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CHANGES IN IMMUNOLOGICAL REACTIVITY IN BACTERIAL-IMMUNE INFLAMMATION OF THE PERIODONTIUM UNDER THE CONDITION OF USING DIFFERENT TYPES OF REMOVABLE DENTURES

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Key words: *removable denture, base materials, acrylic denture, nylon denture, immunoglobulins, circulating immune complexes, periodontitis*
Ключові слова: *знімний протез, базисні матеріали, акриловий протез, нейлоновий протез, імуноглобуліни, циркулюючі імунні комплекси, пародонтит*

Abstract. Changes in immunological reactivity in bacterial-immune inflammation of the periodontium under the condition of using different types of removable dentures. Poliukhovych Yu.I., Demkovych A.Ye. *The purpose of this work was to study the effect of acrylic and nylon bases of removable dentures on the content of immunoglobulins and circulating immune complexes in the blood serum of rats with bacterial-immune periodontitis. The experimental animals were divided into 4 groups: I – control (n=10); II – animals with periodontitis on the 30th day of research (n=8); III – animals with periodontitis on the 30th day of research with acrylic bases (n=8); IV – animals with periodontitis on the 30th day of research with nylon bases (n=8). Acrylic bases were made by thermal polymerization of polymethacrylate material, and nylon bases were made from thermoplastic material by the method of pressing under pressure. Experimental bacterial-immune periodontitis was caused by injection of a mixture of microorganisms suspended in egg protein into the periodontal tissue. To enhance the immune response, rats were simultaneously administered Freund's complete adjuvant. This procedure was repeated on the 14th day. On the 30th day, blood serum was collected. The concentration of immunoglobulins A, M, G was determined by immunoturbidimetric method, circulating immune complexes – by precipitation method using photometry. The results were analyzed using non-parametric statistical methods. On the 30th day of periodontitis development, the level of IgA, M and G increased compared to the control by 2.70 times ($p<0.001$); 1.25 times ($p<0.01$) and 1.28 times ($p<0.01$), respectively. After fixation of acrylic bases, the content of IgA, M and G also increased by 2.48 times ($p<0.001$); 1.19 times ($p<0.001$) and 1.13 times ($p<0.01$), respectively. The use of acrylic bases led to a decrease in immunoglobulins compared to results without dentures. The use of nylon plastic contributed to an increase in the level of all classes of immunoglobulins, but they were lower compared to the data without prosthetics. Determination of IgA and M with nylon prosthetics showed their decrease compared to acrylic bases. The indicators of circulating immune complexes under prosthetics were higher when using acrylic bases than with nylon dentures. During the development of inflammation in the periodontium, a response of the innate link of the cellular immune system to prosthetics with acrylic and nylon structures was observed, which affects the nature of the development, course and completion of the inflammation.*

Реферат. Зміни імунологічної реактивності при бактеріально-імунному запаленні пародонта за умови використання різних типів знімних протезних конструкцій. Полюхович Ю.І., Демкович А.Є. *Метою цієї роботи було вивчення впливу акрилових та нейлонових базисів знімних протезів на вміст імуноглобулінів та циркулюючих імунних комплексів у сироватці крові щурів при бактеріально-імунному пародонтиті. Піддослідних тварин розподілили на чотири групи: I – контроль (n=10); II – тварини з пародонтитом на 30-ту добу дослідження (n=8); III – тварини з пародонтитом на 30-ту добу дослідження з акриловими базисами (n=8); IV – тварини з пародонтитом на 30-ту добу дослідження з нейлоновими базисами (n=8). Акрилові базиси виготовляли шляхом термічної полімеризації з поліметакрилатного матеріалу, а нейлонові – з термопластичного матеріалу методом пресування під тиском. Експериментальний бактеріально-імунний пародонтит викликано шляхом ін'єкційного введення суміші мікроорганізмів, суспендованих у яєчному протеїні, у тканини пародонта. Для посилення імунної відповіді одночасно вводили щурам повний ад'ювант Фрейнда. Цю процедуру повторювали на 14-ту добу. На 30-ту добу робили забір сироватки крові. Концентрацію імуноглобулінів A, M, G визначали імунотурбодиметричним*

способом, циркулюючих імунних комплексів – методом преципітації за допомогою фотометрії. Результати аналізували за допомогою непараметричних статистичних методів. На 30-ту добу розвитку пародонтиту рівень IgA, M та G зріс порівняно з контролем у 2,70 раза (p<0,001); 1,25 раза (p<0,01) і 1,28 раза (p<0,01) відповідно. Після фіксації акрилових базисів вміст Ig класів A, M та G також виявився збільшеним у 2,48 раза (p<0,001); 1,19 раза (p<0,001) та 1,13 раза (p<0,01) відповідно, відносно контролю. Використання акрилових конструкцій приводило до зменшення вмісту імуноглобулінів порівняно з показниками без протезів. Використання нейлонової пластмаси сприяло підвищенню рівня всіх класів імуноглобулінів, але вони були нижчими порівняно з даними без протезування. Визначення вмісту IgA та IgM при нейлоновому протезуванні показало їх зменшення порівняно з акриловими базисами. Показники циркулюючих імунних комплексів, за умови протезування, були вищими при використанні акрилових базисів, ніж при використанні нейлонових протезів. Під час розвитку запалення в пародонті спостерігалася відповідь вродженої ланки клітинної імунної системи на протезування акриловими та нейлоновими конструкціями, що впливає на характер розвитку, перебіг і завершення запального процесу.

In inflammatory processes in periodontal tissues, an excessive response of the immune system to bacterial antigens is often observed. This leads to the inflammatory process, destruction of bone and connective tissue, as well as to the progression of periodontitis and increased activity of lipoperoxidation, which leads to the accumulation of peroxidation products, as well as to the depletion of the body's antioxidant system reserves, and this causes the development of hyperenzymemia and the accumulation of toxic substances [1].

The humoral link of adaptive immunity is one of the key systems of the body's defense against infections and consists in the synthesis of specific antibodies to combat antigens. The main cells of humoral immunity are B lymphocytes, which after contact with the antigen turn into plasma cells and begin to produce antibodies (immunoglobulins) [2]. The protection of periodontal tissues from bacteria that cause inflammatory processes in soft tissues and bone is provided by three main classes of immunoglobulins – IgA, IgM, IgG [3].

The oral mucosa has its own autonomous immune system, which functions independently of the general immunity. Many immune reactions occur in the oral cavity, providing natural protection and maintaining homeostasis [4, 5]. The epithelial layer of the mucosa contains a significant number of immunocompetent cells, in particular neutrophils, which emerge from the vessels of the lamina propria and retain up to 90% of their activity on the surface of the epithelium [6]. The use of removable dentures can cause morphological changes in the oral mucosa, and the rate and nature of these changes largely depend on the reactivity of the patient's body [7]. The state of local immunity plays an important role at all stages of treatment with removable dentures, and changes in the levels of immunoglobulins in saliva are also noted during inflammatory processes in the tissues of the oral cavity [8]. The study of cellular factors of innate immunity allows us to objectively assess the process of adaptation to removable dentures and the quality of this adaptation [9].

The mechanisms of oral cavity protection can be conditionally divided into two main groups. The first group consists of nonspecific factors of innate immunity, which act on a variety of foreign microorganisms regardless of their species. The second group includes specific components of the adaptive branch of the immune system, which act on certain types of microorganisms and provide more targeted protection [10].

Nonspecific protection of the oral cavity is implemented using two mechanisms. The first is mechanical, which includes cleansing and washing of the mucous membrane during eating, removal of microorganisms with saliva, and adhesion (attachment) of microorganisms to the epithelium. The second mechanism is biological, provided by the action of various biologically active substances and processes [11, 12]. Studies have shown that in dental diseases, in particular periodontitis, there is a decrease in the activity of all protective factors of the oral cavity [13].

The purpose of this work: to study the influence of acrylic and nylon bases of removable dentures on the content of immunoglobulins and circulating immune complexes in the blood serum of rats with bacterial-immune periodontitis.

MATERIALS AND METHODS OF RESEARCH

The experiments were conducted on clinically healthy male white rats weighing 150-200 g, which were kept in vivarium conditions in compliance with sanitary standards and principles of good laboratory practice (GLP).

The experimental animals were randomly selected and divided into four groups: Group I - intact animals, control (n=10); Group II – animals with periodontitis on the 30th day of the study (n=8); Group III – animals with periodontitis on the 30th day of the study with acrylic bases (n=8); Group IV – animals with experimental periodontitis on the 30th day of the study with nylon bases (n=8).

Dentures were manufactured using standard methods: acrylic bases by thermal polymerization of polymethacrylate material "Villacryl H Plus" ("Zhermack", Poland) [14.], and nylon bases - by compression molding of thermoplastic material "Vertex ThermoSens" ("Vertex", Netherlands) [15]. Orthopedic structures were designed in such a way as not to

cover the occlusal surfaces of the teeth and were fixed on both central incisors of the lower jaw.

Experimental bacterial-immune periodontitis in experimental animals was induced by injection of a mixture of microorganisms (Staphylococcus aureus and Streptococcus hemolyticus), suspended in egg-white, into the periodontal tissue. The components of the cell wall of gram-positive bacteria are lipoteichoic acids, peptidoglycan, lipoproteins, which are triggers for the development of inflammation through toll-like receptors 2. These molecules are key in recognizing pathogens and launching the innate immune system. To enhance the immune response, rats were simultaneously administered complete Freund's adjuvant. This procedure was repeated on the 14th day of the experiment to confirm the effectiveness of induction and chronicity of periodontitis of bacterial-immune origin [16]. On the 30th day, the experimental animals were euthanized by exsanguination under anesthesia using sodium thiopental, and blood serum was collected.

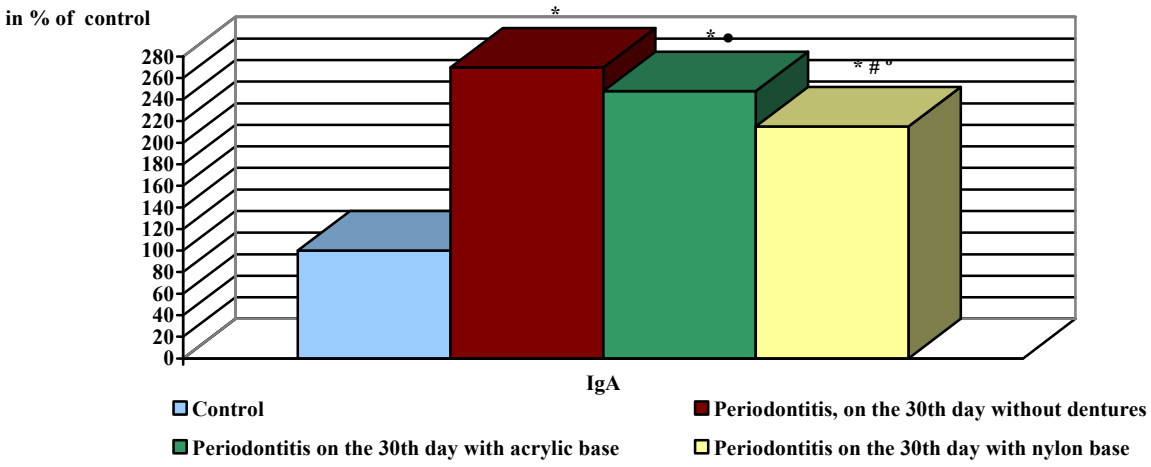
All procedures were carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the "General Ethical Principles of Animal Experiments" (Kyiv, 2001). The study was approved by the Bioethics Commission of the I.Ya. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No. 78 dated August 18, 2024) [17].

The concentration of immunoglobulins A, G, M in blood serum was determined by the immunoturbometric method using reagents from Dialab GmbH (Austria). This method is based on the interaction of antibodies with antigens present in the sample, resulting in the formation of an antigen-antibody complex, and changes in adsorption are measured turbodimetrically at the endpoint [18]. The level of circulating immune complexes was estimated by the precipitation method with polyethylene glycol-6000 with subsequent photometric analysis on a SF-46 spectrophotometer at a wavelength of 450 nm [19].

The results were analyzed using nonparametric statistical methods in the STATISTICA 10.0 software (StatSoft, USA) (License AGAR909E415822FA). To perform statistical processing of the obtained results, the analysis of variation series was used – calculation of the arithmetic mean and its standard error (M and m). The reliability of differences between independent quantitative variables with a normal distribution was assessed using the Mann-Whitney U-test using nonparametric characteristics and criteria: median (Me), interquartile range (25%; 75%) – 25 and 75 percentiles, respectively, for comparison of the studied samples. The critical value of the level of statistical significance (p) for all types of analysis was taken <5% (p<0.05) [20].

RESULTS AND DISCUSSION

IgA is the main immunoglobulin of mucous membranes, it prevents the attachment of bacteria to the gingival epithelium, which reduces their spread deep into the tissues [21]. The results of our studies showed that in experimental animals on the 30th day of the development of experimental periodontitis, the level of IgA in the blood serum increased by 2.70 times (p<0.001) compared to the control group of rats (Fig. 1).



* – significant difference with the intact group of animals (p<0.001); # – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without dentures (p<0.001); ● – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without the use of bases (p<0.01); ° – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day with the use of acrylic bases (p<0.01).

Fig. 1. Changes in IgA concentration under the conditions of bacterial-immune periodontitis development and use of prosthetic bases (in % of control)

During this observation period, similar changes were recorded in the levels of immunoglobulins of classes M and G, which increased by 1.25 times ($p<0.01$) and by 1.28 times ($p<0.01$), respectively, compared with the indicators of control animals.

In experimental animals, in whom acrylic bases of dentures were fixed on the 30th day, the content of immunoglobulins of classes A, M and G was also increased by 2.48 times ($p<0.001$); by 1.19 times ($p<0.001$) and by 1.13 times ($p<0.01$), respectively, relative to the intact group of rats (Table).

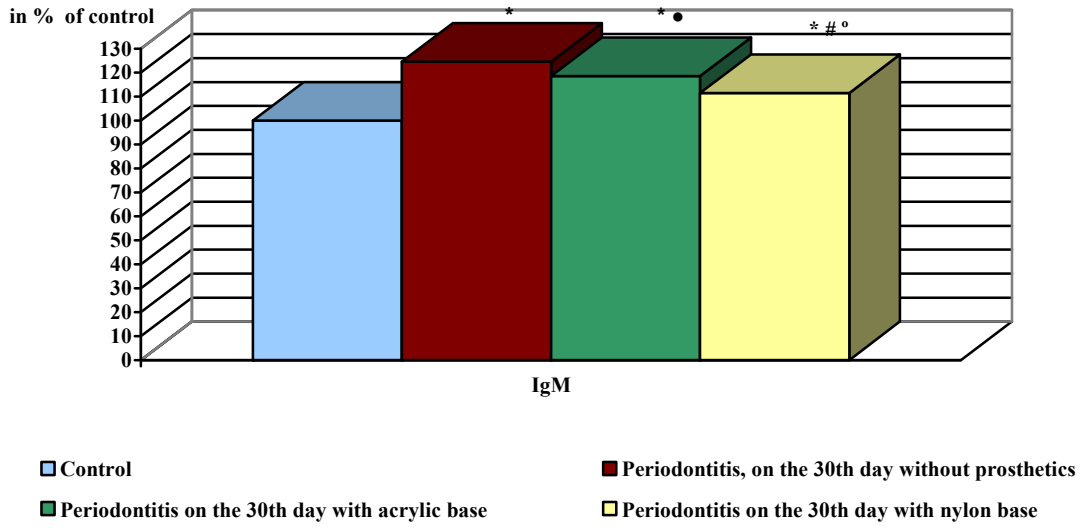
The use of a thermoplastic nylon plastic base in experimental bacterial-immune periodontitis led to an increase in the level of IgA by 2.15 times ($p<0.001$) relative to the indicators of intact rats, which was less, compared to the results of animals with an inflammatory process in periodontal tissues on the 30th day of the experiment without prosthetics by 1.25 times ($p<0.001$). Compared to the group with bases made of polymethacrylate material, the concentration of this immunoglobulin decreased by 1.15 times ($p<0.01$).

Changes in the indicators of the humoral component of adaptive immunity in the blood serum of experimental animals with experimental bacterial-immune periodontitis and under the condition of fixation of prosthetic bases (M±m)

Research conditions and indicators	Control (intact group)	Animals with experimental bacterial-immune periodontitis		
		without prosthetics	acrylic base	nylon base
Duration of research (days)	-	30	30	30
Number of rratsькість щурів	10	8	8	8
Immunoglobulin A, (g/L)	1.59±0.06	4.29±0.06 $p_1<0.001$	3.94±0.07 $p_1<0.001$; $p_2<0.01$	3.42±0.08 $p_1<0.001$; $p_2<0.001$; $p_3<0.01$
Immunoglobulin M, (g/L)	6.46±0.08	8.05±0.04 $p_1<0.001$	7.66±0.11 $p_1<0.001$; $p_2<0.05$	7.20±0.05 $p_1<0.001$; $p_2<0.001$; $p_3<0.01$
Immunoglobulin G, (g/L)	6.54±0.04	8.39±0.06 $p_1<0.001$	7.36±0.23 $p_1<0.01$; $p_2<0.01$	8.06±0.09 $p_1<0.001$; $p_2<0.05$; $p_3>0.05$
CICs, (c.u.)	48.20±1.29	92.38±0.82 $p_1<0.001$	88.63±1.31 $p_1<0.001$; $p_2<0.05$	80.75±0.67 $p_1<0.001$; $p_2<0.001$; $p_3<0.01$

Notes: p_1 –statistical significance of differences with the intact group of animals; p_2 – statistical significance of differences with the group of animals with bacterial-immune periodontitis on the 30th day without prosthetics; p_3 – statistical significance of differences with the group of animals with bacterial-immune periodontitis on the 30th day with the use of acrylic bases.

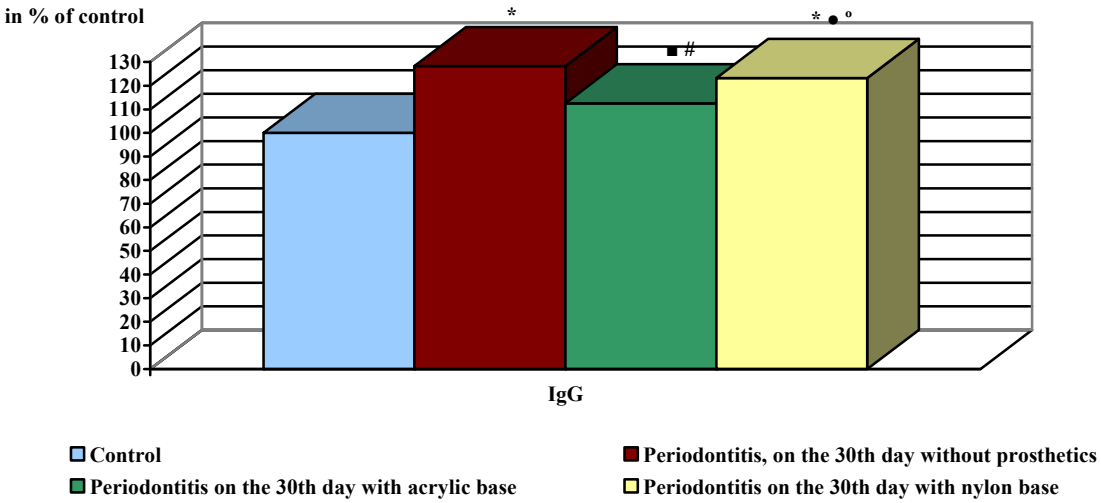
Determination of the concentration of IgM in blood serum, which was used to assess the state of the immune reaction during inflammation of periodontal tissues under the conditions of removable nylon prosthetics, showed that on the 30th day with this type of bases its concentration changed similarly to IgA, that is, it decreased, but with less severity (by 1.06 times; $p<0.01$) compared to the group with acrylic bases. Immunoglobulin M contributes to a rapid immune response to infection, which is important in the early stages of inflammatory diseases [22]. Comparing IgM levels in animals with nylon dentures, it should be noted that they were lower than those in rats with bacterial-immune periodontitis on the 30th day of the experiment without prosthetic structures (1.12 times; $p<0.001$), exceeding the control by 1.12 times ($p<0.001$) (Fig. 2).



* – significant difference with the intact group of animals ($p<0.001$); # – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without prosthetics ($p<0.001$); ● – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without the use of bases ($p<0.05$); º – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day with the use of acrylic bases ($p<0.01$).

Fig. 2. Changes in IgM concentration under the conditions of bacterial- immune periodontitis development and use of prosthetic bases (in % of control)

After determining the level of immunoglobulin G concentration in the blood serum of animals with nylon prosthetics and periodontitis, it was noted that on the 30th day of the experiment this indicator exceeded the results of the control group of animals (1.23 times; $p<0.001$) and was higher in comparison with the values obtained in rats with acrylic bases, but, these data were statistically insignificant ($p>0.05$). However, the IgG content in this group was lower than the values of animals with an inflammatory process in the periodontium without prosthetics (1.04 times; $p<0.05$) (Fig. 3). An increase in the concentration of immunoglobulin G in the blood serum is a reflection of the activation of phagocytosis and promotion of the elimination of microorganisms that have entered the tissues of the periodontal complex [23].



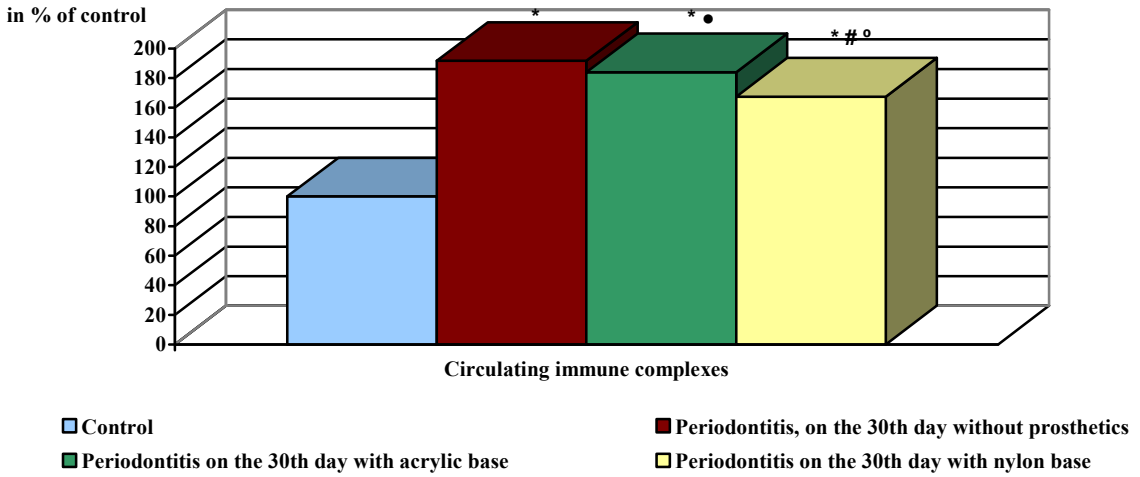
* – significant difference with the intact group of animals ($p<0.001$); ■ – significant difference with the intact group of animals ($p<0.01$); # – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without prosthetics ($p<0.01$); ● – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without the use of bases ($p<0.05$); º – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day with the use of acrylic bases ($p>0.05$).

Fig. 3. Changes in IgG concentration under the conditions of bacterial-immune periodontitis development and use of prosthetic bases (in % of control)

Circulating immune complexes (CICs) are compounds that are formed in the blood when antigens bind to antibodies. They are part of the body's immune response to foreign agents, such as bacteria, viruses, or other antigens [24]. These complexes are usually removed from the body by phagocytosis, but under certain conditions, their excessive accumulation or insufficient removal can lead to the development of pathological conditions [25].

Analyzing changes in the indicators of circulating immune complexes in the blood serum of animals with bacterial-immune inflammation of the periodontium, it should be noted that on the 30th day of the experiment, their concentration significantly exceeded (by 1.92 times; $p<0.001$) the indicators in the animals of the control group. It should be noted that these data were also higher than the indicators that were in rats on the 30th day with periodontitis with prosthetics with removable dentures, by 1.04 times ($p<0.05$) – when using acrylic bases and by 1.14 times ($p<0.001$) – when using nylon bases, respectively (Table).

The indicators obtained in experimental animals that had fixed dentures, indicated a significant increase in CICs in blood serum compared to the data in the intact group both when using polymethacrylate bases and when using bases made of thermoplastic nylon plastic, namely, by 1.84 times ($p<0.001$) and by 1.68 times ($p<0.001$), respectively. Comparing the CICs indicators during this observation period under prosthetic conditions, it should be noted that they were 1.10 times ($p<0.01$) higher when using acrylic bases than the indicators obtained in animals with nylon dentures (Fig. 4).



* – significant difference with the intact group of animals ($p<0.001$); # – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without prosthetics ($p<0.001$); ● – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day without the use of bases ($p<0.05$); º – significant difference with the group of animals with bacterial-immune periodontitis on the 30th day with the use of acrylic bases ($p<0.01$).

Fig. 4. Changes in the concentration of circulating immune complexes under the conditions of bacterial-immune periodontitis development and the use of prosthetic bases (in % of control)

A decrease in the level of immunoglobulins, especially IgA and IgG, during the use of removable dentures in the presence of bacterial-immune periodontitis may indicate a decrease in the effectiveness of protecting periodontal tissues from bacterial infections. This subsequently leads to more active colonization of tissues by pathogens, which contributes to chronic inflammation.

With excessive formation or insufficient removal of circulating immune complexes, they can accumulate in tissues and blood vessels, which causes inflammatory processes and damage to the periodontal complex. This phenomenon is often observed in autoimmune diseases. Although CICs provide protection for the body, their excess or disruption of the mechanisms of their removal can contribute to the development of pathological processes [26].

Therefore, a disruption of the functioning of the immune system, especially in the production of immunoglobulins, contributes to the development of periodontitis due to weakened tissue protection from infections and an uncontrolled inflammatory reaction, which leads to destruction of the periodontium. According to the literature [27], in some forms of periodontitis, the immune system can attack its own periodontal tissues, which leads to their destruction. These autoimmune processes can occur due to

disorders in the regulation of immunoglobulin production and lymphocyte activation.

Removable prosthetic structures increase the risk of developing pathogenic microflora in the oral cavity due to their effect on the biofilm and the surrounding environment. However, the data obtained, on the contrary, showed a decrease in humoral defense in periodontitis with prosthetics. The presence of denture bases in the oral cavity changed the nature of the inflammatory process and led to a decrease in systemic manifestations of inflammation. Perhaps the dentures changed the balance of the microflora, creating conditions for less aggressive bacteria, even if their total number remained high [28]. This change led to a decrease in innate immune defense, despite the fact that local problems arose with the accumulation of food under the dentures and oral hygiene.

The data obtained are important for understanding the impact of dentures on the development and course of periodontitis and can be used to improve methods of treatment and prevention of periodontal diseases in patients with removable dentures.

CONCLUSIONS

1. During the development of the simulated inflammatory process in the periodontal complex, an increase in the phagocytic activity of granulocytes in the blood is observed, which is reflected in the increase in the activity of the B-cell link of adaptive immunity.

2. The response of the innate cellular immune system to prosthetics with acrylic and nylon structures during experimental bacterial-immune inflammation of periodontal tissues in animals is an important pathogenetic factor that affects the nature of the development, course and completion of the inflammatory process.

3. Fixation of prosthetic bases made of different types of plastics under the condition of experimental bacterial-immune periodontitis led to a disruption of an adequate immune response, which in turn was manifested by a decrease in systemic manifestations of the inflammatory process in the blood serum.

4. The use of prosthetic bases of different types under the conditions of bacterial-immune inflammation of the periodontium affects the indicators of cellular adaptive immunity towards a decrease in the dynamics of the inflammatory process in the periodontal complex.

Contributors:

Poliukhovych Yu.I. – writing – initial draft, resources, conceptualization, methodology;

Demkovych A.Ye. – writing – review and editing, data curation

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