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ABSTRACT

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IMMUNOLOGICAL REACTIVITY CHANGES IN PERIODONTITIS OF BACTERIAL-IMMUNOGENIC GENESIS UNDER THE CONDITIONS OF USE OF VARIOUS METAL CROWNS

Introduction. Metal non-removable orthopedic structures that are fixed in the oral cavity are subject to biological destruction, which affects the mechanisms of regulation of both specific and nonspecific tissue resistance. This can cause a decrease in local immunity. The aim of this work was to investigate the disruption of the humoral link of the immune system in experimental periodontitis of bacterial-immune genesis under the condition of using different types of metal crowns.

Methods. In experimental animals, experimental periodontitis was modeled by injecting into the periodontal tissues a suspension containing a mixture of microorganisms (*Staphylococcus aureus* and *Streptococcus hemolyticus*) based on egg white. The study of disorders of the humoral immune system was carried out by determining the content of immunoglobulins of classes A, M and G and the amount of circulating immune complexes in the blood serum on the 30th day of the experiment both without and with stamped and cast crowns fixed in the oral cavity.

Results. The results of the study showed that the development of periodontitis of bacterial-immune genesis is accompanied by the activation of the humoral link of immunity, which is manifested by an increase in the levels of immunoglobulins A, M, G and circulating immune complexes in the blood serum. The use of fixed prosthetics affected the immune response of the body: cast crowns caused a higher level of immunoglobulins compared to stamped crowns, however, prosthetics were generally associated with a decrease in humoral immunity compared to the group of animals that had only an inflammatory process without prosthetics.

Conclusion. The increase in the level of immunoglobulins and circulating immune complexes on the 30th day in animals with periodontitis confirms the activation of the humoral link of immunity. The

use of metal fixed prosthetic structures affects the immunological status of the body, and cast crowns caused more pronounced changes compared to stamped ones.

Key words: non-removable prosthetics, stamped crowns, cast crowns, inflammation, periodontitis, humoral immunity, immunoglobulins, circulating immune complexes.

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ЗМІНИ ІМУНОЛОГІЧНОЇ РЕАКТИВНОСТІ ПРИ ПАРОДОНТИТІ БАКТЕРІАЛЬНО-ІМУННОГО ГЕНЕЗУ ЗА УМОВИ ВИКОРИСТАННЯ РІЗНИХ ТИПІВ МЕТАЛЕВИХ КОРОНОК

Вступ. Металеві незнімні ортопедичних конструкції, які зафіксовані в порожнині рота, піддаються біологічній деструкції, що впливає на механізми регуляції як специфічної, так і неспецифічної резистентності тканин. Це може спричинити зниження місцевого імунітету. Метою даної роботи було дослідити порушення гуморальної ланки імунної системи при експериментальному пародонтиті бактеріально-імунного генезу за умови використання різних типів металевих коронок.

Методи. У піддослідних тварин експериментальний пародонтит моделювали шляхом ін'єкційного введення в тканини пародонта суспензії, що містила суміш мікроорганізмів (*Staphylococcus aureus* і *Streptococcus hemolyticus*) на основі яєчного білка. Дослідження порушень гуморальної ланки імунної системи проводили за допомогою визначення вмісту імуноглобулінів класів А, М і G та кількості циркулюючих імунних комплексів у сироватці крові на 30-ту добу експерименту як без, так і з зафіксованими в ротовій порожнині штампованими та суцільнолитими коронками.

Результати та їх обговорення. Результати дослідження показали, що розвиток пародонтиту бактеріально-імунного генезу супроводжується активацією гуморальної ланки імунітету, що проявляється підвищенням рівнів імуноглобулінів А, М, G та циркулюючих імунних комплексів у сироватці крові. Використання незнімного протезування впливало на імунну відповідь організму: суцільнолиті коронки спричиняли вищий рівень імуноглобулінів порівняно з штампованими коронками, однак протезування загалом асоціювалося зі зниженням показників гуморального імунітету порівняно з групою тварин, які мали лише запальний процес без протезування.

Висновок. Зростання рівня імуноглобулінів та циркулюючих імунних комплексів на 30-ту добу в тварин з пародонтитом, підтверджує активацію гуморальної ланки імунітету. Застосування металевих незнімних протезних конструкцій впливає на імунологічний статус організму, причому суцільнолиті коронки викликали більш виражені зміни порівняно з штампованими.

Ключові слова: незнімне протезування, штамповані коронки, суцільнолиті коронки, запалення, пародонтит, гуморальний імунітет, імуноглобуліни, циркулюючі імунні комплекси.

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ABBREVIATIONS

Ig – immunoglobulin

CICs – circulating immune complexes

INTRODUCTION

Metal elements of fixed orthopedic structures fixed in the oral cavity are subject to biological destruction, which affects the mechanisms of regulation of both specific and nonspecific tissue resistance. This can cause a decrease in local immunity [1]. This is manifested by changes in lysozyme activity, the number of neutrophils in saliva and the level of immunoglobulins, in particular secretory immunoglobulin A [2]. Experimental studies have revealed molecular immunological mechanisms that ensure the recognition of metal ions by T-lymphocytes. This process can cause the development of allergic reactions and increased sensitivity to the material of the fixed prosthetic structure [3].

The humoral component of adaptive immunity plays an important role in the development and course of periodontitis, as it is responsible for the specific immune response that helps to detect and neutralize pathogens that cause inflammation in periodontal tissues [4]. The main elements of the humoral response are antibodies produced by B lymphocytes, as well as various cytokines and other soluble molecules that regulate the activity of immune cells [5]. In the case of periodontitis, changes in the humoral response can significantly affect the course of the disease, contributing to both its progression and the body's defense against infection [6–7].

Therefore, antibodies produced by B-lymphocyte activation may play an important role in the pathogenesis of periodontitis [8]. In particular, specific antibodies against bacteria that are etiological agents of the disease may participate in neutralizing these pathogens, but in conditions of chronic inflammation, their excess or imbalance may have the opposite effect [9]. For example, autoantibodies produced as a result of impaired immune tolerance may attack the body's own tissues, in particular gingival tissues, increasing inflammation and damage to periodontal structures [10].

Despite extensive studies revealing the molecular mechanisms of the influence of dental prostheses on the periodontal complex, and confirming the high sensitizing effect of metal ions of fixed structures on the human body, the issue of further studying the influence of relevant materials on the humoral link of the immune system remains relevant [11].

The aim of this work was to investigate the disruption of the humoral component of the immune system in experimental periodontitis of bacterial-immune genesis under the condition of using different types of metal crowns.

MATERIAL AND METHODS

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 – 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, which were fixed with stamped crowns, n = 8; Group IV – animals with periodontitis on the 30th day of the study, which were fixed with cast crowns, n = 8.

To manufacture fixed structures, impressions were first taken from the central incisors of the lower jaw using silicone impression material “Speedex”. Crowns were manufactured using conventional methods, stamped – by stamping standard sleeves from the company “Medtechnika” (Ukraine) [12], solid-cast – from cobalt-chromium alloy “Argeloy N.P. Supreme” (“ARGEN”, USA) by casting method [13]. The manufactured orthopedic structures were designed in such a way that they did not cover the occlusal surfaces of the teeth and were fixed simultaneously on both central incisors. Composite-reinforced glass ionomer cement “RelyX Luting 2” (3M ESPE, USA) was used to fix stamped and cast crowns.

Experimental bacterial-immune periodontitis in experimental animals was induced by injecting 0.01 ml of a mixture of microorganisms (*Staphylococcus aureus* and *Streptococcus hemolyticus*) at a dose of 4 CFU directly into the periodontal tissue. Components of the cell wall of gram-positive bacteria, such as lipoteichoic acids, peptidoglycan, and lipoproteins, act as triggers of the inflammatory process through toll-like receptors 2, providing pathogen recognition and activation of innate immunity mechanisms. To enhance the immune response, experimental 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 bacterial-immune periodontitis [14]. On the 30th day of the experiment, experimental animals were euthanized by exsanguination under general anesthesia with sodium thiopental. After that, blood serum was collected for analysis of indicators of oxidative processes and peroxidation products.

The principle of the method for determining the content of immunoglobulins of classes A, M and G in blood serum was based on their ability to precipitate from whole blood serum using reagents at different pH values and ionic strength. The protein-buffer complexes that were formed changed the optical density of the medium, which was photometered on a photoelectrocolorimeter with a blue light filter against water [15]. The amount of circulating immune complexes in blood serum was determined by the precipitation method with a polyethylene glycol-6000 solution using photometry on a spectrophotometer SF-46 at a wavelength of 450 nm [16].

All experimental procedures were performed in accordance with the provisions of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and the “General Ethical Principles for Experiments on Animals” (Kyiv, 2001) [17]. 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. 77 dated August 18, 2024).

Data analysis was performed using nonparametric statistical methods in STATISTICA 10.0 software (StatSoft, USA). To process the obtained results, analysis of variation series was used, which included the calculation of the arithmetic mean and its standard error (M and m). To assess the significance of differences between independent quantitative variables with a normal distribution, the Mann-Whitney U-test, based on nonparametric characteristics, was used. All analyses were performed with a critical level of statistical significance (p) of less than 5% ($p < 0.05$).

RESULTS AND DISCUSSION

The humoral component of adaptive immunity includes the regulation of immunoglobulin production, in particular immunoglobulin A (IgA), which is the main antibody in the mucous membranes, in particular in the oral cavity [18]. The results of the conducted studies showed that in animals with periodontitis of bacterial-immune genesis, on the 30th day of the experiment, the level of immunoglobulin A in the blood serum increased by 2.67 times ($p < 0.001$) compared to the intact group (Table 1, Fig. 1). This indicates a significant activation of the humoral response of the body in response to the inflammatory process developing in the periodontal tissues and is a sign of activation of local immunity.

Table – Dynamics of indicators of the humoral component of adaptive immunity in the blood serum of white rats with periodontitis and with the use of crowns ($M \pm m$)

Initial data and study design	Control intact animals	White rats with experimental periodontitis of bacterial-immune genesis		
		Without prosthetics	Stamped crowns	Cast crowns
Duration of the experiment (days)	-	30	30	30
Number of animals	10	8	8	8
Immunoglobulin A (g/l)	1.58 ± 0.06	4.21 ± 0.05 $p_1 < 0.001$	2.93 ± 0.06 $p_1 < 0.001; p_2 < 0.001$	3.41 ± 0.09 $p_1 < 0.001; p_2 < 0.001; p_3 < 0.01$
Immunoglobulin M (g/l)	6.40 ± 0.10	7.95 ± 0.04 $p_1 < 0.001$	6.77 ± 0.08 $p_1 < 0.05; p_2 < 0.001$	7.52 ± 0.07 $p_1 < 0.001; p_2 < 0.01; p_3 < 0.001$
Immunoglobulin G (g/l)	6.57 ± 0.03	8.37 ± 0.07 $p_1 < 0.001$	7.40 ± 0.09 $p_1 < 0.001; p_2 < 0.001$	7.71 ± 0.09 $p_1 < 0.001; p_2 < 0.001; p_3 < 0.05$
CICs (c.u.)	47.30 ± 0.78	91.88 ± 1.01 $p_1 < 0.001$	68.88 ± 1.01 $p_1 < 0.001; p_2 < 0.001$	72.13 ± 0.58 $p_1 < 0.001; p_2 < 0.001; p_3 < 0.05$

Notes: p_1 – significance of differences relative to control animals; p_2 – significance of differences relative to animals with experimental periodontitis on the 30th day without the use of crowns; p_3 – significance of differences relative to animals with experimental periodontitis on the 30th day with stamped crowns

Analyzing the IgA indicators, it is possible to note an increase in its level under the conditions of using stamped crowns against the background of periodontitis in the experiment. The content of immunoglobulin A in the blood was by 1.85 times higher ($p<0.001$) compared to the control group of rats, which indicates the activation of the humoral response as a result of the inflammatory process. However, this indicator turned out to be lower compared to the level of IgA in the group of animals with inflammation of the periodontal complex without prosthetics on the 30th day (by 1.44 times; $p<0.001$).

In animals with cast prosthetics, the content of immunoglobulin A also increased in the blood serum – by 2.16 times ($p<0.001$) compared to intact animals, which is an activation of the immune response in the context of periodontitis and the body's attempts to compensate for the negative impact of infectious agents and reduce damage to gum tissue.

However, it is important that during the fixation of cast crowns and the development of periodontitis, a decrease in IgA levels was observed by 1.24 times ($p<0.001$) compared to the group of animals that had periodontitis of bacterial-immune genesis on the 30th day, but without prosthetics (Fig. 1).

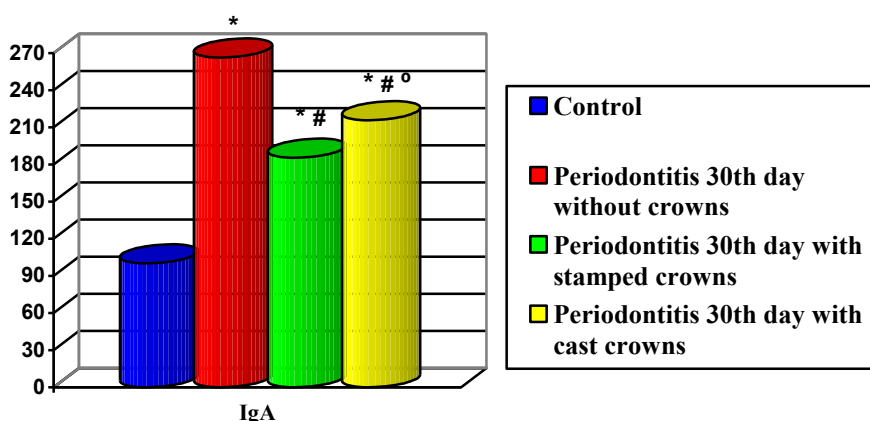


Figure 1 – Changes in the content of immunoglobulin A in blood serum under the conditions of experimental periodontitis development and the use of crowns (in % of control)

Notes: * – significance of differences relative to intact animals ($p<0.001$); # – significance of differences relative to animals with periodontitis on the 30th day without prosthetics ($p<0.001$); ° – significance of differences relative to animals with periodontitis on the 30th day with stamped crowns ($p<0.01$)

Comparing the immunoglobulin A levels in prosthetics with different types of metal crowns, the IgA level was significantly higher in the group of animals that received cast structures compared to the group of animals with stump crowns. In particular, the IgA level was by 1.16 times higher ($p<0.01$) compared to the group with stamped crowns.

During the experimental study, similar changes in the concentration of immunoglobulins of classes M and G were recorded in animals with periodontitis that were not subjected to prosthetics. In particular, the level of IgM increased by 1.24 times ($p<0.001$), and the concentration of IgG – by 1.27 times ($p<0.001$) compared to the intact group of animals, which is evidence of the activation of the humoral immune response in response to bacterial-immune damage to the periodontium, which confirms the important role of these immunoglobulins in the pathogenesis of the inflammatory process (Table 1, Fig. 2, 3).

In animals that underwent fixation of stamped dental crowns, a significant increase in the level of

immunoglobulins of classes M and G was observed on the 30th day of the experiment. In particular, the concentration of IgM increased by 1.06 times ($p<0.05$), and the level of IgG increased by 1.13 times ($p<0.001$) compared to the indicators of the intact group.

The use of stamped steel crowns caused changes in the level of immunoglobulin M in the blood serum compared to the indicators of animals with periodontitis without fixed prosthetics on the 30th day of the experiment. In particular, a 1.17-fold decrease in IgM concentration ($p<0.001$) was found under prosthetic conditions.

In animals with cast crowns on the background of experimental periodontitis of bacterial-immune genesis, an increase in the level of IgM was observed by 1.18 times ($p<0.001$) compared to the indicators of intact rats (Fig. 2), which was less, compared with the results of animals with generalized periodontitis on the 30th day of the experiment without the use of crowns by 1.06 times ($p<0.01$). In comparison with the group with stamped fixed structures, the concentration of this

immunoglobulin increased by 1.11 times ($p<0.001$). Therefore, fixed prosthetics contributes to the modification of the immune response due to the influence of prosthetic structures on systemic mechanisms of immunoregulation.

After determining the level of immunoglobulin G concentration in the blood serum of animals with cast metal crowns and periodontitis, it was found that on the 30th day of the study this indicator was higher compared

to the intact group of animals, exceeding the level by 1.17 times ($p<0.001$). The use of cast crowns caused changes in the concentration of IgG compared to the indicators of animals that did not undergo prosthetics on the 30th day of the experiment. The level of IgG in the group with crowns was lower by 1.09 times ($p<0.001$), which may indicate certain features of the influence of prosthetic structures on the humoral response of the body.

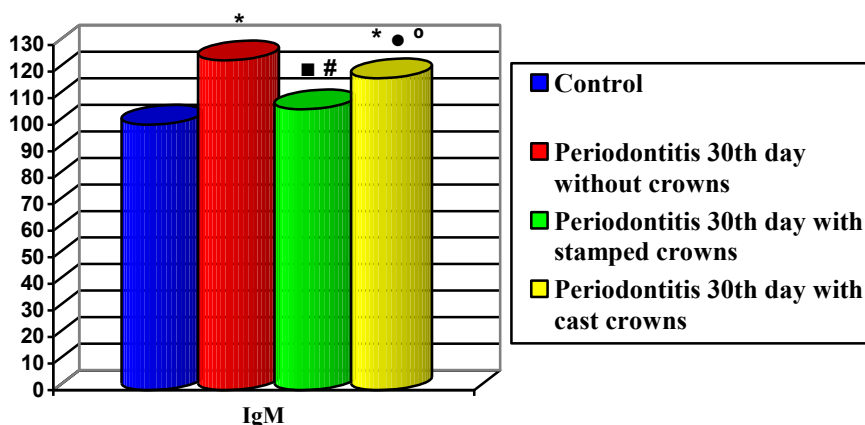


Figure 2 – Changes in the content of immunoglobulin M in blood serum under conditions of experimental periodontitis development and use of crowns (in % of control)

Notes. * – significance of differences relative to intact animals ($p<0.001$); ■ – significance of differences relative to intact animals ($p<0.05$); # – significance of differences relative to animals with periodontitis on the 30th day without prosthetics ($p<0.001$); • – significance of differences relative to animals with periodontitis on the 30th day without prosthetics ($p<0.01$); ° – significance of differences relative to animals with periodontitis on the 30th day with stamped crowns ($p<0.001$)

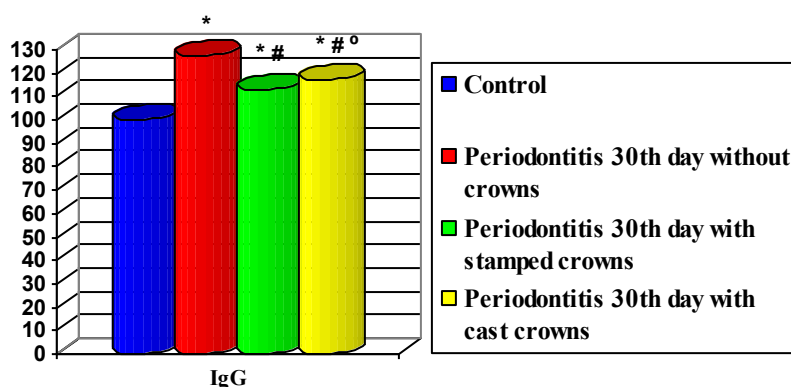


Figure 3 – Changes in the content of immunoglobulin G in blood serum under conditions of experimental periodontitis development and use of crowns (in % of control)

Note: * – significance of differences relative to intact animals ($p<0.001$); # – significance of differences relative to animals with periodontitis on the 30th day without prosthetics ($p<0.001$); ° – significance of differences relative to animals with periodontitis on the 30th day with stamped crowns ($p<0.05$)

A study of IgG concentration in periodontal tissue inflammation under conditions of cast crowns compared to the group with stamped structures showed that the IgG level changed similarly to IgM, i.e. an increase was

observed, but with less pronounced intensity, by 1.04 times ($p<0.05$) (Fig. 3).

Circulating immune complexes (CICs) are important markers of the immune response, formed by the binding

of antigens to specific antibodies [8]. They play a significant role in the development of inflammatory processes in the periodontium, contributing to the activation of the complement system, the recruitment of phagocytes and the release of pro-inflammatory mediators [19].

Analysis of the dynamics of the level of circulating immune complexes in the blood serum of experimental animals with bacterial-immune periodontitis demonstrated their significant increase on the 30th day of the study. In particular, it was found that the concentration of CICs exceeded the corresponding indicators in control animals by 1.94 times ($p<0.001$). Higher values of this indicator were also recorded compared to groups of rats with periodontitis that underwent prosthetics with removable structures. In animals with fixed stamped crowns, the level of CIC was increased by 1.33 times ($p<0.001$), and when using

solid-cast crowns – by 1.27 times ($p<0.001$), which indicates the influence of the type of prosthetics on the intensity of the immune response (Table 1).

The results of the study demonstrate a significant increase in the level of circulating immune complexes in the blood serum of animals that underwent fixation of metal crowns, compared with the intact group. In particular, the use of stamped steel crowns contributed to an increase in this indicator by 1.46 times ($p<0.001$), and the use of cast fixed structures – by 1.53 times ($p<0.001$). Analyzing the CICs indicators in animals with fixed prosthetics, it was found that their level under the condition of fixation of cast crowns exceeded similar values in animals with stamped crowns by 1.05 times ($p<0.05$), which indicates differences in the influence of different types of prostheses on the immune response of the body (Fig. 4).

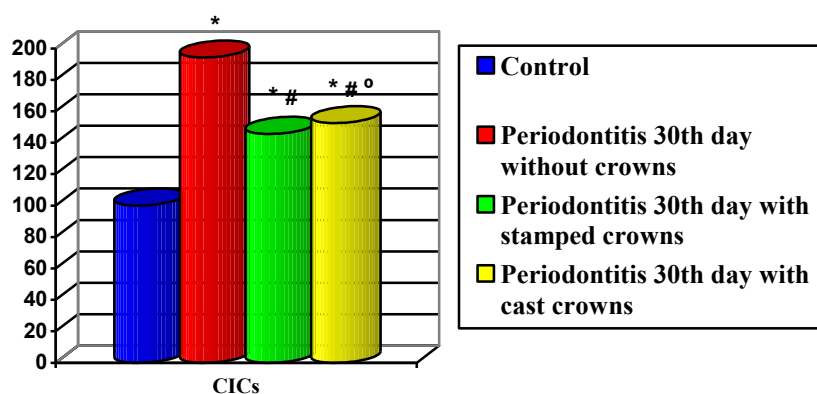


Figure 4 – Changes in the content of circulating immune complexes in blood serum under conditions of experimental periodontitis development and use of crowns (in % of control)

Notes: * – significance of differences relative to intact animals ($p<0.001$); # – significance of differences relative to animals with periodontitis on the 30th day without prosthetics ($p<0.001$); ° – significance of differences relative to animals with periodontitis on the 30th day with stamped crowns ($p<0.05$)

IgA protects the mucous membranes from microbial invasion, forming a barrier on the surface of the gingival mucosa. Disruption of its production can contribute to increased susceptibility to infections and promote the development of inflammatory processes in periodontal tissues [20]. A decrease in IgA levels was observed during the fixation of cast crowns and the development of periodontitis, which may indicate a decrease in the intensity of the inflammatory reaction in the tissues of the periodontal complex after the fixation of such crowns. A decrease in IgA levels in this case may be the result of a certain regulatory effect, in which the body tries to limit excessive activation of the immune response, which can lead to a decrease in the inflammatory process and an improvement in the condition of the tissues [21].

Comparing the immunoglobulin A levels in prosthetics with different types of metal crowns, the IgA level was higher in the group with cast crowns. Perhaps this is a sign that the use of cast structures activated a more intense humoral immune response, which may be associated with different mechanisms of interaction of the crown material with periodontal tissues. Such differences in IgA levels may be due to the physicochemical properties of crown materials, as well as their influence on local immune processes in periodontal tissues, which requires further studies to clarify these mechanisms.

Immunoglobulin M is the first antibody synthesized in response to antigenic stimulation, activates the complement system and promotes the elimination of pathogens by agglutination and opsonization [22].

Increased IgM levels in periodontitis may be a marker of a systemic inflammatory response and indicate increased antigenic stimulation due to excessive accumulation of bacterial toxins in the periodontal complex [23]. The use of stamped crowns probably has a less pronounced effect on IgM levels, which is probably due to the better biomechanical characteristics of these structures and reduced irritation of periodontal tissues compared with cast crowns.

Immunoglobulins of class G are the main components of humoral immunity, which play an important role in protecting the body from bacterial and viral infections. Changes in their levels in the blood serum can indicate the activity of the inflammatory process, the intensity of the immune response and the degree of antigenic stimulation [24].

The use of different types of metal crowns affected the immunological status of the body, in particular the level of immunoglobulin G, which indicates an enhanced immune response. Fixation of stamped structures led to a moderate decrease in IgG levels compared to the group without prosthetics, which is probably associated with some stabilization of the inflammatory process in the periodontium. However, IgG levels remained elevated compared to intact animals, which may indicate the preservation of immune activation against the background of periodontitis [25].

Increased serum CICs levels in periodontitis may indicate chronic antigenic stimulation due to bacterial toxins and tissue autoantigens [26]. Conditions of fixed prosthetics, in particular the use of metal crowns, may also affect the dynamics of CIC, causing changes in local and systemic immune responses. Depending on the type of construction, both an increase and a decrease in CICs levels are possible, which may be associated with the adaptive mechanisms of the body and the characteristics of the inflammatory process [27].

Increased concentration of circulating immune complexes in blood serum is an important marker of immune inflammation, reflecting excessive activation of

the humoral component of the immune system. High levels of CICs can have negative consequences for the periodontium, which can lead to the development of autoimmune and chronic inflammatory processes, the deposition of these complexes in the walls of blood vessels and tissues can cause complement-dependent damage, which disrupts microcirculation and tissue trophism [28]. The interaction of immune complexes with Fc-receptors of phagocytes stimulates the release of pro-inflammatory mediators, which contributes to the chronicity of the inflammatory process.

Therefore, an imbalance in the production of immunoglobulins, in particular IgM and IgG, may play a key role in the pathogenesis of periodontitis. A decrease in the level of protective antibodies may cause insufficient neutralization of bacterial toxins, which contributes to the spread of infection. At the same time, excessive activation of humoral immunity and the accumulation of circulating immune complexes may cause chronic inflammation, damage to blood vessels and periodontal tissues [29].

CONCLUSIONS

The increase in the level of immunoglobulins and circulating immune complexes on the 30th day in animals with periodontitis confirms the activation of the humoral link of immunity. The use of metal fixed crowns affects the immunological status of the body, modulating the inflammatory response. Comparison between the types of crowns showed a difference in the effect on the humoral response, with cast crowns causing a more pronounced activation of IgA compared to stamped crowns, although this indicator was somewhat lower than in animals without prosthetics. Higher levels of circulating immune complexes with metal crowns may indicate an increased inflammatory reaction in the periodontium. Stamped crowns demonstrate a lower effect on the level of immune complexes, which may be a consequence of their better biomechanical characteristics and reduced local irritation of periodontal tissues.

PROSPECTS FOR FUTURE RESEARCH

Future studies should be aimed at clarifying the effects of different types of fixed metal crowns on lipoperoxidation indices, the body's antioxidant defense system, and cytokine status.

AUTHOR CONTRIBUTIONS

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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A – Work concept and design,

B – Data collection and analysis,

C – Responsibility for statistical analysis,
 D – Writing the article,
 E – Critical review,
 F – Final approval of the article.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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