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## CRYSTALLOGRAPHIC FEATURES OF ORAL FLUID IN YOUNG PEOPLE WITH GINGIVITIS

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### Abstract

**Introduction.** Oral fluid is involved in maintaining the homeostasis of hard and soft tissues of the oral cavity. Therefore, it can indicate the changes in both the local environment and the whole organism. Recently, the crystallographic features of oral fluid have been actively studied. Therefore, this paper is devoted to the possibility of using crystallographic patterns of oral fluid for the early diagnosis of periodontal diseases in young people under 30 years of age.

**The purpose of the research** is to study the crystallographic picture of oral fluid in subjects aged 18-23 years with gingivitis.

**Materials and methods.** Subjects were divided into two groups: main — with chronic catarrhal gingivitis (28) and control (32). The main group consisted of two subgroups — with mild (18) and moderate (10) gingivitis. The degree of gingivitis was determined using the papillary-marginal-alveolar index (PMA) in Parma modification (1960). The study of oral fluid was performed using the method of wedge-shaped dehydration. The type of crystallization was determined according to the method of Leus P. A. modified by Dubrovina L. A. (1989).

**Results and discussion.** The type II crystallization of oral fluid was observed in the majority of individuals in both groups. Type II of the main group was characterized by massive crystals and minor crystals, smaller in size, and of different shapes. Type III was detected only in subjects with gingivitis. 75,00 % of representatives with mild gingivitis have had type II of crystallization patterns. Again, type III of oral fluid facies was observed only in moderate gingivitis.

**Conclusions.** Dependence of the crystallization type of oral fluid on the severity of the inflammatory process can be used in the development of methods for early diagnosis and prevention of periodontal pathology progression.

**Key words:** gingivitis; periodontium; saliva; crystallization.

**Introduction.** Saliva is a mucoserous biological fluid secreted by three pairs of large salivary glands, as well as small glands of the lips, tongue, palate, and cheeks. It consists of almost 99% water and contains various electrolytes (sodium, potassium, calcium, magnesium, chlorides, bicarbonates, phosphates), proteins (enzymes, immunoglobulins, other antimicrobial factors, glycoproteins, traces of albumin, some oligo- and polypeptides), glucose and nitrogen compounds (urea and ammonia). All these components interact with each other and thus provide the functions of saliva. Oral fluid is a collection of secretions of salivary glands, gingival fluid, transudate from the oral mucosa, food debris, components of

the oral microbiota, desquamated epithelial cells, immune and blood cells, mucus from the nasal cavity and pharynx, as well as traces of medicines [1,2].

Saliva, and therefore oral fluid, is essential for oral health. It is involved in maintaining the homeostasis of the hard tissues of the teeth and periodontium, providing physical and chemical metabolic processes in the oral cavity. The properties and composition of oral fluid can change in response to various exogenous and endogenous factors. For example, environmental factors, somatic diseases, inflammatory and inflammatory-destructive processes have a significant impact. Thus, oral fluid can be the subject of analysis to identify both the causes, risk factors, and pathogenetic mechanisms of various pathologies, and to identify signs of these same pathologies [1,3–5].

Such research in the field of dentistry has intensified. Many papers are devoted to the study of crystallographic features and mineralizing potential of oral fluid in children and adolescents with different degrees of caries activity. These include the study of concomitant pathology or adverse environmental factors impact [4–6]. There are data on the use of this method for the diagnosis and prediction of treatment outcomes in adolescents and individuals over 30 years of age with gingivitis, generalized periodontitis, as well as at different stages of implant treatment [7–11]. Therefore, we consider it expedient to study the possibility of using crystallographic patterns of oral fluid for early diagnosis of periodontal diseases in young people under 30 years of age.

**Purpose of the research.** To study the crystallographic picture of oral fluid in subjects aged 18-23 years with gingivitis.

**Materials and methods.** The research was conducted at the Laboratory of Psychophysiological Research of the Department of Physiology, Bioethics and Biosafety of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Certificate № 003/18) and the Department of Therapeutic Dentistry of the same university. The work adhered to the bioethical norms of the World Medical Association Declaration of Helsinki "Ethical Principles of Medical Research Involving Human Subjects" (adopted by the 18th World Medical Association General Assembly, amended in October 2013), the International Code of Medical Ethics, and the laws of Ukraine confirmed by the decision of the commission on bioethics of I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, protocol №59 dated June 5, 2020.

60 subjects aged 18-23 studying at Ternopil National Medical University were selected for the study. The main group includes 28 students with chronic catarrhal gingivitis. Representatives of this group were also divided into subgroups according to the severity of gingivitis: with mild gingivitis — 18 people and with moderate — 10. The degree of gingivitis was determined using the papillary-marginal-alveolar index (PMA) in Parma modification (1960). The control group consisted of 32 young people with healthy periodontium. All study participants did not have concomitant chronic pathology.

The oral fluid was taken from the bottom of the mouth with a sterile pipette in the morning, at least two hours after a meal. The study was performed using the method of wedge-shaped dehydration. Three drops of oral fluid were applied to a glass slide and dried at room temperature and minimum ambient mobility. A drop with the most clearly formed structure was chosen for interpretation. Saliva samples were examined and photographed using a Granum L30 trinocular microscope, a Granum DC 1300 camcorder, and Image Driving Software at  $10 \times 4$  and  $10 \times 10$  magnifications. The type of crystallization was determined according to the method of Leus P. A. modified by Dubrovina L. A. (1989) [4,12]:

- type I — a clear pattern of elongated crystal-prismatic structures that have fused and occupy the entire surface of the droplet;
- type II — individual dendritic crystal-prismatic structures of a smaller size than in type I in the center of the drop;
- type III — throughout the drop, there is a large number of isometrically located structures of irregular shape.

Statistical processing of the obtained data was performed using the licensed statistical software package StatPlus 6 (AnalystSoft Inc., license number 11114110), as well as IBM SPSS Statistics Subscription Trial. An exact Fisher-Freeman-Halton test and Pearson's chi-square test were used to analyze the parameters with nominal scales.

**Results and discussion.** Examination of oral fluid samples showed that type II crystallization of oral fluid was observed in the majority of healthy individuals. A quarter of cases matched the type I. Type III of the crystallographic picture was not recorded at all (Fig. 1).

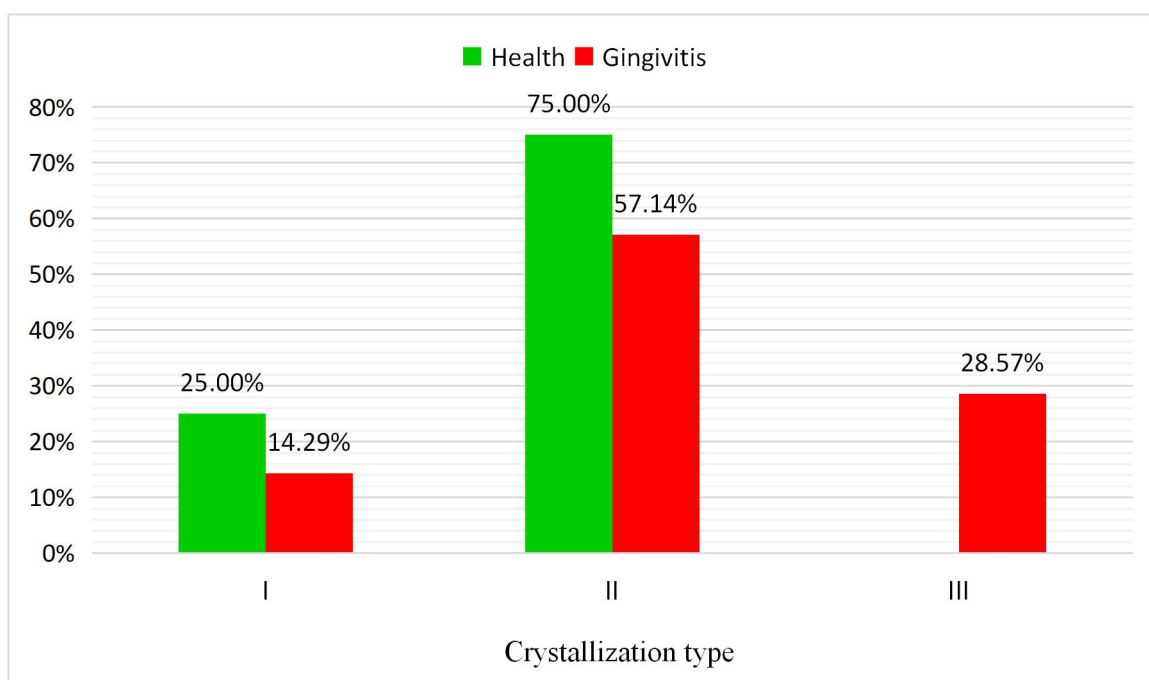


Figure 1. Crystallographic patterns of oral fluid in the study groups

Similarly, in young people with gingivitis, the largest percentage belonged to type II crystallization — a little more than half of the cases. This is 17.86% lower compared to periodontally healthy subjects. However, almost a third of the samples of oral fluid in the main group matched the type III facies. Type I crystallographic picture was the least common among people with gingivitis, namely 10.71% lower compared to students with periodontal health (Fig. 1).

Statistical analysis confirmed the dependence of the distribution of crystallization types of oral fluid on the condition of periodontal tissues ( $p = 0.0027$ ). In the further study of the data, a significant dependence of the distribution of I and III types, as well as II and III types of facies was recorded ( $p = 0.0047$  and  $p = 0.0039$ , respectively). This may indicate a significant probability of detecting inflammatory periodontal disease in subjects with type III crystallographic picture of oral fluid.

When comparing the data in the subgroups of subjects with varying degrees of gingivitis severity, it was found that the majority of oral fluid samples of subjects with mild gingivitis matched type II crystallization. In slightly less than a quarter of cases, type I was registered. At the same time, only types II and III of the crystallographic picture were found in young people with moderate gingivitis. The majority had type III, and only one-fifth of

those surveyed had type II. It should be noted that type III crystallization of oral fluid was recorded only in the main group with moderate gingivitis (Fig. 2).

The dependence of oral fluid crystallographic picture on the severity of gingivitis was proved statistically ( $p = 0.00001$ ).

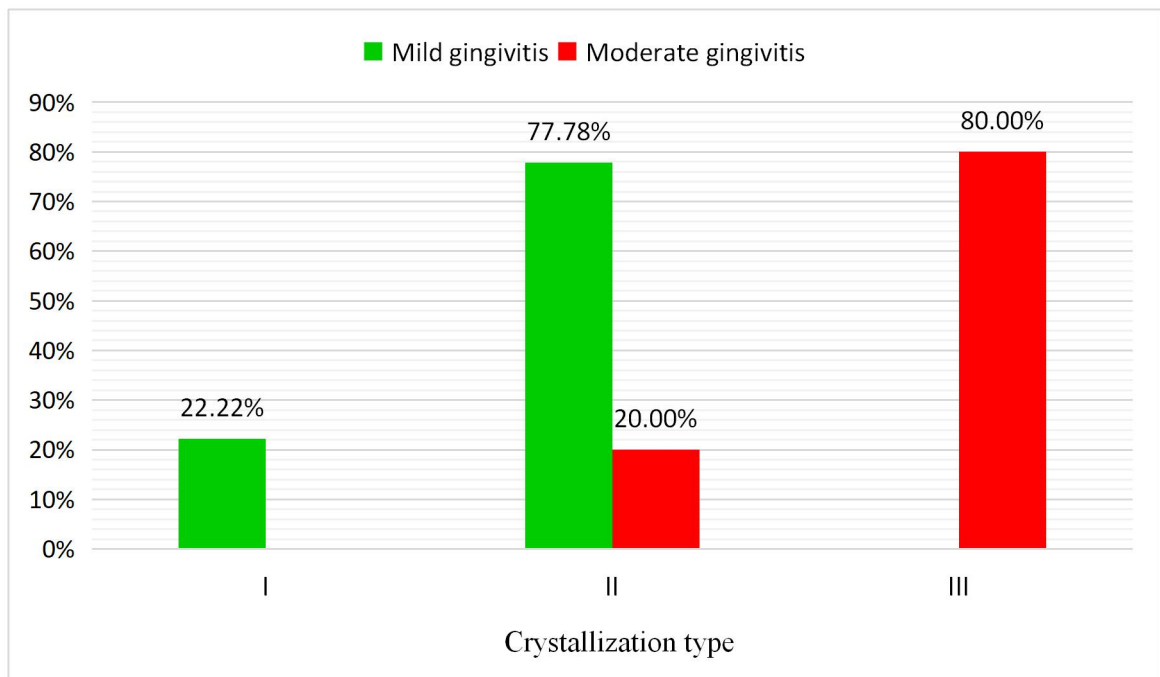


Figure 2. Crystallographic patterns of oral fluid depending on the severity of gingivitis

In addition, some differences between the main and control groups in I and II types of crystallographic patterns were detected. In general, in the samples of oral fluid in gingivitis was observed greater crystal thickness and greater diversity of structure. In type I, they filled a larger area, had flat extensions at the ends. Asymmetry of growth of 2nd and 3rd order crystals was also observed. Several patterns of crystallization in type II were identified. In the control group — a grid of smaller than in type I crystals of less clear structure, fused in any order. In most samples of this type, a large number of inclusions between the crystals were found. In the representatives of the main group type II was characterized by the presence of mainly massive crystals, between which minor crystals, smaller in size and of different shapes, were seen. There were variants of the facies, in which the central crystallized part occupied a small area and contained long large asymmetric crystals growing from the periphery (Fig. 3).

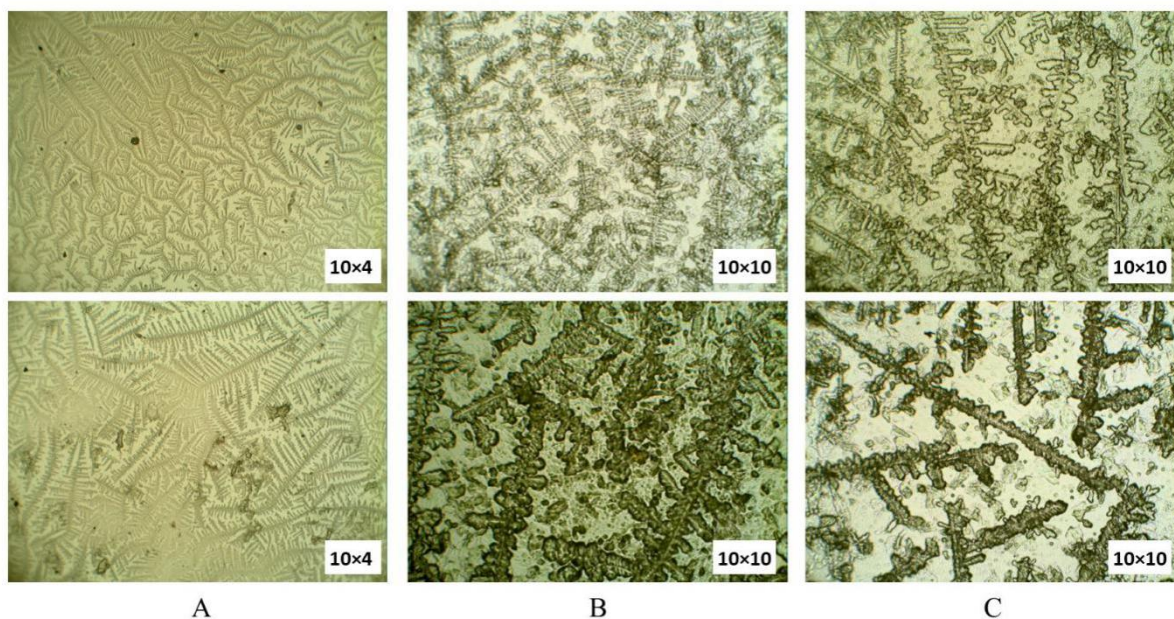


Figure 3. Samples of oral fluid facies (magnification is indicated in the lower right corner): top — control group, bottom — main group; A — I type, B and C — II type

There were also rare cases where almost the entire facies was covered with crystals with central symmetry — in the form of "flowers", "stars" (Fig. 4). According to Bulkina N. V, such features are found in generalized periodontitis [8]. This suggests that the characteristics of the facies can help to recognize the preclinical stage of the destructive process or a predisposition to the development of periodontitis.

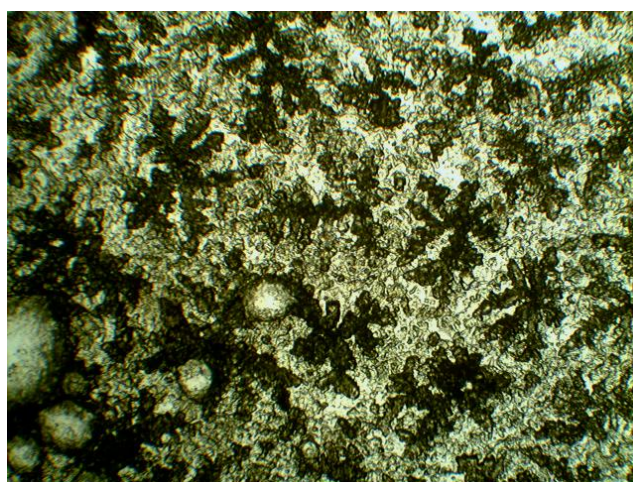


Figure 4. Crystals with central symmetry in subjects with gingivitis (collection 10 × 10)

Such results indicate a significant heterogeneity of oral fluid facies. Some authors believe that this is due to the physicochemical characteristics of saliva (amount of protein, viscosity, pH) [8,13]. According to many studies, the very phenomenon of facies formation, as well as the diversity of its structure, is based on complex molecular and physical mechanisms [14–16]. The increase in the number of protein components and the decrease in the viscosity of the oral fluid causes a violation of the structure of the crystals, thickening of the main trunks and processes, as well as the formation of many irregular crystallization centers or their complete absence. Changes in these characteristics may be caused by various external and internal factors or might be a predictor of disease progression, which requires further study.

**Conclusions.** Thus, the characteristic features of the crystallographic picture of oral fluid in young people with different conditions of periodontal tissues were established. In particular, the dependence of the crystallization type of oral fluid on the severity of the inflammatory process was determined, which can be used in the development of methods for early diagnosis and prevention of periodontal pathology progression.



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**Authors' contributions:**

1. Research designing: Author1, Author2, Author3, Author4, Author5
2. Conducting experiments: Author1, Author2, Author3, Author4
3. Writing the manuscript: Author1, Author2, Author3, Author4
4. Final approval of the version to be published: Author1, Author2, Author3, Author4, Author5