadomości Lekarskich" mieli szerszy ogląd możliwości, jakie stwarza NAWA, pozwolę sobie przybliżyć naszą ofertę, która pozwala nie tylko na wyjazdy zagraniczne do najlepszych ośrodków badawczych zlokalizowanych na całym świecie naukowcom na różnym etapie kariery, ale także daje możliwość szerokiej współpracy instytucjonalnej pomiędzy polskimi a zagranicznymi jednostkami naukowymi. Taką możliwość daje program Akademickie Partnerstwa Międzynarodowe ukierunkowany na współpracę instytucjonalną w obszarze badań naukowych, działalności wdrożeniowej lub kształcenia czy program Katamaran, finansujący tworzenie i realizację wysokiej jakości wspólnych studiów II stopnia prowadzących do uzyskania wspólnego lub podwójnego dyplomu.

Na szczególną uwagę środowiska medycznego zasługuje Program im. Prof. Franciszka Walczaka, nazywanego w środowisku ojcem chrzestnym polskiej elektrofizjologii. Program ten NAWA realizuje wspólnie z Ministerstwem Zdrowia. W jego ramach doktoranci i naukowcy z obszaru kardiologii, onkologii, alergologii lub chorób zakaźnych mogą otrzymać stypendia na trwający od trzech do sześciu miesięcy wyjazd do najlepszych ośrodków medycznych zlokalizowanych na terenie Stanów Zjednoczonych Ameryki. Natomiast reprezentantów innych specjalności w obszarze nauk medycznych bądź wszystkich zainteresowanych wyjazdem do dobrego ośrodka w innym niż USA kraju, NAWA zaprasza do Programu im. Mieczysława Bekkera, który otwarty jest na wszystkie dziedziny nauki, a także na wszystkie państwa i oferuje wyjazdy trwające nawet do 24 miesięcy.

Pragnę podkreślić, że Agencja oferuje także stypendia na przyjazdy naukowców z zagranicy do polskich jednostek naukowych. Korzystając z tego, że Polska dysponuje znakomitymi laboratoriami, szczególnie w zakresie *life sciences*, a w wielu jednostkach językiem codziennej pracy naukowej jest język angielski, chcemy zaprosić do naszego kraju obiecujących naukowców z całego świata, aby wzmacniać potencjał polskich jednostek i rozwijać współpracę międzynarodową oraz naukę, która przecież nie zna podziałów na granice państw. I tak na przykład w Programie im. Stanisława Ulama przyznajemy stypendia pozwalające zrealizować w Polsce staż podoktorski. Wkrótce na krótsze wymiany zaprosimy do nas także doktorantów. Liczymy, że dla naukowych środowisk polonijnych będzie to dobra okazja, by skierować do Polski swoich doktorantów czy młodych naukowców.

Nie mogę też nie wspomnieć o sztandarowym programie NAWA, jakim są Polskie Powroty. Program skierowany jest do naukowców polskiego pochodzenia, którzy przez ostatnie lata rozwijali kariery poza granicami Polski. Agencja stwarza im szansę powrotu do kraju i kontynuowania kariery naukowej na bardzo dobrych warunkach finansowych. Finansuje także koszty ich przeprowadzki i utworzenia w Polsce grupy badawczej. To duża szansa nie tylko dla naukowców myślących o powrocie do kraju, ale przede wszystkim dla polskich jednostek, które mogą zaprosić do swoich zespołów wybitnych naukowców polskiego pochodzenia i skorzystać z ich wiedzy i nieocenionych doświadczeń.

W pierwszych kilkunastu miesiącach naszego funkcjonowania NAWA ogłosiła 19 nowych programów adresowanych do studentów (w tym z USA), doktorantów, naukowców i instytucji nauki, a także dla osób zainteresowanych językiem i kulturą polską (organizujemy kursy letnie, mamy 85 lektorów języka polskiego na całym świecie). Trudno tę działalność streścić w krótkiej wypowiedzi prasowej, dlatego zapraszamy zainteresowanych do kontaktu z nami i odwiedzania strony Agencji (https://nawa.gov.pl/).

Chciałbym się też odnieść do podniesionej przez Pana Profesora Wszołka i wspólautorów w ich *Liście* idei mentoringu. NAWA jest młodą, rozwijającą się instytucją. W chwili obecnej dopiero nasi nieliczni stypendyści zakończyli realizację swoich stażów bądź projektów. Wielu rozpocznie wyjazdy w najbliższych tygodniach czy miesiącach. Z myślą o naszych alumnach oraz przyszłych stypendystach planujemy stworzyć platformę współpracy oraz sieć kontaktów do instytucji

czy też indywidualnych, doświadczonych naukowców, którzy będą zainteresowani przyjmowaniem stypendystów programów NAWA. Mamy nadzieję, że szczególnie naukowcy, którzy powracają z pobytów zagranicznych, a także ci, którzy przyjeżdżają do Polski w ramach programów NAWA, będą ambasadorami umiędzynarodowiania nauki w polskich jednostkach naukowych i potrzebnych do tego zmian.

Sądzę też, że naturalnym środowiskiem do budowania relacji mentoringowych mogą być tworzone w polskich uczelniach szkoły doktorskie. Mają one na celu podwyższenie jakości kształcenia na studiach doktoranckich, którą rozumieć należy także w kontekście umiędzynarodowiania. Jesteśmy otwarci na współpracę z ośrodkami polonijnymi, a także zagranicznymi uczelniami, które prowadzą studia polonistyczne. Bardzo cenimy realizowaną już współpracę z Fundacją Kościuszkowską; cieszymy się także z nawiązanych kontaktów z Federacją Polonijną Organizacji Medycznych. Zachęcamy wszystkich zainteresowanych do dołączenia do grona ekspertów NAWA. Dzięki nim możemy w pełni postawić na jakość i finansować najlepsze w danej dziedzinie wnioski.

Działając aktywnie w zarysowanych obszarach, mamy nadzieję na zbudowanie sieci kontaktów z naukowcami i instytucjami naukowymi, która z czasem może przekształcić się w bazę mentorów.

Z nadzieją na dalszą owocną współpracę
Łukasz Wojdyga
Dyrektor Narodowej Agencji Wymiany Akademickiej

 © Wydawnictwo Aluna

RESPONSE TO THE LETTER TO THE EDITOR OF "WIADOMOSCI LEKARSKIE"
ODPOWIEDŹ NA LIST DO REDAKTORA "WIADOMOŚCI LEKARSKICH"

PROGRAMY EDUKACYJNE DLA POLAKÓW CHCĄCYCH STUDIOWAĆ/PODNOSIĆ KWALIFIKACJE ZA GRANICĄ

Unislawa Williams[1], Janusz Kasina[2], Zbigniew K. Wszolek[3]
[1]DEPARTMENT OF POLITICAL SCIENCE, SPELMAN COLLEGE, ATLANTA, GA, USA
[2]FEDERACJA POLONIJNYCH ORGANIZACJI MEDYCZNYCH, STOCKHOLM, SWEDEN
[3]DEPARTMENT OF NEUROLOGY, MAYO CLINIC FLORIDA, JACKSONVILLE, FL, USA

Szanowny Panie Redaktorze,

Dziękujemy bardzo za przekazanie nam listu Dyrektora Narodowej Agencji Wymiany Akademickiej (NAWA), Pana Łukasza Wojdygi odpowiadającego na nasz list do Redaktora Wiadomości Lekarskich opublikowany w numerze 6/2019 [1, 2].

Cieszymy się bardzo, iż NAWA, pomimo tak krótkiego funkcjonowania – zaledwie 20 miesięcy, była w stanie rozwinąć tak wiele programów naukowych dla polskich naukowców na ich różnych szczeblach zawodowej kariery, a także dla naukowców zagranicznych, w tym też dla polonijnych. Te programy w miarę ich dalszego rozwijania i dojrzewania z cała pewnością będą cieszyły się dużym zainteresowaniem i okażą się wielkim sukcesem.

Chcielibyśmy tylko krótko zwrócić uwagę na dwa aspekty wymiany naukowej, które dla organizacji takich jak NAWA, także i tych funkcjonujących w innych krajach, są podstawą ich sukcesów, a to: *networking* i *mentoring* (Ryc. 1).

Networking pełni istotną funkcję w nawiązaniu kontaktów naukowych pomiędzy naukowcami z różnych krajów świata. Internet w ogromnym stopniu przyczynił się do połączenia nie tylko indywidualnych naukowców z różnych krajów świata pracujących nad podobnymi zagadnieniami, ale także nad powiązaniem całych grup badawczych. W magazynach medycznych coraz częściej widzi się artykuły opisujące badania naukowe, w których nawet kilka konsorcjów badawczych jest wyszczególnianych w tytułach prac. Systemowe podejście do tego zagadnienia leży w najlepszym interesie NAWA.

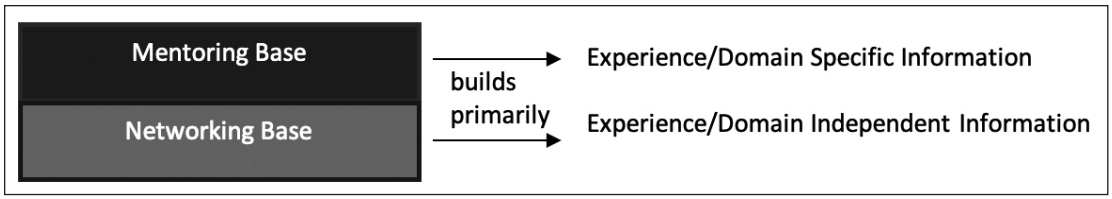
Mentoring jest w naszej opinii drugim ważnym zagadnieniem, o którym wspomnieliśmy w naszym Liście [1] i cieszymy się bardzo, iż Pan Wojdyga odniósł się do tego tematu tak pozytywnie. Bardzo nam było miło spotkać przedstawiciela NAWA, pana Pawła Kurzyńskiego, w czasie obrad X Kongresu Polonii Medycznej i III Światowego Zjazdu Lekarzy Polskich w czerwcu 2019, w Gdańsku. Pan Kurzyński spotkał się z licznymi polonijnymi naukowcami z wielu krajów świata, reprezentującymi nauki medyczne. Miał możliwość przedstawić NAWA, jej założenia i cele. Cieszy nas też bardzo, iż NAWA już nawiązała współpracę z Fundacją Kościuszkowską w USA i Federacją Polonijnych Organizacji Medycznych.

Pozostaje nam tylko życzyć NAWA wielu dalszych sukcesów i jeszcze raz podkreślić, iż jako Polacy mieszkający poza granicami kraju jesteśmy dumni z istnienia i działalności NAWA, działalności tak bardzo potrzebnej i ważnej.

PIŚMIENNICTWO

1. Williams U, Kasina J, Wszolek ZK. Programy edukacyjne dla Polaków chcących studiować i podnosić kwalifikacje za granicą. Wiad Lek 2019;72:1163-1164.
2. Wojdyga L. Odpowiedź na list otwarty. Wiad Lek 2019;72: 1592-1593.

CORRESPONDING AUTHOR
Zbigniew K. Wszolek, M.D.
Department of Neurology
Mayo Clinic Florida
4500 San Pablo Road
Jacksonville, FL 32224, USA
Tel: 904-953-7229
fax: 904-953-0757
e-mail: Wszolek.zbigniew@mayo.edu



Ryc. 1. Budowanie bazy wiedzy poprzez tworzenie sieci *mentoring* i *networking*. Baza danych mentorów pozwoli NAWA na wybór recenzentów złożonych projektów, monitorowanie jakości ich pracy, a petentom ubiegającym się o finansowanie z NAWA ułatwi potencjalny wybór mentora. Poprzez *networking* NAWA może mieć możliwość komunikowania się z dużą grupa naukowców z wielu dziedzin i z wielu krajów świata szybko i efektywnie w celu np. informowania o programach NAWA i *vice versa* naukowcy mogą informować NAWA o potrzebach, trendach naukowych i innych. *Mentoring* i *Networking* wymagają stworzenia odpowiednich platform elektronicznych, aby mogły działać skutecznie i sprawnie.