ORIGINAL ARTICLE

FUNCTIONING OF NO-CYCLE IN THE ORAL FLUID IN CHILDREN OF PRIMARY SCHOOL AGE WITH TYPE 1 DIABETES MELLITUS IN THE TREATMENT OF CHRONIC CATARRHAL GINGIVITIS

DOI: 10.36740/WLek202203116

Olga V. Sheshukova, Iryna O. Kuz, Vitalii O. Kostenko, Oleg Ye. Akimov, Alina I. Maksymenko, Olena A. Pysarenko, Natalia A. Lyakhova
POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

The aim: To determine the activity of NO-synthase and arginase in oral fluid in children with type 1 diabetes mellitus and to evaluate the efficacy of the treatment scheme we elaborated in the treatment of chronic catarrhal gingivitis.

Materials and methods: 82 children were examined, they were divided into groups by presence of gingivitis and diabetes mellitus. NO-synthase (NOS) activity was determined in oral fluid by the difference in nitrite concentration before and after incubation. The arginase activity was determined in oral fluid by the difference in the concentration of L-ornithine before and after incubation.

Results: Use our treatment scheme in children with chronic catarrhal gingivitis and type 1 diabetes mellitus lead to a change in the polarization of oral macrophages towards the predominance of M2 polarization in 1 month. The polarization of macrophages changed to the predominance of M1 polarization activity in 1 year.

Conclusions: We have elaborated a scheme for the treatment of chronic catarrhal gingivitis in children with type 1 diabetes mellitus. It normalizes the polarization of oral macrophages caused by exposure to chronic catarrhal gingivitis as a local pathogenetic factor.

KEY WORDS: type 1 diabetes mellitus, macrophages, gingivitis, iNO-synthase, arginase

Wiad Lek. 2022;75(3):654-658

INTRODUCTION

Diabetes mellitus is one of the most common metabolic diseases. According to the International Diabetes Federation (IDF), the number of people with diabetes will exceed 500 million by 2030. Local changes in periodontal tissues in persons with diabetes mellitus are characterized by increased production of reactive oxygen species, and pro-inflammatory cytokines, which are based on the accumulation of glycation products and their active interaction with receptors [1, 2]. An increase in the level of pro-inflammatory cytokines leads to the development of oxidative stress and stimulation of further inflammation in periodontal tissues. One of the central roles in the development of inflammation is played by macrophages. They have either pro-inflammatory M1 or anti-inflammatory M2 phenotype. It depends on the microenvironment [3-5].

There is a possibility of transformation of the M1 phenotype into M2 by changing the spectrum of stimulating cytokines. One of the differences between M2 and M1 phenotypes is that they metabolize L-arginine differently. M2 macrophages transform L-arginine into L-ornithine and polyamines, while M1 macrophages produce nitric oxide and L-citrulline. It is proved that the determination of the expression of the inducible isoform of the nitric oxide synthase enzyme (iNOS) makes it possible to assess the polarization of macrophages towards "classically" activated M1 phenotype with the significant formation of nitric oxide and other proinflammatory cytokines and mediators. It is believed that the activity of iNOS characterizes the features of polarization and differentiation of macrophages into different types, namely, M1 or M2 [6-8].

A better understanding of the etiological factors and pathogenetic mechanisms of periodontal diseases in diabetes mellitus is one of medical and healthcare priorities [9-11], which will enable to elaborate more effective approaches for the prevention and treatment of oral diseases.

THE AIM

The aim of this study is to determine the activity of NO-synthase and arginase in oral fluid in children of primary school age with type 1 diabetes mellitus and to evaluate the efficacy of the treatment scheme we elaborated in the treatment of chronic catarrhal gingivitis.

MATERIALS AND METHODS

We examined 82 children (from six to twelve years old), including 56 children with type 1 diabetes mellitus and 26 children without somatic diseases.

According to our dental examination, all the patients were divided into the following subgroups:

Group 1 - 13 children with clinically healthy periodontium and no comorbidities, this group was a control one;

Group 2 - 13 children of children without comorbidities and with chronic catarrhal gingivitis (CCG);

Group 3 - 26 children with type 1 diabetes mellitus without signs of periodontitis;

Group 4 - 30 children with type 1 diabetes mellitus who had chronic catarrhal gingivitis.

We studied the PMA index (papillary–marginal–alveolar index) modified by Parma (1960). General NO-synthase (gNOS) activity was determined in oral fluid by the difference in nitrite concentration before and after incubation. Activities of constitutive (cNOS) and inducible (iNOS) isoforms of NOS were determined with the usage of selective iNOS inhibitor (Aminoguanidine hydrochloride) The arginase activity was determined in oral fluid by the difference in the concentration of L-ornithine before and after incubation in phosphate buffer solution containing L-arginine [12].

The findings obtained were statistically processed using Microsoft Office Excel 2016 software pack. The distribution was checked by the Shapiro-Wilk test. The arithmetic mean (M), the representativeness error of the mean (m), and the significance level of the differences in the mean values (p) were calculated. The t-test for paired samples was used to compare values. The difference was considered statistically significant at $p<0.05$.

We taught all 82 children how to perform oral hygiene routine properly. We recommended using soft toothbrushes, toothpaste, and mouth rinse with extracts of medicinal plants "BIOMED SENSITIVE" (STS Holding Group LTD, Bulgaria) for all the participants [13]. We treated chronic catarrhal gingivitis in children of group 2 following the protocols in the specialty "Pediatric Dentistry" approved by the Ministry of Health of Ukraine.

The management of the children with chronic catarrhal gingivitis and type 1 diabetes mellitus (group 4) combined therapeutic and hygienic measures. Following the examination and diagnosis confirmation, the children received professional oral hygiene and educated how to keep oral hygiene properly. The one-month course included our recommendation to use the toothpaste mentioned above and the mouth rinse with grape extract. The children were also prescribed to take the antioxidant "Askorutin" (LLC Agrofarm, Ukraine) 1 tablet twice a day for 1 month [14]. To normalize oral mocrobiota, we recommended the patients to rinse the mouth with an antiseptic solution "Sangiva" (JSC Pharmaceutical firm "Darnitsa", Ukraine) twice a day for 5 days and to take the probiotic "BioGaia Prodentis" (BioGaia AB, Sweden) 1 tablet once a day for 10 days. The evaluation of treatment results was carried out in immediate follow up period, a month since the completion of the full course of treatment for chronic catarrhal gingivitis, and in the remote follow-up period, in 3, 6, and 12 months.

The scientific materials meet the rules of humane treatment of patients in accordance with the requirements of the Tokyo Declaration of the World Medical Association, international recommendations of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, Laws of Ukraine, orders of the Ministry of Health of Ukraine.

RESULTS

Using the standard treatment complex in non-diabetic children with chronic catarrhal gingivitis leads to a decrease in iNOS activity and an increase in arginase activity in oral fluid one month after treatment, according to our results (Table I).

The concentration of nitrites does not change statistically significantly. It evidences an increase in reparative processes and a decrease in pro-inflammatory processes. It is also confirmed clinically, namely, the PMA index is 20.52% before treatment, and it decreases to 12.22% a month after treatment (Fig. 1).

The activity of iNOS and the concentration of nitrites in the oral fluid of the above-mentioned group of children increases compared to pre-treatment in 3 months after using the standard treatment complex. The activity of arginases does not change statistically significantly. The activity of iNOS and nitrite concentration increase, arginase activity decreases after 6 months. There is a change in the biochemical parameters of oral fluid in the group of non-diabetic children with chronic catarrhal gingivitis 1 year after the start of the standard treatment complex. Thus, the activity of iNOS decreases, and the activity of arginases increases compared to pre-treatment data, the concentration of nitrites stays unchanged. Thus, there is a decrease in the pro-inflammatory component and an increase in reparative function. These data coincide with the data of the clinical research, namely, the PMA index before treatment was 20.52%, and a year later it decreased to 13.35%. The use of the above-mentioned personal hygiene products (a soft toothbrush, toothpaste and rinse aid with grape extract) in children with healthy gums and type 1 diabetes leads to increased arginase activity and nitrite concentration in oral fluid in 1 month. It evidences the strengthening of reparation processes (Table II). The activity of iNOS is not statistically significant.

The iNOS activity and nitrite concentration increase, and arginase activity decreases compared to pre-treatment values in 3 months. The iNOS activity, arginase, and nitrite concentration increased in 6 months. The iNOS activity and nitrite levels in the oral fluid increased compared to pre-treatment data in 1 year after using the soft toothbrush, toothpaste, and rinse with grape extract, which was recommended by us. And the activity of arginases, respectively, did not change statistically significantly compared with pre-treatment activity.

We noted a statistically significant decrease in iNOS activity and a statistically significant increase in arginase activity and nitrite concentration in oral fluid in children with type 1 diabetes mellitus and chronic catarrhal gingivitis in 1 month after using of treatment complex elaborated by us. (Tab. III). This fact evidences an increase in reparative

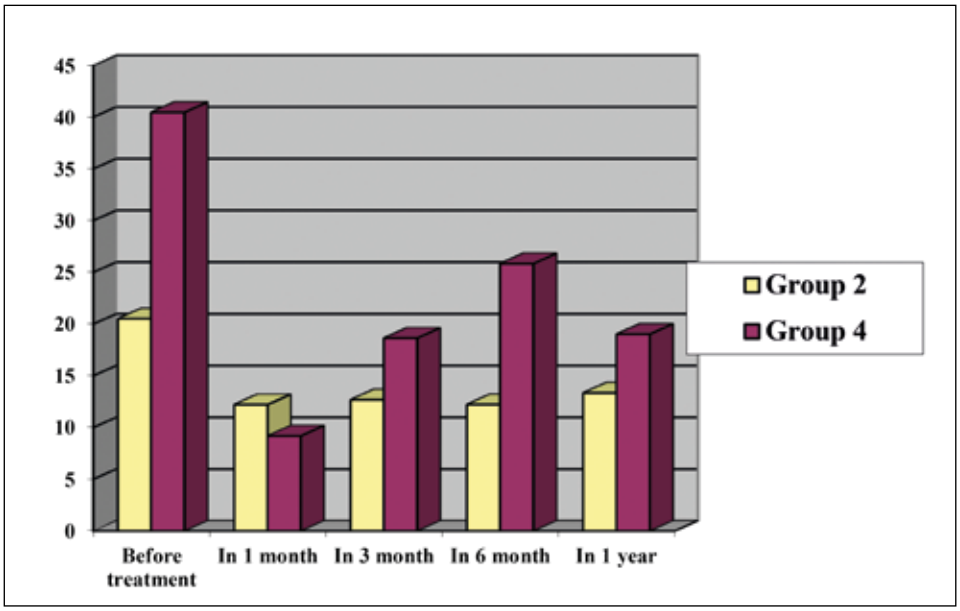

Fig. 1. The PMA index in Parma modification (1960) in non-diabetic children with chronic catarrhal gingivitis (group 2) and children with chronic catarrhal gingivitis and type 1 diabetes mellitus (group 4).

Table I. The influence of standard treatment complex on the biochemical parameters of oral fluid in non-diabetic children with chronic catarrhal gingivitis n = 13

Term of sampling of oral fluid	Biochemical parameters of the oral fluid				
	gNOS, μmol/min. per g of protein	cNOS, μmol/min. per g of protein	iNOS activity, μmol / min per g of protein	Nitrite concentration, nmol / l	Arginase activity, μmol / min per g of protein
Before treatment	0.55±0.02	0.09±0.001	0.46±0.02	2.50±0.20	0.67±0.02
After 1 month	0.34±0.01*	0.09±0.002	0.30±0.01*	2.01±0.10	0.92±0.02*
In 3 months	0.66±0.08	0.09±0.002	0.57±0.07	3.84±0.37*	0.57±0.05
In 6 months	1.43±0.13*	0.11±0.008*	1.22±0.13*	6.30±0.80*	0.34±0.04*
After 1 year	0.40±0.01*	0.09±0.002	0.29±0.01*	2.27±0.10	1.09±0.02*

Note: * - the difference is statistically significant when compared with the indicators before treatment ($p<0.05$).

Table II. The influence of preventive measures on the biochemical parameters of oral fluid in children with healthy gums and type 1 diabetes mellitus, (M ± m). n = 26

Term of sampling of oral fluid	Biochemical parameters of the oral fluid				
	gNOS, μmol/min. per g of protein	cNOS, μmol/min. per g of protein	iNOS activity, μmol / min per g of protein	Nitrite concentration, nmol / l	Arginase activity, μmol / min per g of protein
Before treatment	0.87±0.07	0.11±0.001	0.76±0.07	1.93±0.15	0.42±0.03
After 1 month	0.65±0.04*	0.09±0.001*	0.64±0.04	3.24±0.23*	0.78±0.04*
In 3 months	1.75±0.05*	0.17±0.002*	1.72±0.05*	10.98±0.22*	0.24±0.01*
In 6 months	1.18±0.08	0.14±0.006*	0.93±0.09	9.04±0.53*	0.63±0.04*
After 1 year	1.37±0.08*	0.13±0.005*	1.20±0.07*	8.15±0.57*	0.43±0.03

Note: * - the difference is statistically significant when compared with the indicators before treatment ($p<0.05$).

processes and reduction of pro-inflammatory processes. Clinical manifestations are consistent with laboratory data. The PMA index was 40.47% (moderate degree of inflammation) before treatment in this group of children, and it decreased more than 4 times to 9.2% (mild degree of inflammation) in 1 month (Fig. 1).

We noted the increase in the activity of iNOS and concentration of nitrites, and also decrease in activity of arginases in 3 months and 6 months after using the treatment complex developed by us in this group of children. The iNOS activity did not change statistically significantly, arginase activity and nitrite concentration increased statistically significantly compared to pre-treatment values in 1 year. We can conclude that the treatment complex elaborated by us, enhances the repair process (it is evidenced by the increase in arginase activity) even in 1 year. But the activity of iNOS and the concentration of nitrites stayed stable and did not change after a year. We believe that it is a consequence of the presence of type 1 diabetes mellitus in these children and, consequently, systemic inflammation in the body as a whole.

Table III. The influence of the treatment complex elaborated by us on the biochemical parameters of oral fluid in children with type 1 diabetes mellitus and chronic catarrhal gingivitis, (M ± m). n = 30n = 30

Term of sampling of oral fluid	Biochemical parameters of the oral fluid				
	gNOS, µmol/min. per g of protein	cNOS, µmol/min. per g of protein	iNOS activity, µmol / min per g of protein	Nitrite concentration, nmol / l	Arginase activity, µmol / min per g of protein
Before treatment	0.75±0.04	0.13±0.004	0.62±0.04	1.34±0.14	0.72±0.03
After 1 month	0.45±0.01*	0.09±0.002*	0.36±0.01*	4.80±0.24*	0.88±0.02*
In 3 months	1.78±0.02*	0.18±0.002*	1.62±0.02*	14.04±0.13*	0.21±0.01*
In 6 months	2.05±0.04*	0.13±0.005	1.92±0.05*	12.62±0.23*	0.22±0.01*
After 1 year	0.72±0.02*	0.13±0.004	0.61±0.02	7.97±0.41*	0.51±0.02*

Note: * - the difference is statistically significant when compared with the indicators before treatment ($p<0.05$).

DISCUSSION

The most indicative terms for the assessment of treatment schemes and prevention measures are 1 month and 1 year according to our research. A decrease in the iNOS activity and an increase in arginase activity and the absence of changes in nitrite concentrations in oral fluid indicates a predominance of anti-inflammatory polarization in non-diabetic children with chronic catarrhal gingivitis in 1 month and in 1 year after using the standard treatment scheme. This fact, together with the clinical condition of the gums, indicates sufficient effectiveness of the standard method of treatment of chronic catarrhal gingivitis.

The preventive complex in children with healthy gums and type 1 diabetes leads to an increase in arginase activity after 1 month, which indicates an increase in reparative processes. However, there are no changes in the activity of arginases, and the activity of iNOS even increases in 1 year. Thus, despite the absence of inflammation, proinflammatory polarization of oral macrophages predominates. Such changes may be associated with the change of the normal oral microbiota to the so-called "diabetic" [15]. It is characterized by the presence of specific pathogenic strains. The increase in nitrite concentration can be considered not as a damaging factor, but as an adaptive response aimed at suppressing bacterial pathogens [16]. It is evidenced by the intact periodontium.

Using our own treatment complex in children with chronic catarrhal gingivitis and type 1 diabetes mellitus leads to a change in the polarization of oral macrophages towards the predominance of M2 polarization in 1 month. It is evidenced by increased arginase activity and a statistically significant decrease in iNOS activity. The polarization of macrophages changes to the predominance of M1 polarization in 1 year. It is evidenced by the decrease in arginase activity, and no changes in the iNOS activity. Thus, taking into account the state of the periodontium in this group of children, we can conclude that changes in biochemical parameters of oral fluid in 1 month are associated with the elimination of chronic catarrhal gingivitis, and changes in biochemical parameters in 1 year are due to systemic pathogenetic factor, namely, type 1 diabetes mellitus. Increasing nitrite concentrations may also be an adaptive response of oral macrophages to diabetes-induced changes in the composition of the oral microflora [17, 18].

CONCLUSIONS

Our treatment complex of chronic catarrhal gingivitis in children with type 1 diabetes mellitus normalizes the polarization of oral macrophages caused by the influence of chronic catarrhal gingivitis as a local pathogenetic factor.

Standard treatment of chronic catarrhal gingivitis in non-diabetic children leads to the predominance of anti-inflammatory polarization of oral macrophages.

The complex of preventive measures for children with type 1 diabetes mellitus and healthy gums preserve the pro-inflammatory polarization of macrophages in the long term but prevent damage to periodontal tissues.

REFERENCES

1. Maksymenko A.I., Sheshukova O.V., Kuz I.O. et al. The level of interleukin–18 in the oral fluid in primary school children with chronic catarrhal gingivitis and type I diabetes mellitus. Wiadomości Lekarskie. 2021;74(6):1336-1341.
2. Kuz I.O., Akimov O.Ye., Kostenko V.O. et al. Functioning of NO-cycle in the saliva of children with type 1 diabetes mellitus. PEP. 2021;78(4):34-39.
3. Burg A.R., Tse H.M. Redox-sensitive innate immune pathways during macrophage activation in type 1 diabetes. Antioxid Redox Signal. 2018;29(14):1373-1398.
4. Liu P.S., Ho P.C. Determining macrophage polarization upon metabolic perturbation. Methods Mol Biol. 2019;1862:173-186. doi: 10.1007/978-1-4939-8769-6_13.
5. Orliaguet L., Dalmas E., Drareni K. et al. Mechanisms of macrophage polarization in insulin signaling and sensitivity. Front Endocrinol (Lausanne). 2020;11:62. doi: 10.3389/fendo.2020.00062.
6. Rendra E., Riabov V., Mossel D.M. et al. Reactive oxygen species (ROS) in macrophage activation and function in diabetes. Immunobiology. 2019;224(2):242-253. doi: 10.1016/j.imbio. 2018.11.010.
7. Thapa B., Lee K. Metabolic influence on macrophage polarization and pathogenesis. BMB Rep. 2019;52(6):360-372. doi: 10.5483/BMBRep.2019.52.6. 140.
8. Tugal D., Liao X., Jain M.K. Transcriptional control of macrophage polarization. Arterioscler Thromb Vasc Biol. 2013;33(6):1135-44. doi: 10.1161/ ATVBAHA.113.301453.
9. Lyakhova N.A., Kasinets S.S. The preexposure prophylaxis of stomatological diseases among the population of ukraine in the practice of the family doctor and the pediatrician. Wiadomosci Lwkarskie. 2017;70 (3): 470–473.
10. Lyakhova N.A. Analysis of risk factors of orthodontic pathology: literature review. Wiadomosci Lekarskie. 2018;.71 (5):1084–1088.

11. Sheshukova O.V., Trufanova V.P., Polishchuk T.V. et al. Monitoring of efficiency of dental caries management in children's temporary teeth throughout Poltava oblast. Wiad Lek. 2018;71(3): 761-767.
12. Yelins'ka A.M., Akimov O.Ye., Kostenko V.O. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. Ukr.Biochem.J. 2019; 91 (1): 80-85.
13. Frombaum M. Antioxidant effects of resveratrol and other stilbene derivatives on oxidative stress and NO bioavailability: Potential benefits to cardiovascular diseases. Biochimie. 2012;94(2):269-276.
14. Gegotek A., Jarocka-Karpowicz I., Skrzydlewska E. Cytoprotective efect of ascorbic acid and rutin against oxidative changes in the proteome of skin fibroblasts cultured in a three-dimensional system. Nutrients. 2020;12:1074. doi: 10.3390/nu12041074.
15. Lv W., Graves D.T., He L. et al. Depletion of the diabetic gut microbiota resistance enhances stem cells therapy in type 1 diabetes mellitus. Theranostics. 2020;10(14):6500-6516. doi: 10.7150/thno.44113.
16. Zhou L.N., Bi C.S., Gao L.N. et al. Macrophage polarization in human gingival tissue in response to periodontal disease. Oral Dis. 2019;25(1):265-273. doi: 10.1111/odi.12983.
17. Yang D., Wan Y. Molecular determinants for the polarization of macrophage and osteoclast. Semin Immunopathol. 2019;41(5):551-563. doi: 10.1007/s00281-019-00754-3.
18. Zhang C., Han X., Yang L. et al. Circular RNA circPPM1F modulates M1 macrophage activation and pancreatic islet inflammation in type 1 diabetes mellitus. Theranostics. 2020;10(24):10908-10924. doi: 10.7150/thno.48264.

This study is conducted as a part of the research project "Investigation of pathogenetic mechanisms of the most common childhood diseases, improvement of their diagnosis and treatment", the Department of Pediatrics № 2, Poltava State Medical University, registration number 0117U004683 (2017-2021) and a part of the research project "The development of methods for the treatment of inflammatory pathology of the maxillofacial area aimed at the polarization of macrophage subpopulations", the Research Institute of Genetic and Immune Fundamentals of Pathology and Pharmacogenetics, Poltava State Medical University, registration number 0117U005251 (2017-2021).

ORCID and contributionship:
Olga V. Sheshukova: 0000-0002-4739-4890 [A, F]
Iryna O. Kuz: 0000-0003-0287-4783 [B, D]
Vitalii O. Kostenko: 0000-0002-3965-1826 [A, F]
Oleg Ye. Akimov: 0000-0002-4958-3695 [B, C]
Alina I. Maksymenko: 0000-0002-9791-0873 [A]
Olena A. Pysarenko: 0000-0001-8376-7124 [E]
Natalia A. Lyakhova: 0000-0003-0503-9935 [C]

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

CORRESPONDING AUTHOR
Iryna O. Kuz
Poltava State Medical University
23 Shevchenko st., 36000 Poltava, Ukraine,
tel: +380684616494
e-mail: kuz.irina.irinovna@gmail.com

Received: 14.06.2021
Accepted: 10.02.2022

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article