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# INVESTIGATION OF HUMORAL IMMUNITY INDICATORS OF THE ORAL CAVITY IN PATIENTS DEPENDING ON THE DEGREE OF GENERALIZED CHRONIC PERIODONTITIS

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The purpose of this research was to investigate the level of oral humoral immunity factors in patients depending on the degree of generalized chronic periodontitis. The study involved 60 patients. The material for the study was unstimulated oral fluid. Determination of lysozyme activity was carried out by the nephelometric method of V.G. Dorofeychuk. The level of sIgA in the oral fluid was identified by enzyme-linked immunosorbent assay according to the manufacturer's instructions. The correlation coefficient (Pearson's r) was computed to determine if a relationship exists between the levels of sIgA and lysozyme with the degree of generalized chronic periodontitis. It has been established an increase in the level of immune factors in the oral fluid of patients with generalized chronic periodontitis development of II and III degree. The lowest level of these indices was recorded in patients with generalized chronic periodontitis of sIgA and lysozyme in the oral fluid of patients with the degree of generalized chronic periodontitis of sIgA and lysozyme and sIgA and lysozyme in the oral fluid of patients with the degree. We have established an inverse correlation between the levels of sIgA and lysozyme in the oral fluid of patients with the degree of generalized chronic periodontitis of sIgA and lysozyme in the oral fluid of patients with the degree of generalized chronic periodontitis of sIgA and lysozyme in the oral fluid of patients with the degree of generalized chronic periodontitis affects the level of humoral immune factors in the oral fluid and has an inverse correlation with them.

**Keywords:** generalized chronic periodontitis, lysozyme, humoral immune factors, sIgA.

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# ВИВЧЕННЯ ПОКАЗНИКІВ ГУМОРАЛЬНОГО ІМУНІТЕТУ РОТОВОЇ ПОРОЖНИНИ У ПАЦІЄНТІВ В ЗАЛЕЖНОСТІ ВІД СТУПЕНЮ ХРОНІЧНОГО ГЕНЕРАЛІЗОВАНОГО ПАРОДОНТИТУ

Метою даного дослідження було вивчити рівень факторів гуморального імунітету ротової порожнини у пацієнтів у залежності від ступеню хронічного генералізованого пародонтиту. В дослідженні прийняли участь 60 пацієнтів. Матеріалом для дослідження була ротова рідина. Визначення активності лізоциму проводили нефелометричним методом за Дорофейчук В.Г. Рівень sIgA в ротовій рідині визначали за допомогою імуноферментного аналізу. З метою визначення наявності зв'язку між рівнем sIgA та лізоциму зі ступенем хронічного генералізованого пародонтиту визначали коефіцієнт кореляції (г-Пірсона). Встановлено збільшення рівня імунних факторів в ротовій рідині хворих з І ступенем хронічного генералізованого пародонтиту. За умов розвитку хронічного генералізованого пародонтиту II та III ступеню спостерігали прогресуюче достовірне зниження лізоциму та sIgA. Нами встановлено обернений кореляційний зв'язок між рівнем sIgA та лізоциму в ротовій рідині пацієнтів зі ступенем хронічного генералізованого пародонтиту. Отже, ступінь перебігу хронічного генералізованого пародонтиту впливає на рівень гуморальних факторів імунітету в ротовій рідині та має з ними обернений кореляційний зв'язок.

Ключові слова: хронічний генералізований пародонтит, лізоцим, гуморальні фактори, sIgA.

The work is a fragment of the research project "Mechanisms of pathogenic factors impact on the dental status of persons with somatic pathology, ways of their correcting and blocking", state registration No. 0115U001138.

According to statistics, nowadays the vast majority of the world's population has periodontal tissue diseases. They significantly worsen the standard of living of people and lead to a number of problems related to the deterioration of general health and socio-economic welfare and quality of life as well [2, 7].

It is well known that periodontitis is a chronic polyetiological plaque-associated inflammatory disease characterized by progressive destruction of periodontal tissues and subsequently can lead to tooth loss [3, 11]. According to the literature data, periodontitis can cause tooth loss 5 times more often than caries and its complications [1].

However, despite the indisputable fact of the dominance of microorganisms in the development of periodontitis, scientists in recent decades have pointed to a number of exo- and endogenous factors, the role of which in the pathogenesis of inflammatory diseases of periodontal tissue should not be underestimated [9, 12, 13]. Thus, factors of natural immunity of the oral cavity are able to maintain the constancy of the ecological balance of microorganisms in dental plaque, preventing the reproduction of pathogenic species. For this very reason, it is essential to study the factors of the oral cavity that regulate the metabolism of oral tissue and are able to enhance or reduce the pathogenic potential of microorganisms and their waste products [6].

Factors of local immunity of the oral cavity play an important role in the complex mechanism of development of the inflammatory process in periodontal tissues. This is because the chronic course of the disease is accompanied by microbial sensitization and a decrease in both general and local resistance of the body [8]. Therefore, a thorough study of the state of local humoral factors of the oral cavity in patients with

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generalized chronic periodontitis (GCP) will expand modern understanding of the pathogenetic development mechanisms of this disease and create prerequisites in finding new approaches to medicamentous correction of the pathological condition in periodontal tissues.

**The purpose** of the study was to examine the level of humoral immunity factors of the oral cavity in patients depending on the degree of generalized chronic periodontitis.

**Materials and methods.** The study involved 60 patients of the middle age group according to the WHO standards (mean age 52±8 years) who were treated at the Department of Therapeutic Dentistry of UMSA and periodontal office of ME "Poltava Regional Dental Center – Dental Clinical Polyclinic" of the Poltava Regional Council during 2015–2020. Patients were divided into 4 groups, depending on the degree of GCP. The first group included 15 patients who were diagnosed with GCP of I degree based on the results of instrumental and laboratory research methods; the second group consisted of 15 patients with GCP of II degree constituted the third group of supervision. The comparison group included 15 people who showed no signs of periodontal disease.

All patients were consulted by a family doctor at their place of residence, in order to exclude concomitant somatic pathology. Patients with chronic diseases in the acute exacerbation stage, neurological diseases, HIV and diabetes mellitus were excluded from the study.

The study material was unstimulated oral fluid, which was collected in sterile eppendorfs during maximal saliva secretion in the morning (10–11 a.m.) on an empty stomach in a volume of 2–3 ml by spitting. Before taking oral fluid samples, the factors influencing the secretion of salivary glands (physical activity, smoking, chewing gum) were excluded. Each patient signed a personal consent form before taking the material. The research was carried out in accordance with the Helsinki Declaration of the World Medical Association on the ethical principles of medical research involving human subjects.

The state of non-specific humoral protection of the oral cavity was assessed by determining the activity of lysozyme with the nephelometric method of V.G. Dorofeychuk using a museum strain of test culture M. lysodeicticus [5].

The level of sIgA in the oral fluid of patients was determined using a specific enzyme-linked immunosorbent assay kit (Elabscience, USA) according to the manufacturer's instructions. The results were obtained using a spectrophotometer Humareader (Germany) with a wavelength of 450 nm.

Data obtained from patients were subjected to statistical processing using the standard software packages "SPSS 16.0" and "Microsoft Excel 2016" with the calculation of the arithmetic mean (M), arithmetic mean error ( $\pm$  m) and the significance test (p). The presence of differences between the studied indicators was assessed by the Student's t-test. The results were considered significant at p <0.05, highly significant at p <0.01.

To identify the connection between sIgA and lysozyme level with the degree of generalized chronic periodontitis (GCP), correlation coefficient (Pearson's r) was determined, the absolute value of which characterized the bonding force. The correlation relationship was considered to be very weak at Pearson's correlation coefficient <0.2, weak – at <0.5, middle – at <0.7, high at – <0.9 and very high – at such indices >0.9.

**Results of the study and their discussion**. The difference of indices of humoral immunity in the oral liquid of patients with different degree of generalized chronic periodontitis (GCP) was determined. There was observed a 1.5 times increase of sIgA level in the oral liquid of patients with I degree of GCP compared to the index of patients with intact parodontium (p<0.05). At the same time, under the conditions of the development of GCP of the II and III degrees, progressing significant decrease of sIgA was observed. Therefore, the average number of the investigated index in the oral liquid of the patients with GCP of II degree of severity was lower in 1.6 times compared to patients of the experimental group. The lowest level of sIgA was determined of III degree of GCP, which was twice lower than the index in periodontitis-free patients (p<0.05).

Table 1

Indices of local humoral immunity of the ora	l cavity in patients with	generalized chronic	periodontitis (M±m)
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	Comparison group	I degree of GCP	II degree of GCP	III degree of GCP
sIgA, g/l	0.348±0.02	0.521±0.01	0.218±0.03*	0.174±0.03*
Lysozyme, µg/ml	16.47±0.12	19.22±0.17	13.69±0.21	10.01±0.16**
NT	1. 6.4 1.66 6.14	C T A 1 1	1. 4 1 64	

Note: \*- reliability of the difference of indices of sIgA level compared to the index of the comparison group, p<0.05; \*\*- reliability of the difference of indices of lysozyme level compared to the index of the comparison group, p<0.01.

The same tendency was observed when determining the lysozyme level in the oral liquid of patients with generalized chronic periodontitis. Under the conditions of Idegree of generalized chronic

periodontitis, number of lysozyme increased relatively to the comparison group of patients, however no statistically reliable differences between defined groups were detected. On average, the lysozyme level of patients with I degree of the disease was higher than normal values. By contract to this, the sharp decrease of lysozyme number in the oral liquid of patients with II and III degrees in 1.2 and in 1.7 times was established correspondingly with data obtained from patients with intact paradontium.

Because of investigations, it has been detected the consistency of the indices decrease of the humoral immunity, which have been investigated, with progressing of the severity degree of generalized chronic periodontitis. Inverse correlation relationship between the sIgA and lysozyme level in the oral liquid in patients with the degree of generalized chronic periodontitis was established (Picture1, 2). Therefore, indices of the humoral immunity of the oral cavity in patients with aggravating degree of generalized chronic periodontitis have decreased. Hence, correlation coefficient (Pearson's r) for number of sIgA in the oral cavity and the degree of severity was -0.85, and it indicated the middle correlation relationship between data of indices.



Fig. 1. The correlation between the level of sIgA in the oral liquid and the degree of generalized chronic periodontitis in patients (n=45)

There has been identified the high correlation between lysozyme content in the oral liquid of patients and the degree of GCP, so correlation coefficient (Pearson's r) has indicated such index (-0.98).



Fig. 2 The correlation between lysozyme level in the oral liquid and degree of GCP in patients (n=45)

Microbiocenosis disruption of the oral cavity is an integral to the development of infectious and inflammatory diseases. Its permanency is supported with immune system and depends on the number of unfavorable factors, which decrease protective mechanisms of the organism. It is known, key role in the immune protection is associated with nonspecific factors, which function as strong barrier and react on the presence of the foreign body in the oral cavity. That's why, it is presently topical to develop various approaches to the diagnostics and evaluation of patients' treatment quality with GCP [4, 10].

Antibacterial the properties of the oral fluid are related with the presence of significant amounts of lysozyme, bactericidal action of which is due to its ability to hydrolyze glycosaminoglycans in polysaccharides of cell membranes of microorganisms. Along with antibacterial activity lysozyme possesses pronounced immunomodulatory activity. One of the most important components of the mucosal barrier is sIgA, which is synthesized in the lymphoid tissue. By determining the level of sIgA in pathology of periodontal tissues, it is possible to assess the state of the barrier properties of the oral mucosa. This is true for dentistry and other medical specialties related to the study of the state of the mucous membranes.

Consistently, in patients with I degree of GCP the increase of lysozyme and sIgA level has been determined. Excessive accumulation of dental plaque and its imbalance of gram-negative microflora is the

"triggering mechanism" in the development of periodontal diseases. Therefore, the increase of bacteria assists in the secretion of local protective factors in the oral cavity [10]. Inflammation of the gums occurs under the influence of the waste products of microorganisms of the microbial plaque, which consist of toxins, enzymes and antigenic material. As a result for local damage, a cascade of changes in the microvasculature is activated, aimed at eliminating the damaging agent. This, in fact, explains the increase in immune factors in the oral fluid at I degree of GCP.

However, with process chronicity, there has been identified the decrease of organism reactivity and, respectively, the level of immune components. Consequently, as called "vicious loop" has been occurred and it assists in decreasing the immune factors and, subsequently, their insufficiency aggravates the course of indicated diseases [4, 5]. Similar phenomena were observed in patients who were involved in the investigation. But, during the development of II and III degrees of GCP, lysozyme and sIgA indices significantly have been decreased. This explains the high inverse correlation between lysozyme and sIgA content in the oral liquid of patients and the degree of GCP.

With a severe degree of the disease, a breakdown of adaptive mechanisms occurs, which is manifested in the form of a lack of response of macrophages and lymphocytes, a decrease in the functional properties of neutrophils, inhibition of the processes of secretion of sIgA at the local level.

Studies of the chemical composition of the oral liquid, determining factors of non-specific and specific protection of oral cavity allow solving problems as well diagnostics as choice of tactics rational treatment of patients.

#### Conclusion

There has been identified the increase of local indices of the immunity of the oral cavity in patients of Idegree of GCP. At the same time, during the development of II and III degrees of GCP, lysozyme and sIgA level in the oral liquid of the patients has been decreased. There is strong inverse correlation relationship between sIgA and lysozyme level in the oral liquid of patients and the degree of GCP. Namely, with the aggravation GCP degree in patients, decrease of indices of the humoral immunity of the oral cavity has been occurred.

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