Morphological features of enamel in fluorosis of different degrees of severity
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The occurrence of dental fluorosis is facilitated by a violation of enamel mineralization caused by fluorides, which enter the human body in excess during its development and formation and have a toxic effect on enamel blasts. However, the molecular mechanisms involved in the pathogenesis of fluorosis are not fully understood. Enamel formation is a complex process involving cell proliferation and differentiation through epithelial-mesenchymal sequential secretion of matrix proteins, tissue-specific transport of ions including calcium and fluoride, and precipitation and alignment of enamel crystals through interactions between organic and inorganic molecules. Understanding the morphological features of enamel changes during fluoride intoxication of the human body in the endemic region allows us to clearly understand the need for a comprehensive solution to this medical and social problem. The aim was to study the morphological features of enamel in fluorosis in residents of the endemic region of Ukraine, in particular the Poltava region. The work examines different groups of teeth (both intact and affected by fluorosis) removed for orthodontic or clinical indications in men and women aged 17 to 40 years. Morphological signs were studied first on native, and later on histochemically stained sections. It was established that the violation of the structure of the enamel layer of the teeth in mild and severe fluorosis is characterized by both partial and complete violation of the movement of the enamel prisms with signs of destruction. Fragmentation and homogeneity throughout the entire thickness were found in some areas of the enamel. When evaluating histochemically stained sections of teeth affected by fluorosis, it was established that dystrophic changes in the enamel structure and accumulation of acidic glycosaminoglycans in the lesions are more characteristic of mild and moderate forms. Under the conditions of a severe form of fluorosis, complete destruction of the prisms, fragmentation of the lamella, homogenization of areas on the entire enamel layer, which is due to the uneven distribution of acidic mucopolysaccharides, have been established. Morphologically and histochemically dystrophic changes in the areas of the affected enamel are confirmed by uneven distribution and accumulation of acidic glycosaminoglycans. An assessment of the effect of fluoride intoxication on the state of tooth enamel was carried out, which will allow to expand the possibilities of preventive measures for related specialists, as well as to create and develop additional treatment methods that will contribute to the improvement of physical and aesthetic indicators of teeth. dental health.

Keywords: enamel structure, fluorosis, glycosaminoglycans, mineralization.
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[29]. As a result, fluorosis of teeth and bones and general hyperfluorosis of the body develops [21, 23, 27, 28]. However, the toxic effect of hyperfluorosis requires further study, in particular, the relationship between tooth morphogenesis and the development of fluorosis [20].

The primary effect of fluoride ion is related to its interaction with calcium, magnesium, iron, copper ions, alkaline groups of biopolymers. In the secondary case, there is activation of adenylate cyclase and calcium messenger systems, inhibition of protein synthesis and a number of enzymes. Tertiary action is determined by a change in the hormonal balance [5, 10]. With the accumulation of excess fluoride in the hard tissues of bones and teeth, in addition to the normal fluorination of hydroxyapatite with the formation of strong crystals of isomorphic monofluorapatite (possible centers of crystallization), hyperfluorination occurs with the appearance of calcium phosphate and calcium fluoride crystals that are close to amorphous, also poorly soluble in water, but such coming from the enamel prism system due to the lack of isomorphism to hydroxyapatite or monofluorophosphate [25, 35]. The primary mineralization of enamel takes place in the early period of its formation. Secondary mineralization is due to the influx of minerals from the blood through the dentinal tubules, its zones - Schreger lines, lamellae and prismless enamel. Tertiary mineralization begins after teeth erupt due to the entry of substances from the oral fluid, its structure is the organization of hydroxyapatite crystals in the striae of Retzius. According to the opinion of Gasyuk A. P., the development of dental fluorosis occurs at the stages of primary and secondary mineralization [8]. At the stage of tertiary mineralization, the enamel of those teeth whose morphogenesis led to susceptibility to the action of fluoride is affected [15, 17, 18].

The purpose of this work is to study the morphological features of enamel in fluorosis in residents of Ukraine, in particular, the Poltava region.

Materials and methods

The work is a fragment of the initiative research work of the Poltava State Medical University: "Development of pathogenetic prevention of pathological changes in the oral cavity of people with internal diseases" (state registration number 0121U108263), completion date 2021-2025. The design of the study excluded the possibility of identifying individuals. Ethical standards, patient rights and confidentiality were not violated. The research was conducted in accordance with ethical and moral-legal requirements and does not contradict the basic bioethical norms of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine (1977), the relevant provisions of the WHO and the laws of Ukraine (Minutes of the meeting of the Commission on Biomedical Ethics of the Poltava State Medical University № 201 dated 27.01.2022).

The object of our morphological study were incisors, canines, premolars and molars of the upper and lower jaws, which were removed according to orthodontic (or clinical) indications in male and female persons aged 17 to 40 years. In the set, seven teeth are intact, 20 teeth were affected by fluorosis: six of them - a mild form, six - a moderate form, and eight teeth - a severe form.

The classification recommended by WHO is used, according to which 5 forms of dental fluorosis are distinguished: doubtful, very mild, mild, moderate and severe fluorosis.

A visual assessment of the extracted teeth was carried out, their preparation for the production of grindings, the formation of thick and thin grindings (30-50 microns and 100-200 microns). Morphological features were first studied on native, and later on histochemically stained sections. The staining technique was as follows: the slides were immersed in a 10% solution of NH4Cl and placed in a thermostat at a temperature of 37 °C for 6-12 hours, after which they were immersed for 15-30 minutes in an alcohol solution of Sudan III. Subsequently, they were rinsed in distilled water and immersed in a solution of iodine for 5-10 minutes, washed again with two to three portions of distilled water and stained with Schiff's reagent for 10-30 minutes. Then it was washed again with water for 10 minutes and stained with alcin blue solution for 10-30 seconds. After washing, the sections were dehydrated, illuminated and fixed in polyoxyme. A MBS-9 binocular magnifier (magnification x16, x32) and a Rathawow M-79053 light microscope (with image magnification from 80 to 252 times) were used to examine the slices.

PAS, Alcian blue, and Sudan III histochemical stains were used to determine the presence and changes in the distribution of lipoproteins, glycosaminoglycans, and proteoglycans.

Results

The obtained results indicate that the morphological pathohistological picture is characteristic of mild, moderate and severe forms of fluorosis. Study of slides of intact teeth and those affected by mild fluorosis yielded the following results.

On the surfaces of intact teeth, bundles of enamel prisms form longitudinal and transverse Hunter-Schreger bands (Fig. 1). Fissures, grooves, and pits contain histochemically stained PAS-positive fibrous structures surrounded by alcin-positive material. The enamel-dentine boundary is defined by a blue-green colored line. Enamel plates are contoured with a less saturated, but rather bright blue color. Enamel prisms from the enamel-dentine border to the surface of the enamel are followed by intense blue rod color, less saturated inter-rod gaps - light blue color. Under conditions of high magnification, it was established that bundles of enamel prisms, which were intensively colored in blue, alternated with inter-rod zones, where the intensity of coloring was much lower.
In contrast to the slides of intact teeth, changes in the enamel of the slides of teeth affected by mild fluorosis are observed. On native and histochemically stained sections, there is a thickening of the cuticular layer, the pattern of lamellae becomes more pronounced, an uneven color and increased transparency of enamel appear, which can be explained by atypical architecture of enamel prisms and a shift in the directionality of the Retzius striae (Fig. 2).

PAS + Alcian blue positive fibrous structures are morphologically determined. When visually evaluating the macropreparation (extracted teeth, from which grindings were made), these are areas of noticeable white dots or spots. When analyzing the slides under high magnification of the microscope, the enamel prism changes its color, so we can assume that the process of mineralization of the enamel is increasing. This happens due to the redistribution of acidic glycosaminoglycans (Fig. 3).

Visually present brown mottling of the enamel, that is, moderate fluorosis of the teeth, has a slightly different morphological picture.

Clinically, dental fluorosis of a moderate degree is characterized by destructive changes in the enamel and the presence of brown spots. Morphologically moderate form of fluorosis is characterized by the following. Dotted pigmentation of the enamel surface can be traced on the section, which spreads from the outer border, sometimes to the entire thickness, almost to the enamel-dentine border (Fig. 4). The morphological picture demonstrates the presence of foci of destruction of bundles of enamel prisms, their partial homogenization or complete destruction. The orientation of the course of the enamel prisms is partially or completely lost. This phenomenon is characterized by the blue coloration of areas of accumulation of acidic glycosaminoglycans.
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Discussion

Enamel is a unique tissue in vertebrates, its epithelial ameloblast cells secrete a number of matrix proteins that are broken down by enzymes during enamel mineralization. Any genetic or environmental disturbance will result in certain recognizable defects. Thus, depending on the specificity of the changes, a teratogenic event can be established retrospectively. Advances in this field allow the use of enamel defects as tools for the diagnosis of molecular disorders. The multifunctionality of enamel peptides is currently identified from their chemical role in mineralization to cellular signaling, which is the source of specific innovations in regenerative medicine [16]. Some studies discuss the current state of enamel tissue engineering and a new perspective on the future possibilities of regeneration of this unique tissue [22]. The analysis of numerous literary sources contains both experimental and clinical studies of the toxic effect of fluoride on the human body or animals. Animal models contribute to clinical applications by addressing disease pathogenesis. Rodent models are used to study dental fluorosis, as rat incisors erupt continuously and all stages of enamel development are present [32]. In general, fluoride promotes damage to other tissues, particularly chondrocytes, by regulating autophagy and apoptosis. This indicates that with long-term fluoride contamination and blocking of chondrocyte development, exposure to fluoride is a risk factor for cartilage development [34]. A certain percentage of researchers of the biochemical composition of enamel and the role of the protein matrix at the stages of mineralization confirm the conditionality of destructive changes and track these relationships in their experimental works [15, 28].

Analysis of caries indicators of permanent teeth in children, taking into account endemic features, demonstrates the connection of this disease with fluorosis. That is why, depending on the nature of the region, scientists offer various preventive measures [1, 2].

M. A. Buzalaf et al. [6] believe that despite the causal relationship between fluoride exposure and dental fluorosis, other factors likely account for its severity. There are observations that certain ethnic groups are more susceptible to this disease. Therefore, studies were carried out on the genetic susceptibility of different species of rodents to dental fluorosis, which aimed to evaluate the overall profile of apatite crystals in the enamel matrix of mice susceptible (strain A/J) or resistant (strain 129P3/J) to dental fluorosis by means of analysis with using atomic force microscopy.

The formation of tooth enamel is a change of successive stages, starting with presecretory, then secretory, through a short transition to the maturation stage, which is followed by apoptosis of ameloblasts and eruption of teeth in the oral cavity [9].

D. V. Kalashnikov et al. [13] determined the peculiarities of the process of enamel biomineralization in different

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Fig. 5. Fluorosis, severe form. Homogeneous areas of cavernous structures. 1 - enamel prism destruction; 2 - cavernous structures. Staining: Sudan III + PAS + Alcian blue. Light microscope Rathenow M-79053, x252.

At high magnification of the microscope, the morphological picture was as follows. Areas of brown spotting were clearly defined. Visually intact areas are morphologically changed, because according to their location, the enamel prisms have gait disturbances. The outer enamel layer is structurally broken, the enamel layer from the enamel-dentine border contains only isolated bundles of enamel prisms. Both the outer and inner layers of enamel were painted blue from light to saturated color, which indicated the content of acidic mucopolysaccharides.

Severe fluorosis (total pigmentation, with areas of enamel destruction) was morphologically characterized by an uneven distribution of acidic mucopolysaccharides. Some areas of the enamel have sharply expressed violations of the structure.

After examination of slides of teeth with severe fluorosis under a polarizing microscope, morphological changes in the structure of the enamel were established: in the surface layer of the enamel, areas of total pigmentation and destruction had a heterogeneous structure. In the deep enamel layers of the native slides with areas of enamel destruction towards the enamel-dentine border, there was an intensification of the pattern: both Hunter-Schreger bands and striae of Retzius. Areas of enamel destruction are areas of structureless zones of an amorphous substance. The direction of movement of the enamel prisms was completely destroyed, uniform homogeneous areas with the formation of cavernous structures were traced throughout the thickness of the enamel (Fig. 5). These areas were located next to the fragmented lamellae, the enamel prisms there were partially or completely destroyed.

Thus, the morphological study of both native tooth slides and histochemically stained ones established changes in the enamel structure depending on the period of the toxic effect of fluoride on the body.
anatomical areas of the tooth. E. C. Küchler et al. [14]
established that the polymorphism of the nonomogenin enamel matrix genes is associated with dental fluorosis.
When conducting a quantitative study of the pore volume fraction of human fluorotic enamel filled with resin infiltrate, it was established that organic substances, tightly bound water and air remained in the enamel pores after impregnation with resin [31]. Under the condition of different clinical conditions of the hard tissues of the tooth, the morphological structure of the enamel has certain features. This feature may be associated with a decrease in enamel density, which is associated with a decrease in the number of prisms in non-carious tooth pathology and an increase in the spaces between them [33].

Summarizing the results of our morphological study, it should be noted that there is a negative effect of excess fluoride for the body in general and for tooth enamel in particular. The study of both native and histochemically stained sections shows the morphological changes that develop in enamel during fluoride intoxication of different duration and intensity.

The basis of our results were the studies of Aoba T. [3], in which it is stated that fluoride is involved in many aspects of the formation of calcium phosphate in vivo and has a huge impact on both the process and the nature and properties of the formed mineral. In the process of enamel mineralization during amelogenesis, it is assumed that free fluoride ions in the liquid phase accelerate the hydrolysis of the acidic precursor and increase the driving force for the growth of the apatite mineral. However, an excess of fluoride leads to abnormal formation of enamel, slowing down the maturation of tissues.

The work of Robinson C. and co-authors [26] contains a description of the effect of fluoride on biological processes, which is reflected in the formation of tooth tissues, especially tooth enamel. Attention is primarily focused on the mechanisms that, if disturbed, can lead to dental fluorosis. The turbidity characteristic of fluoridated enamel is the result of incomplete formation of apatite crystals. Matrix proteins associated with the mineral phase, which are usually decomposed and removed to allow for final crystal growth, are retained to some extent in the fluorinated fabric. Concentrations of fluoride and magnesium increase here, and carbonates decrease. The morphology of the crystal surface at the nanolevel changes, as does the functional morphology of ameloblasts at the maturation stage. The inclusion of fluoride in enamel apatite leads to the formation of more stable crystals. Such changes in crystal chemistry and morphology, including stronger ionic and hydrogen bonding, also lead to stronger binding of matrix proteins and proteolytic enzymes. This leads to a decrease in degradation and an increase in the content of protein components in mature tissue. This is most likely the cause of porous fluorine enamel, as matrix protein removal is necessary for normal crystal growth. To resolve the question of the role of cellular changes and the exact causes of protein retention, more detailed studies of changes in cell function, effects on specific types of proteins, and nanochemistry of apatite crystal surfaces are needed.

According to Gil-Bona A. and Bidlack F. B. [9] enamel-specific proteins and proteases are crucial for the correct formation of enamel. Recent proteomic analyzes have identified many other proteins whose roles in enamel formation remain to be elucidated. Although the exact protein composition of healthy tooth enamel is still unknown, it is clear that the amount and composition of organic material is different in damaged enamel. Why these differences affect both the pre-eruption mineralization process and the properties of erupted teeth will become clear when proteomics protocols are adjusted for inter-species variability, tooth size, sample size and ephemeral organic content of forming teeth. A summary of current knowledge and published data on the proteomics of healthy and diseased tooth enamel, including advances in forensic applications and animal disease modeling, highlights how recent proteomics discoveries contribute to our understimation of the complexity and temporal changes in extracellular matrix composition during tooth enamel formation.

On the slides of visually unchanged teeth, the structure of the enamel has increased clarity of the course of the enamel prisms and Retzius striae. An uneven distribution of acidic glycosaminoglycans and neutral mucopolysaccharides is determined. Homogenization of alcian-positive masses and PAS-positive fibrous structures is determined in the affected areas. Histochemical examination shows a change in the color of homogeneous amorphous areas, which is traced from the outer surface to the enamel-dentine border of the tooth section.

The morphological changes found on the slides are obviously caused by the toxic effect of excess fluoride in one or another period of mineralization of the teeth. Excessive intake of fluoride to the body during the period of primary and secondary mineralization is especially harmful for tooth enamel. In the affected areas, fluoride continues to act even after teething, as a result - areas of total pigmentation and destruction (erosive-destructive form of fluorosis), because in the weakened area, fluoride continues to act even after teething. In the case of fluoride intoxication, only during tertiary mineralization (coming from saliva), the surface layers of the enamel are affected by fluorosis (doubtful, very weak, weak).

The results of morphological studies are a justification for further scientific developments and the introduction of new treatment methods in dentistry, in particular those involving the adhesive technique of tooth surface restoration.

Conclusions

1. In the area of endemic fluorosis, in the absence of preventive measures for the population during the period of primary, secondary or tertiary mineralization of tooth enamel, fluoride intoxication can lead to irreversible structural consequences.
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2. Changes can be manifested starting from atypical architecture of enamel prisms and a violation of the directionality of Retzius striae to the appearance of areas of enamel destruction - structureless zones of amorphous substance, cavens, in which the direction of movement of enamel prisms over the entire thickness of enamel is completely destroyed.

3. Factors that have a direct impact on the morphogenesis of fluorosis during all stages of mineralization can increase or, conversely, decrease the severity of this endemic disease.

4. In the areas affected by fluorosis, the predominance of dystrophic and necrotic changes causes varying degrees of disruption of the architecture of the entire enamel layer. The spread of degenerative processes in the enamel has a tendency to spread both to the surface of the lesion and to the depth.

5. For the prevention of dental fluorosis, it is extremely important to understand the toxic effect of fluoride on ameloblasts during a certain period of enamel mineralization and to eliminate or maximally weaken the pathogenic effect of the factor.

References
МОРФОЛОГІЧНІ ОСОБЛИВОСТІ ЕМАЛІ ПРИ ФЛЮОРОЗІ РІЗНОГО СТУПЕНЯ ВАЖКОСТІ

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Виникнення флюорозу зубів сприяє порушення мінералізації емалі, які викликані фтором, що надходять до організму людини в надлишкових кількостях в період її розвитку та формування тканинно-специфічного транспорт іонів, включаючи кальцій і фторид.

Метою роботи стало вивчити морфологічні особливості змін емалі при флюорозі в урізах, що потребувають виявлення та вивчення, які викликані фторидами, які токсично впливають на енамелобласті.

Розуміння морфологічних особливостей емалі значною мірою залежить від генетичних факторів, які сприяють розвитку цих змін.

Для вивчення морфологічних особливостей емалі при флюорозі використано нативний матеріал, отриманий при експериментальних дослідженнях.

За умов тяжкої форми флюорозу встановлено, що відомість змін емалі при флюорозі є складним процесом, що включає вибірку та диференціацію клітин через епітеліально-мезенхіматальну перетворення матриць, які включують кальцій і фторид.

Заключна частина роботи подає можливі шляхи впливу фтору на емалі, які потребують додаткового вивчення та створення додаткових лікувальних методів для покращення фізичних та естетичних властивостей емалі.

Ключові слова: структура емалі, флюороз, гілоксамілозгілі, мінералізація.
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Author’s contribution
Marchenko A. V. - conceptualization, methodology, writing of the original draft.
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