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ABSTRACT

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INFLUENCE OF FLUORIDE ON DENTAL TISSUES: MINI-REVIEW AND OWN RESEARCH

Dental fluorosis is a general medical problem, common in many countries of the world. The clinical signs of fluorosis are localized in the maxillofacial area and are of great importance for the quality of life of patients (aesthetics, impaired chewing function, etc.). People are exposed to fluorides not only through food and water, but also from industrial and pharmaceutical products. Fluoride compounds are also used in dentistry to prevent dental caries. Excessive consumption of fluoride-containing products in children can lead to poisoning or fluorosis. According to WHO, the problem of dental fluorosis remains relevant.

Research objective: to characterize the structural changes in teeth enamel with different manifestations of fluorosis.

Materials and methods: the study was conducted on the basis of Poltava State Medical University (Ukraine). Content of fluoride in the drinking water of Karliv's and Mashiv's districts of Poltava region is 7–12 mg/l. Dental fluorosis was diagnosed in 58 patients of 25–44 years. For orthodontic and surgical indications, 18 fluorotic and intact teeth were removed. With the help of scanning electronic microscopy, the enamel surface structure was studied, and penetration of ammonia silver into enamel and dentin was assessed using light microscopy.

Results and discussion: according to the results of scanning electron microscopy, in mild and moderate cases, an inhomogeneous structure of the enamel is determined, the density of prisms in the rows is disturbed. In severe fluorosis, deep deformations and significant destruction of the enamel prisms occur. After impregnation with a solution of ammonia silver in intact teeth, a strip of uniform accumulation of the dye was observed in the surface layer of the enamel along the entire perimeter of the crown. In moderate fluorosis, the dye penetrated into the surface and deep layers of the enamel, and in severe fluorosis, into the deep layers of

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the enamel and beyond the enamel-dentine boundary. Enamel damage is localized on the vestibular and oral surfaces of the tooth. Histological examination confirmed that fluorinated enamel has a porous structure. This leads to rapid pigmentation of the surface layer of the enamel due to the diffusion of exogenous dyes.

Conclusions: the microstructure and chemical composition of hard tooth tissues reflect processes associated with the pathology of teeth and the whole organism. Structural changes in tooth enamel with various manifestations of fluorosis indicate a violation of the architectonics of enamel prisms and interprismatic substance**.**

Keywords: fluorosis, microstructure, enamel, penetration, tooth.

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ВПЛИВ ФТОРУ НА ТКАНИНИ ЗУБА: МІНІ-ОГЛЯД І ВЛАСНЕ ДОСЛІДЖЕННЯ

Флюороз зубів є загальномедичною проблемою, поширеною в багатьох країнах світу. Клініка флюорозу локалізується в щелепно-лицевій ділянці та має важливе значення у формуванні якості життя пацієнтів (естетика, порушення функції жування тощо). Люди піддаються впливу фторидів не лише через їжу та воду, але й з промислових і фармацевтичних продуктів. Сполуки фтору також використовуються в стоматології для профілактики карієсу зубів. Надмірне споживання фторвмісних продуктів у дітей може призвести до отруєння організму або флюорозу. За даними ВООЗ проблема флюорозу зубів залишається актуальною.

Мета дослідження: охарактеризувати структурні зміни емалі зубів при різних проявах флюорозу.

Матеріали і методи: дослідження проводили на базі Полтавського державного медичного університету (Україна). Вміст фтору в питній воді Карлівського та Машівського районів Полтавської області становить 7–12 мг/л. Флюороз зубів діагностовано у 58 пацієнтів віком 25–44 років. З ортодонтичних та хірургічних показань видалено 18 флюорозних та інтактних зубів. За допомогою скануючої електронної мікроскопії досліджували структуру поверхні емалі, а за допомогою світлової мікроскопії вивчали проникнення аміачного срібла в емаль і дентин.

Результати і обговорення: за результатами скануючої електронної мікроскопії при легкому та середньому визначається неоднорідна структура емалі, порушується щільність призм у рядах. При важкому флюорозі відбуваються глибокі деформації та значне руйнування призм емалі. Після імпрегнації розчином аміачного срібла в інтактних зубах спостерігалася смужка рівномірного накопичення барвника в поверхневому шарі емалі по всьому периметру коронки. При середньому флюорозі барвник проникав у поверхневі та глибокі шари емалі, а при тяжкому флюорозі – у глибокі шари емалі та за емалево-дентинну межу. Пошкодження емалі локалізовано на вестибулярній та оральній поверхнях зуба. Гістологічне дослідження підтвердило, що фториста емаль має пористу структуру. Це призводить до швидкої пігментації поверхневого шару емалі за рахунок дифузії екзогенних барвників.

Висновки: мікроструктура і хімічний склад твердих тканин зуба відображають процеси, пов'язані з патологією зубів і всього організму. Структурні зміни емалі зуба з різними проявами флюорозу свідчать про порушення архітектоніки емалевих призм і міжпризматичної субстанції.

Ключові слова: флюороз, мікроструктура, емаль, проникнення, зуб.

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INTRODUCTION

Dental fluorosis is a common pathology in many countries of the world [1, 2]. The clinic of fluorosis is localized in the maxillofacial area and is important in shaping the quality of life of patients (aesthetics, dysfunction of chewing, formation of the musculoskeletal system) [3, 4, 5, 6].

The deterioration of environmental living conditions, the development of the chemical industry, and the consumption of water and food in endemic regions with high fluoride content contribute to the excessive accumulation of fluoride in the body [7, 8].

Humans are exposed to various levels of fluoride intake, not only through food and water, but in industrial and pharmaceutical products and other sources.

Fluorine compounds are also used in dentistry to prevent dental caries. Excessive intake of fluoride-containing products in children can lead to body poisoning or fluorosis. There have been variability in the prevalence and severity of dental fluorosis over the past 30 years.

Many of the changes caused by fluoride are related to cell-matrix interactions during tooth formation and mineralization [9, 10, 11, 12].

Conducted epidemiological, morphological, and laboratory studies confirm the toxicity of fluoride to human and animal tissues [3]. The results obtained serve as the basis for the prevention and treatment of dental fluorosis [13, 14]. According to World Health Organization (WHO), the problem of dental fluorosis remains relevant [1].

Purpose of the study: to characterize structural changes in tooth enamel with various manifestations of fluorosis.

Dental Fluorosis Clinic

Fluoride toxicity can be manifested either as dental fluorosis or skeletal fluorosis. As an early sign of dental fluorosis the teeth lose their shiny appearance with development of chalk-white spots. Later these white patches become yellow and sometimes brown or black and in severe cases, loss of enamel gives the teeth a corroded appearance. Detailed studies on enamel changes occurring in human dental fluorosis is a must to understand the pathogenic mechanisms of fluoride action on tooth structure resulting in mottling (Fig. 1).

Clinical diagnosis of dental fluorosis was carried out according to the WHO classification. WHO recommends using the Dean criteria, proposed in 1934 and modified in 1942, according to which 5 forms of dental fluorosis are distinguished [15, 16].

Figure 1 – Patient K., 25 years old. Severe dental fluorosis

Questionable fluorosis (0,5 point) – in case of questionable fluorosis, barely noticeable white dots or spots are found on the enamel.

Very mild fluorosis (1 points) – very mild fluorosis is characterized by the presence of white, opaque spots occupying less than 25% of the labial surface of the tooth.

Mild fluorosis (2 points) – with mild fluorosis, white, opaque spots are more common, but the affected area is no more than 50% of the tooth surface. Atypical placement of enamel prisms is accompanied by increased enamel permeability.

Moderate fluorosis (3 points) – moderate dental fluorosis has abrasion of the enamel, destructive changes corresponding to brown spots on the teeth that distort its surface.

Severe fluorosis (4 points) – all enamel surfaces areaffected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic

sign of this classification is discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded- like appearance.

This classification is still the “gold standard” and is used in clinical research of patients.

MATERIALS AND RESEARCH METHODS

The study was conducted on the basis of Poltava State Medical University (Ukraine). Content of fluoride in drinking water Karliv’s and Mashiv’s districts of Poltava region is 7–12 mg/l [17].

The patients were examined according to WHO recommendations [18]. The clinical diagnosis of dental fluorosis was made according to the WHO classification. The form of fluorosis was determined by the two most affected teeth using a scoring system (Dean's criteria). 358 men and women with non-carious dental lesions were examined. Dental fluorosis was diagnosed in 58 patients.

Fluorous and intact teeth were removed for orthodontic and surgical reasons in individuals aged 25-44 years. 18 teeth were examined: 6 teeth with mild fluorosis (3 incisors, 3 premolars), 6 teeth with severe fluorosis, 6 intact teeth.

In order to eliminate methodological errors, only those intact teeth in which there were no cracks, erosions, or destruction of enamel were selected for the study.

At the first stage of the work, structural changes in the enamel surface were studied using scanning electron microscopy (SEM). At the second stage of the work, the process of penetration of ammonia silver into the structures of enamel and dentin was studied.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the local ethics committee of the participating institution. Informed consent from the patients was obtained for the study. The authors declared no conflicts of interest.

Preparation of sections of hard dental tissues

Longitudinal sections of hard dental tissues were made manually. 50 sections of incisors and premolars, intact and affected by fluorosis, were obtained. Sections were obtained at three different points of the transversal plane. To ensure that midsagittal sections of incisors would be obtained, the more apical 1/3 of the tooth was removed. Then, the remaining incisal 2/3 were hand-ground, so that a longitudinal section reaching the central aspect of the tooth was achieved, on the basis of the anatomical aspect of the pulp cavity. At this point, the other side of the tooth was exposed to sand paper, so that this side would be ground until a midsagittal 100 mm section was obtained. Thereafter, the section was polished and cleaned for quantitative polarized light microscopy and scanning electron microscopy analyses.

Method for studying structural changes in the enamel surface using scanning electron microscopy

Extracted teeth were cleaned of blood clots in 3% hydrogen peroxide solution, washed with running water and stored in 10% (neutral) formaldehyde solution. An organic film on the surface of the enamel prevents a detailed study of the structure of enamel prisms. The samples were prepared by removing the organic film (cuticle): the enamel was etched with 0.1 N hydrochloric acid – exposure 30 seconds. The tooth crown was cleaned of cuticle residues, washed in running water and dried.

The enamel relief was studied using scanning electron microscope SEM-100U.

Method for studying the permeability of the enamel layer using light microscopy

To determine the routes of penetration of the coloring matter into the thickness of the affected enamel, an ammonia silver solution was prepared [19]. The teeth were immersed in the solution (exposure 48 hours), removed and washed with distilled water.

Longitudinal sections of hard dental tissues were made manually. 50 sections of incisors and premolars, intact and affected by fluorosis, were obtained. Sections were obtained at three different points of the transversal plane.

To obtain midsagittal sections of the incisors, the apical third of the tooth was removed. The remaining two-thirds of the teeth were manually ground in the longitudinal plane. The opposite side of the tooth was then processed with a grinding disc to obtain a midsagittal section of 100 μm thickness. Finally, the sections were polished and rinsed with running water. The sections were prepared for quantitative polarized light microscopy and scanning electron microscopy[20].

To restore ammonia silver in the enamel and dentin layers, teeth were immersed in a 10% formaldehyde solution (exposure 48 hours), then washed with distilled water, and longitudinal sections were made. The depth of impregnation of enamel and dentin was studied on thin sections of teeth using a light binocular stereoscopic microscope (MBS-9).

Table 1 – Corresponding to the magnification of the microscope MBS-9

Increase on the handle scale	Scale division value 0.1mm
	Corresponds to the value on site
0,6	0,17
1	0,1
2	0,05
4	0,025
7	0,014

To estimate linear dimensions, we used an eyepiece with an interchangeable scale and diopter adjustment. The number of scale divisions that fits into the measured area of the object was counted. The result was multiplied by the number indicated in the conversion table, corresponding to the magnification of the microscope (Table 1).

The following histological layers were analysed in the area covering a distance of 100 μm from the enamel surface to the enamel–dentine junction (EDJ): the outer enamel, the superficial 30 μm enamel layer; the middle enamel, the 20 μm layer just beneath the outer layer; and the inner layer, which comprised the innermost 20 μm enamel layer, close to the EDJ.

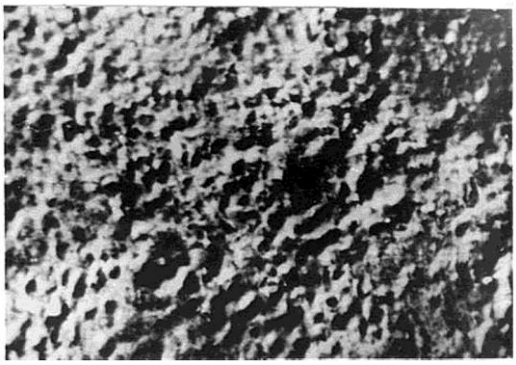

Figure 2 – The granular surface of the enamel of an incisor affected by mild fluorosis. SEM, magnification 1000

Statistical analysis

Statistical data processing was performed on personal computer using Microsoft Excel Office 2007. All numerical data obtained during the survey were processed by mathematical method with calculation of Student's criterion. The indicators were considered reliable at $p<0.05$.

RESULTS

Results of studying structural changes in the enamel surface using scanning electron microscopy

Granular structure of the enamel of teeth affected by mild fluorosis (Fig. 2).

With mild fluorosis, a heterogeneous enamel structure was determined. In most cases, the enamel prisms had an irregular shape, were deformed in the form of depressions and were covered with a mineralized substance. The density of the prisms in the rows was violated. The interprismatic spaces had different widths and heights, which formed a chaotic relief of the enamel surface.

When examining the enamel of teeth affected by moderate fluorosis, a heterogeneous structure of enamel prisms was noted, the size and shape were changed. In some areas of the enamel, the prisms are deformed, the density of placement in the rows is broken. The interprismatic spaces are enlarged and look like depressions or slits of various shapes (Fig. 3).

In severe fluorosis, deep deformations and significant destruction of enamel prisms were observed on the enamel surface. The entire surface is covered with a porous cement-like substance in the form of blocky elevations of various shapes. In certain areas of the relief, depressions and furrows of various sizes are identified (Fig. 3).

Results of studying the permeability of enamel with ammonia silver solution using light microscopy

After impregnation with ammonia silver, a stripe of uniform accumulation of coloring matter along the entire perimeter of the tooth crown was observed on sections of intact teeth in the surface layer of enamel. The depth of penetration of the coloring matter into the surface layer of enamel of intact incisors and premolars did not exceed 194.9±14.20 μm ($p<0.05$) (Fig. 4).

On thin sections of teeth affected by moderate fluorosis, it was revealed that the ammonia silver solution penetrated into the superficial and deep layers of enamel to a depth of 348.56±17.07 μm ($p<0.05$) (Fig. 5).

The localization of areas of enamel damage is noted on the vestibular and oral surfaces of the tooth crown. In the middle third of the tooth crown, ammonia silver penetrates to the enamel-dentin border. Ammonia silver stains the superficial and deep layers of the enamel of the cutting edge and the cervical part of the tooth crown. In these areas, the enamel-dentin border is not stained.

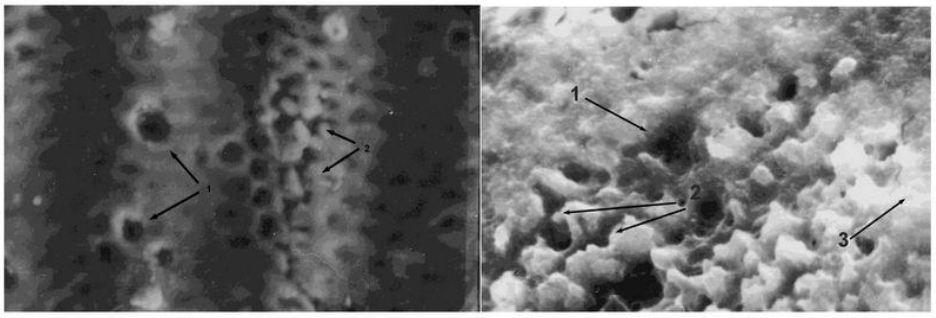

Figure 3 – The enamel surface of an incisor affected by moderate and severe fluorosis. 1 – deformation of prisms; 2 – gaps between deformed prisms; 3 – surface of a mineralized cement-like substance. SEM, magnification 2000

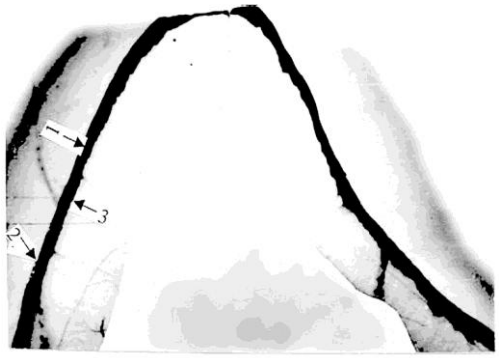

Figure 4 – Accumulation of ammonia silver solution in surface layer of enamel of intact incisor. Longitudinal grinding MBS-9, magnification 28. 1 – continuous strip of uniform penetration of ammonia silver into surface layer of enamel; 2 – enamel surface; 3 – border of strip of painted enamel

On sections of teeth affected by severe fluorosis, the ammonia silver solution predominantly penetrated into the deep layers of enamel – 609.6±68.09 μm ($p<0.05$). In some areas, penetration of ammonia silver beyond the enamel-dentin border into the deep layers of dentin is noted (Fig. 5).

The localization of areas of enamel damage is noted on the vestibular and oral surfaces of the tooth crown. In the middle third of the tooth crown, ammonia silver penetrates beyond the enamel-dentin border. In some areas, staining of the deep layers of dentin is noted.

DISCUSSION

Studying the structure of enamel in various manifestations of fluorosis using electron microscopy showed the severity of structural changes depending on the degree of fluorosis. In the enamel affected by fluorosis, a chaotic arrangement of prisms was found, the interprism spaces were changed, which, perhaps, facilitates the penetration of pigment into the thickness of the dental enamel during fluorosis. Our observations coincide with previous studies by other authors [3, 21].

Thus, the results of the study showed pronounced structural changes in the surface relief and thickness of the affected enamel in dental fluorosis compared to intact teeth. Our observations confirm the data of other authors about the disruption of the internal microarchitecture and density of enamel in severe forms of dental fluorosis and possible changes in its physiological properties (permeability).

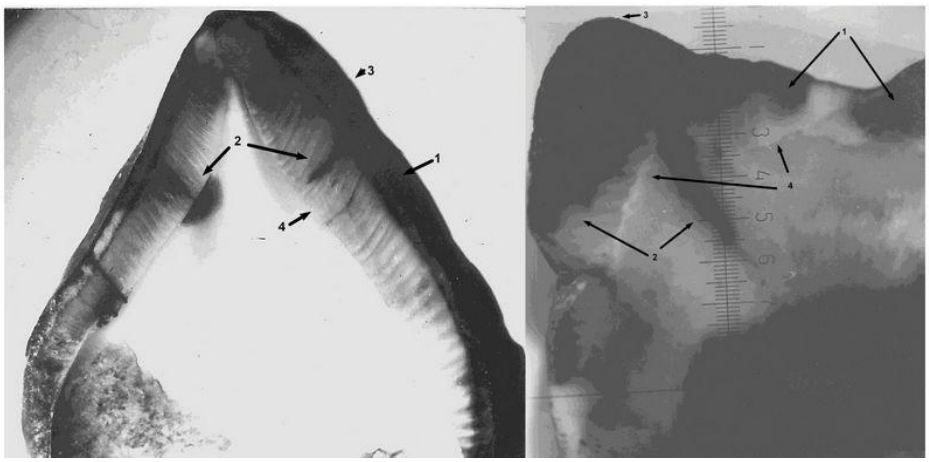

Figure 5 – Longitudinal section of incisor and premolar affected by moderate and severe fluorosis. MBS-9, magnification 28. 1 – layers of enamel painted with ammonia silver; 2 – uneven penetration of ammonia silver into enamel and dentin; 3 – enamel surface; 4 – enamel-dentin border

The results of a study of thin sections of teeth affected by various forms of fluorosis and intact teeth showed that the depth of penetration of the coloring matter depends on the form of fluorosis.

On thin sections of teeth (incisors, premolars), the depth of penetration of the coloring matter into the enamel of teeth affected by moderate fluorosis was 348.56±17.07 μm ($p<0.05$) and was significantly different from the depth of penetration of the coloring matter into the enamel of intact teeth – 194.9 ±14, 20 μm ($p<0.05$).

With moderate fluorosis, ammonia silver penetrates between 1/3 and 1/2 the thickness of the enamel. In severe fluorosis, the coloring matter penetrates to a much greater depth, passing beyond the enamel-dentin border into the dentin.

In severe fluorosis, the penetration depth of the coloring matter was 609.6±68.09 μm ($p<0.05$) and was significantly different from the penetration depth in moderate fluorosis - 348.56±17.07 μm ($p<0.05$).

With mild fluorosis, a heterogeneous enamel structure is determined. In most cases, enamel prisms are

irregularly shaped and deformed into depressions that are covered with a mineralized substance. The density of the prisms in the rows is broken. The interprismatic spaces have different widths and heights, which forms a chaotic relief of the enamel surface.

When examining the enamel of teeth affected by moderate fluorosis, a heterogeneous structure of enamel prisms is noted, their size and shape are changed. In some areas of the enamel, the prisms are deformed, the density of placement in the rows is broken. The interprismatic spaces are enlarged and look like depressions or slits of various shapes and sizes.

In severe fluorosis, the enamel surface has deep deformations and significant destruction of enamel prisms. The entire surface is covered with a porous cement-like substance in the form of blocky elevations of various shapes. In certain areas of the relief, depressions and furrows of various sizes are identified.

The results of the impregnation research method showed uneven penetration of ammonia silver into the layers of destructively altered enamel and dentin during dental fluorosis. With moderate fluorosis, ammonia silver penetrates into the middle third of the tooth crown within the enamel-dentin border. In case of severe fluorosis in certain areas, the coloring matter penetrates into the deep layers of dentin. The depth of penetration of the coloring matter directly depends on the form of dental fluorosis. With dental fluorosis, there is a direct dependence of the depth of penetration of the coloring matter into the layers of enamel and dentin on the form of the disease. Uniform accumulation of coloring matter in the surface layer of enamel occurs only in intact teeth.

Localization of areas of enamel damage is noted from the vestibular and oral surfaces of the tooth crown.

An experimental study confirmed that fluorous enamel has a porous structure. Moreover, the porosity and permeability of enamel increases as the degree of fluorosis increases, which coincides with the data of previous studies.

An experimental study confirmed that fluorous enamel has a porous structure. This leads to rapid pigmentation of the surface layer of enamel due to the diffusion of exogenous dyes.

Pigmentation and destructive changes are more pronounced on the vestibular surface in the middle third of the tooth crown. In our opinion, this is due to the fact that the cutting edge of the frontal group of teeth, the occlusal surface, as well as the oral part of the crown of the teeth are physiologically erased. And the vestibular surface is primarily in contact with the external environment, including dyes.

We agree with the position that dental tissue must be considered as a system characterized by a set of elements interconnected and reacting to environmental changes as a single whole [22, 23, 24].

CONCLUSIONS

The microstructure and chemical composition of hard tissues of teeth reflects complex processes associated with the pathological state of hard tissues of teeth and the whole organism. Our data confirmed the results of previous studies.

The identified structural changes in tooth enamel with different manifestations of fluorosis indicate a violation of the architectonics of enamel prisms and their relative position.

With dental fluorosis, the morphological integrity of the system of hard dental tissues and the function of its individual elements is disrupted in direct dependence on the form of the pathological process.

Further identification of the patterns of pathological processes in the hard tissues of teeth during fluorosis will allow us to better understand the mechanisms of their development and develop tactics for the treatment of non-carious lesions.

PROSPECTS FOR FUTURE RESEARCH

Further research will focus on the treatment of dental fluorosis with the use of remineralizing agents.

AUTHOR CONTRIBUTIONS

Oleksij Kostyrenko: literature search, manuscript preparation, final approval of the version to be published (guarantor). Anatoly Nikolishin: concept, manuscript review. Natalya Kotelevska: data acquisition, manuscript editing. Ella Nikolishyna: design, clinical studies. Nataliya Ilenko: clinical studies, data analysis. Alla Marchenko: experimental studies, statistical analysis. All authors read and approved the final manuscript.

FUNDING

None.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

CONNECTION WITH OTHER SCIENTIFIC RESEARCH WORKS

The work is a fragment of the research work of the Department of Therapeutic Dentistry of Poltava State Medical University (PSMU) “Increasing the effectiveness of prevention and treatment of lesions of hard dental tissues taking into account the mechanisms of pathology development” (State registration number: 0124U005249).

COMPLIANCE WITH ETHICS REQUIREMENTS

The authors declare that all procedures of this research comply with the ethical standards in the Helsinki Declaration of 1975, as revised in 2008.

ARTIFICIAL INTELLIGENCE DISCLOSURE

The authors confirm that no artificial intelligence-based technologies were used in writing or editing the manuscript.

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