ORIGINAL ARTICLE
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THE USE OF PHOTODYNAMIC THERAPY IN THE TREATMENT OF DENTAL CARIES IN CHILDREN OF CONTAMINATED AREAS OF THE ECOSYSTEM OF THE UPPER TYSA REGION

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ABSTRACT

The aim: improving the effectiveness of treatment of dental caries in children using the method of photodynamic therapy.

Materials and methods: The treatment of permanent tooth decay was performed in 35 children of the Upper Tysa region aged 12-15 years. Using the polymerase chain reaction method, the clinical efficacy of photodynamic therapy in the treatment of dentin caries was determined.

Results: During the study, by PCR analysis, dentin caries most commonly revealed genetic markers of DNA of the five most virulent anaerobic bacteria: Prevotella intermedia, Fusobacterium spp., Enterococcus Faecalis, Veilonella spp., Candida albicans. After treatment of the carious cavity by the method of photodynamic therapy with exposure of 30 s – fusobacterium spp. were not detected, the detection rate of prevotella intermedia decreased 3-fold, enterococcus faecalis 3.5-fold, veilonella spp.- 5-fold, and candida albicans- 8-fold; after treatment with carious cavities with 60 s exposure – anaerobic microorganisms were not detected in the investigated samples. The effect of photodynamic therapy with laser exposure of 60 seconds on caries-causing streptococci resulted in their total death, and with exposure of 30 seconds – the frequency of isolated strains decreased several folds.

Conclusions: The use of photodynamic therapy in the treatment of dentine caries is a highly effective and pathogenetically sound method of treatment that provides a significant reduction in the optional and obligate types of cariesogenic microorganisms.

KEY WORDS: laser FotoSan 630, PDT (photodynamic therapy), PCR (polymerase chain reaction), caries

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INTRODUCTION

The relevance, prevalence, effectiveness and prevention of caries and its complications continue to be at the forefront of dental health issues in Eastern Europe, including Ukraine. According to WHO, dental caries in most countries of the world range from 80% to 98% and progress to 100% in low-living countries [1]. Analysis of the structure of carious lesions in children of 15-18 years living in the combined negative effects of factors of natural-technological genesis (Upper Potysia region), according to the ICDAS II system, shows that in children of low biogeochemical zone the caries prevalence is lower, unlike children and the mountain zone where carious cavities on the proximal surfaces are much more prevalent, and the tendency to increase the number of carious cavities is even more pronounced [2]. Despite some advances in the treatment of dental caries, the search for new treatments and treatments remains relevant. Recently, work has emerged on the feasibility of incorporating photodynamic therapy (PDT) as a new strategic direction for the stage of secondary prevention of caries. The use of PDT can be attributed not only to its effect on the vast majority of pathogenic bacteria in the microbial landscape of carious cavities, but also to the activation of microcirculation in the pulp as a result of regulatory action on the microvasculature by laser radiation [3]. The essence of the photodynamic reaction is the formation of free radicals or short-lived forms of singlet (active) oxygen. In terms of photodynamic reactions, it should be noted that singlet oxygen and free radicals are short-lived forms and are inactivated for one millionths of a second, decaying into their original components and thus not posing a risk to other cells [3]. The most important feature of the interaction of biological tissues with molecules of photosensitizing substance is the selective consumption of it only "harmful" to the body cells, which allows to maintain normally functioning and destroy atypical, which is an obstacle to the activity of the whole organism. The type of such "harmful" cells is determined by the hyperactivity of their metabolic activity, which exceeds the normative level of cellular functioning [3, 4]. The level of dental health in children is closely linked to an increase in the relative weight of the risk factors for the formation and progression of diseases of hard and soft tissues of the oral cavity, which is reflected in the structure of the main dental diseases[1].

Table I. The distribution of patients into groups according to the method of antiseptic treatment of carious cavity.

Groups	Method of antiseptic treatment of carious cavity	Number of patients	Number of teeth
№1	Photodynamic therapy: treatment with photosensitizer followed by laser irradiation for 30 s	12	15
№2	Photodynamic therapy: treatment with photosensitizer followed by laser irradiation for 60 s	12	17
№3 (control group)	Traditional treatment: washing with 0.12% solution of chlorhexidine	11	14
Total		35	46

Transcarpathia refers to the climate-geographic zone with a low level of fluoride and iodine in the environment, and as shown by epidemiological surveys «very high» according to WHO criteria, the level of intensity of major dental diseases associated with a deficit in the daily intake of iodine and fluorine [2]. In the domestic and foreign literature there are a sufficient number of publications confirming the clinical effectiveness of the use of photodynamic therapy in dentistry in diseases of periodontal tissues, endodontic treatment, as well as obtaining solutions for irrigation of the oral cavity [5,6,7,8,9]. In recent years, at the same time photoactivating disinfection has been used in dentistry in the treatment of caries and its complications, in periodontics, implantology, in pathologies of the mucous membrane, in maxillofacial surgery [10]. Publications on the use of photodynamic therapy in the treatment of dental caries are isolated [11], some of them are not scientifically substantiated. In this regard, conducting a study to study the effectiveness of the use of photodynamic therapy in this pathology is relevant today.

THE AIM

The purpose was to improve the clinical efficacy of standard dental caries treatment protocols in children of the Upper Tysa region by using photodynamic therapy.

MATERIALS AND METHODS

The clinical study was conducted on the basis of the dental department of the Central District Hospital in Berehovo. There were 35 patients aged 12 to 15 years under clinical observation who were treated for 46 permanent bite teeth; with dentine caries, under the control and written consent of the parents. When making a diagnosis of caries used the conventional classification International Statistical Classification of Diseases and Related Health Problems. The study is based on the main provisions of the GCP ICH and the Helsinki Declaration on Biomedical Research, the Council of Europe Convention on Human Rights and Biomedicine (2007) and the recommendations of the Committee on Bioethics at the Presidium of the National Academy of Sciences of Ukraine (2002). Violations of moral and ethical standards were not found during the study. Tested laboratories are certified. Depending on the method of antiseptic treatment of caries, all patients included in the study were divided into 3 groups (table I). In groups №1 and №2, tooth treatment was performed using photodynamic therapy, in group №3 (control) – traditional antiseptic treatment of carious cavity with 0.12% solution of chlorhexidine. The algorithm of photodynamic therapy was as follows. After the examination and diagnosis, the patient was anesthetized and dissected carious cavity with a turbine tip with the maximum observance of all rules of aseptic and antiseptic. The machining of the hard tissues of the tooth was carried out with cooling due to the constant supply of air and water, in the form of an aerosol, into the treated cavity. After completion of mechanical treatment and isolation of the tooth from saliva in patients of groups №1 and №2 carried out disinfection of the carious cavity by the method of photodynamic therapy. The walls and bottom of the prepared cavity were covered with a photosensitizer FotoSan Agent (toluidine blue) with a uniform layer up to 1 mm, which remained for 30 seconds. Then, irradiation was performed using the FotoSan 630 laser apparatus (CMS Dental, Denmark) in continuous mode, the exposure time was in the group №1 – 30 s, in the group №2 – 60 s. In group No. 3 (control), after the carious cavity preparation, traditional antiseptic treatment with 0.12% chlorhexidine solution was carried out. Further treatment of the teeth in patients in all groups was performed according to the standard technique using adhesive technique and permanent restoration. The sampling of the material was performed by scraping dentin from the walls of the carious cavity with a sterile dental excavator №2. The re-taking of the material was carried out in groups №1 and №2 after photodynamic therapy, after treatment of the cavity with a solution of chlorhexidine. Thus, two groups of dentine sawdust samples taken before and after antiseptic caries treatment were obtained. The obtained dentine sawdust samples were placed in an Eppendorf-type tube containing 500 μl of saline solution, stirred and sent to the AstraDia Microbiology Laboratory. A molecular genetic method for the study of anaerobic microflora – PCR diagnostics in Real time stomatoflora (DK 021: 2015: 33696500-0 UA) was used to detect bacterial DNA marker fragments in the material. The results of laboratory and clinical studies were processed by the methods of variational statistics with determination of the average value, its errors, the Student's t test for multiple comparisons, using Excel (MS Office 2010, Microsoft, USA) and STATISTICA 6.0 (StatSoft, USA). Differences of indicators at significance level $p < 0.05$ were considered statistically significant.

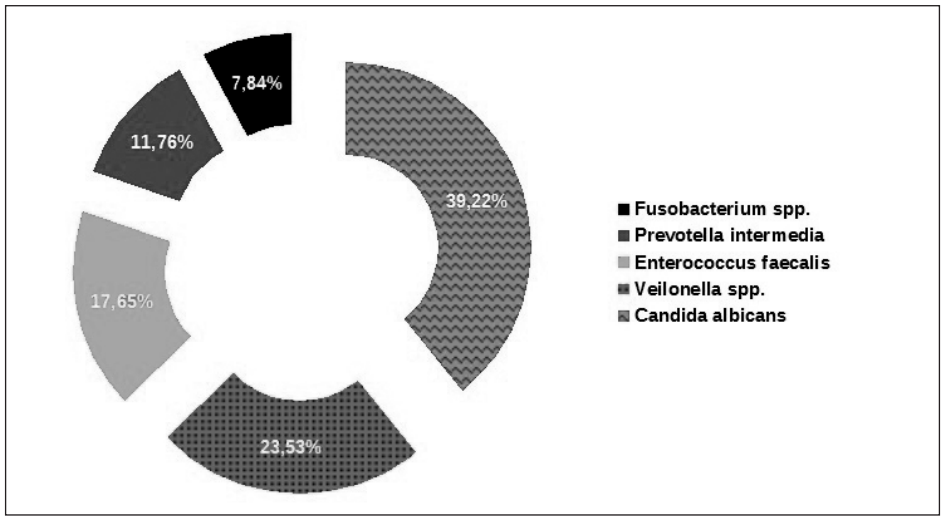

Fig. 1. Comparative PCR detection rate of virulent anaerobic bacteria in dentine caries (n=51)

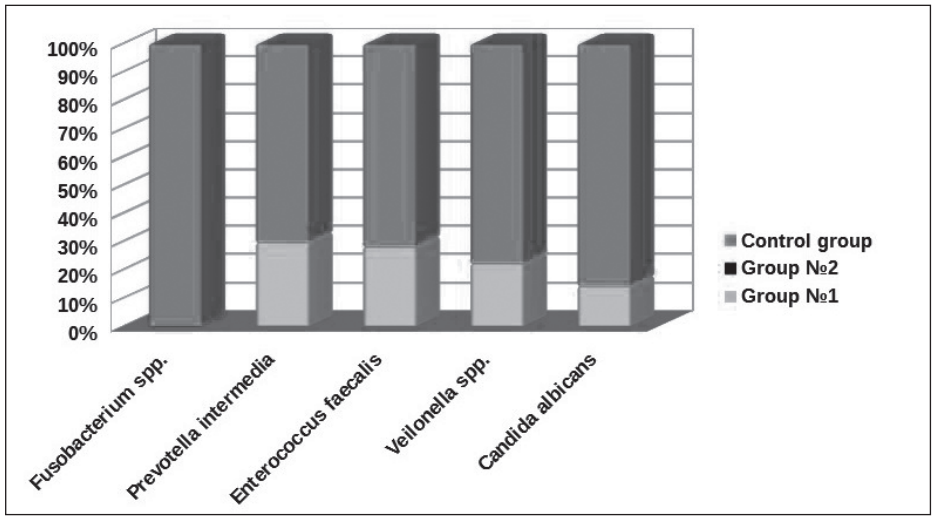

Fig. 2. Comparative frequency of PCR detection of virulent anaerobic bacteria in dental caries after photodynamic therapy.

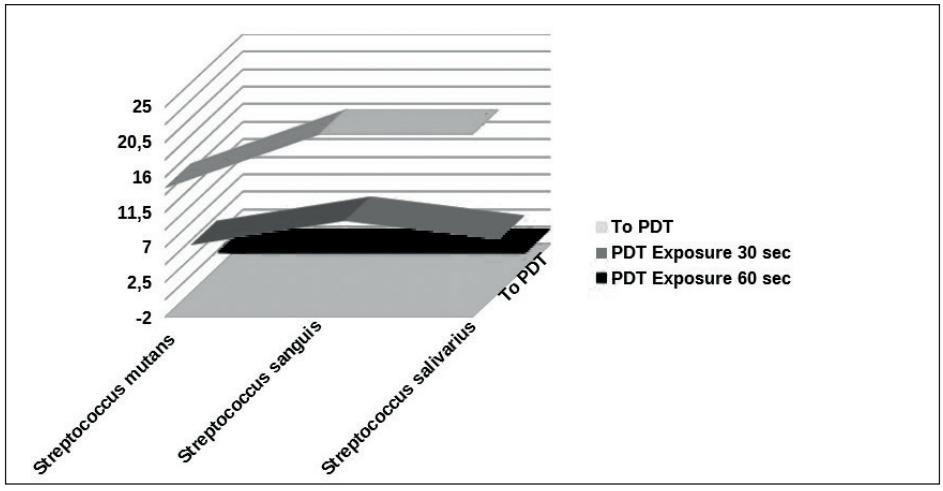

Fig. 3. Frequency of cariesogenic streptococci secretion after photodynamic therapy with exposures of 30 and 60 seconds.

RESULTS

Based on the analysis of the polymerase chain reaction of the microflora associated with the development of dental caries, it was found that pigment-forming DNA was detected in 27 (77.1%) patients. In this case, 7 (21.5%) patients had two types, and 20 (55.6%) had one type of microorganisms. Only 8 (22.9%) patients had no DNA sample of the microorganisms. Such a difference in the average quantitative indicator of the contamination of microorganisms with carious cavity, in our opinion, is explained by the different clinical course of caries. In the course of PCR analysis with dentine caries, genetic markers of the five types of the most virulent anaerobic bacteria were most commonly detected: Prevotella intermedia in 4 (7.84%) cases, Fusobacterium

Table II. Frequency of discharge of carious streptococci in dentine caries

Kind of microorganisms	Selection frequency strains	The titer of the selected strains
Streptococcus mutans	14,64±0,08(p<0,04)	10^5
Streptococcus sanguis	21,53±0,06(p<0,04)	10^6-10^7
Streptococcus salivarius	21,53±0,06(p<0,04)	10^6-10^7

Table III. Frequency of cariesogenic streptococci excretion in dentine caries after administration of 0.12% solution of chlorhexedine.

Kind of microorganisms	Selection frequency strains	The titer of the selected strains
Streptococcus mutans	9,08±0,06(p<0,05)	10^5
Streptococcus sanguis	13,42±0,09(p<0,05)	10^5
Streptococcus salivarius	11,56±0,04(p<0,05)	10^5

spp.-in 6 (11.76%), Enterococcus Faecalis- in 9 (17.65%), Veilonella spp.-in 12 (23.53%), Candida albicans-in 20 (39.22%) cases (Fig. 1). In a clinical study in group №1 after caries treatment with photodynamic therapy with 30 s exposure – Fusobacterium spp. were not detected, the percentage of detection of Prevotella intermedia was 3.92%, Enterococcus faecalis – 5.04%, Veilonella spp.-4.71%, and Candida albicans- 4.9%. In group №2, where caries were treated by photodynamic therapy with 60 s exposure – anaerobic microorganisms were not detected in the studied samples. In group №3 (control), after treatment of the carious cavity with a solution of chlorhexidine, the detection rate of bacteria Prevotella intermedia decreased, compared with the original level, by 9.34%; Fusobacterium spp – 6.31%; Enterococcus faecalis – 12.9%; Veilonella spp. -on 16.62%; Candida albicans at 29.76%. (Fig. 2).

After contaminating carious cavities with bacteria and incubation, teeth were divided into a control group and a test group. Half of the teeth did not under go any intervention and served as the control, where as in the test group the teeth received a solutionof 0.0125 % toluidine blue for 5 min followed by irradiationusing a 50-mW diode laser (Ga–Al–As) at a wave length of 660 nm. Bacterial samples were taken before and after irradiation. The number of colony-forming units was counted and it was concluded that PDT was effective in E.faecalis contamnated carious cavities. These indicators are significantly inferior to those of groups №1 and №2. Thus, after the treatment of carious cavity by photodynamic therapy, the frequency of detection of pathogenic anaerobes was significantly lower than after traditional treatment with a solution of chlorhexidine. The results of the study strongly demonstrate that the technique of photoactivating disinfection successfully destroys anaerobic bacteria with the right combination of the photosensitizer FotoSan Agent (toluidine blue) and an adequate dose of the energy of the red diode laser FotoSan 630 (CMS Dental, Denmark). To determine the antibacterial efficacy of photodynamic therapy for cariesogenic streptococci, we conducted a study of seeding frequency and quantitative ratio of streptococci to caries. In a background study, it was found that Streptococcus sanguis, Streptococcus salivarius were isolated from all carious cavities (100%), and Streptococcus mutans in 68% of cases (Table II).

The effect of photodynamic therapy with laser exposure of 60 seconds on caries-causing streptococci resulted in their total death, and with exposure of 30 seconds – the frequency of isolated strains of Streptococcus mutans decreased from 14,64±0,08 x 10^5(p<0,04) to 4,18 ±0,06 x 10^3(p<0,04), Streptococcus sanguis — from 21,53±0,06(p<0,04) x 10^6-10^7 to 7,28±0,09(p<0,04) x 10^3-10^4, and Streptococcus salivarius — from 21,53±0,06(p<0,04) x 10^6-10^7 to 4,84±0,07(p<0,05) x 10^3(Fig.3).

In the control group, where a 0.12% solution of chlorhexedine was used as an antiseptic for the treatment of carious cavity, there was also a decrease in the frequency of discharge and the number of streptococci, but less pronounced than in patients groups 1 and 2 (Table III).

The results of microbiological studies indicate that the use of photodynamic therapy in the treatment of patients with various forms of caries is a highly effective and pathogenetically sound treatment that provides a significant reduction of optional and obligate types of cariesogenic microorganisms. Based on the study, it can be considered that photodynamic therapy has a more effective effect on infected hard tissues of the tooth, which contributes to a more complete elimination of microorganisms, improve the tight fit of the filling material and prevent the development of secondary caries. Given that the origin and development of caries infectious factor is crucial, the relevance of the study of aspects of photodynamic therapy in this application, in our opinion, is not in doubt.

DISCUSSION

Fonseca et al. [12] have investigated the effects of antimicrobial photodynamic therapy on cariesogenic pathogens by evaluating the decrease in numbers of Enterococcus faecalis colonies in the carious cavities of extracted human teeth. During the clinical study Velichko I. after a carious cavity by photodynamic therapy with laser light irradiation for 30 s percent detection of Veilonella spp. 4.7-fold Enterococcus faecalis – 2.-fold, Candida albicans 6.8-fold, Fusobacterium spp.-fold [11]. Biofilms of Streptococcus intermedius, Streptococcus mutans, Streptococcus salivarius prepared in carious cavities extracted human teeth, have been subjected to photodynamic antimicrobial chemotherapy using

toluidine blue O, a laser diode device emitting at 633 nm. Photoactive disinfection significantly reduced the number of bacteria in repaired dental cavities: the quantitative value of Streptococcus intermedius decreased by 87%, Streptococcus mutans – by 71%, Streptococcus salivarius – by 82%. [4]. The results of the Pierre Adriano Moreno NEVES study showed a statistically significant difference in the number of viable microorganisms before and after PDT application in molars that had been removed for partial removal of carious tissue. Therapy resulted in an average log decrease of 0.61 in the total number of microorganisms, 0.44 in streptococcal mutants and 0.46in Lactobacillus spp. ($p > 0.05$) [13]. However, this therapy presents different challenges on the susceptibility of different microorganisms. Most of the photosensitizers used in PDT are significantly more effective in inactivating Gram-positive bacteria than Gram-negative bacteria, which favors their use against dental caries microorganisms, since these caries lesions typically present the prevalence of Gram-positive strains [14].

CONCLUSIONS

In the study of microbial contamination of the carious cavity with pathogenic flora by PCR diagnostics, the genetic markers of the DNA of the five most virulent anaerobic bacteria were most commonly detected: Prevotella intermedia in 4 (7.84%) cases, Fusobacterium spp.-in 6 (11.76%) %), Enterococcus Faecalis- in 9 (17.65%), Veilonella spp.-in 12 (23.53%), Candida albicans-in 20 (39.22%) cases. When exposed to the red diode laser FotoSan 630 on the microflora of carious cavities of children of the Upper Tysa region with an exposure of 30 seconds, there was a decrease of pathogenic flora in carious cavities by 40%.

The maximum antiseptic effect of treatment of carious cavities was observed with the use of photodynamic therapy with an exposure time of 60 seconds, in which total destruction of pathogenic microflora occurred. The least effective method of antiseptic treatment is the use of 0.12% chlorhexidine, in which the reduction of pathogenic flora occurred by only 20%. The impact of photodynamic therapy with laser exposure for 60 seconds on caries-causing streptococci resulted in their total death. Given that in the pathogenesis of caries infectious factor is crucial, the relevance of the study of aspects of photodynamic therapy may in the near future be a real alternative to traditional methods of antibacterial exposure.

The obtained experimental and clinical data allow us to confirm the possibility and effectiveness of the use of photodynamic therapy in the clinic of therapeutic dentistry, as a new strategic direction of secondary prevention of carious disease.

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

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