MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
SUMY STATE UNIVERSITY
ACADEMIC AND RESEARCH MEDICAL INSTITUTE

Eastern Ukrainian Medical Journal

116, Kharkivska st., Sumy 40007, Ukraine eumj.med.sumdu.edu.ua
e-mail: eumj@med.sumdu.edu.ua ISSN: 2663-5909 (print)/2664-4231 (online)

How to cite: Marfiian OV, Demkovych AYe, Bandrivska OO, Bedenyuk OA. CHANGES IN THE PHAGOCYTIC ACTIVITY OF LEUKOCYTES AND THE LEVEL OF C-REACTIVE PROTEIN AS PATHOGENIC FACTORS OF THE INFLAMMATORY PROCESS IN PERIODONTIUM SECONDARY TO THE USE OF METAL CROWNS. *East Ukr Med J*. 2026;14(2):500-509. DOI: https://doi.org/10.21272/eumj.2026;14(2);500-509

ABSTRACT

Oleh V. Marfiian
https://orcid.org/0009-0007-7782-8278
Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Andrii Ye. Demkovych
https://orcid.org/0000-0001-9823-4283
Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Orysia O. Bandrivska
https://orcid.org/0000-0002-3274-1781
Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

Oleksandr A. Bedenyuk
https://orcid.org/0000-0002-9644-1809
Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

CHANGES IN THE PHAGOCYTIC ACTIVITY OF LEUKOCYTES AND THE LEVEL OF C-REACTIVE PROTEIN AS PATHOGENIC FACTORS OF THE INFLAMMATORY PROCESS IN PERIODONTIUM SECONDARY TO THE USE OF METAL CROWNS

The aim of the study: The aim of this work was to investigate changes in the phagocytic activity of leukocytes and the level of C-reactive protein in experimental periodontitis of bacterial-immune genesis under the condition of using different types of metal crowns.

Materials and methods. The study was conducted on clinically healthy male white rats weighing 150–200 g, which were kept in a vivarium in compliance with sanitary and hygienic standards and requirements of good laboratory practice (GLP). The experimental animals were divided into four groups: Group I – intact animals (n = 10); Group II – animals with periodontitis (30th day of the experiment, n = 8); Group III – animals with periodontitis, which were installed with stamped crowns for periodontitis (30th day of the experiment, n = 8); Group IV – animals with periodontitis, which were installed with cast crowns for periodontitis (30th day of the experiment, n = 8).

Bacterial-immune periodontitis was modeled by injecting a mixture of *Staphylococcus aureus* and *Streptococcus hemolyticus* microorganisms suspended in egg white into periodontal tissues. To produce permanent structures, impressions were taken from the central incisors of the lower jaw of experimental animals using Speedex silicone impression material and crowns were fabricated using standard techniques.

To study the phagocytes activity of leukocytes, it was determined by counting the number of neutrophils in smears and expressed as phagocytic number. The level of C-reactive protein (CRP) in serum was determined by enzyme-linked immunosorbent assay. Data processing was carried out using non-parametric statistical methods in STATISTICA 10.0 software (StatSoft, USA).

Results. Analysis of the dynamics of the phagocytic number of blood granulocytes in animals with periodontitis under the conditions of using cast crowns showed that on the 30th day of the experiment this indicator was significantly higher – by 1,48 times, p<0,001, compared to the intact group. It was also found that the phagocytic number slightly exceeded the value in animals with an inflammatory process without prosthetics by 1.07 times, p<0.05. At the same time, this indicator was lower by 1,07 times, p<0,01, compared to similar data obtained in rats with stamped crowns.

The content of C-reactive protein, on the 30th day of the experiment, in the blood serum of experimental animals with periodontitis of bacterial-immune genesis, exceeded by 1,40 times, p<0.001, the indicators that were in the animals of the intact group. In experimental animals after prosthetics with stamped crowns, the content of CRP was significantly increased compared to the data of the intact and the groups with periodontitis of bacterial-immune genesis – by 1.83 times and by 1.30 times, respectively, p<0,001.

A similar trend was observed in the blood serum of animals with cast structures: the CRP content significantly increased – by 2,14 times, p<0,001, compared to intact animals; by 1,53 times, p<0.001, compared to the group with simulated periodontitis on the 30th day without crowns and by 1,17 times, p<0,01, in animals with fixed stamped structures.

Conclusion. Thus, as a result of the study, it was found that on the 30th day of periodontitis of bacterial-immune genesis, activation of the immune response was observed with a significant increase in phagocytic activity in the experimental groups using fixed dentures, which indicates an increase in the inflammatory reaction, probably due to mechanical irritation or microbial colonization around the dentures. The course of experimental periodontitis was also accompanied by an increase in the level of C-reactive protein in the blood serum, which indicates the presence of an inflammatory process, and the indicators obtained under the conditions of using fixed structures indicate a more adverse effect of integral prosthetics on this link in the development of the inflammatory process in the periodontal complex.

Keywords: unremovable prosthetics, stamped crowns, cast crowns, periodontium, inflammation, periodontitis, phagocytic activity, C-reactive protein.

Corresponding author: *Orysia Bandrivska, Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine, e-mail: bandrivska@tdmu.edu.ua*

РЕЗЮМЕ

Олег Марфіян
https://orcid.org/0009-0007-7782-8278
Кафедра ортопедичної стоматології, Тернопільський національний медичний університет імені І.Я. Горбачевського Міністерства охорони здоров'я України, Тернопіль, Україна

Андрій Демкович
https://orcid.org/0000-0001-9823-4283

ЗМІНИ ФАГОЦИТАРНОЇ АКТИВНОСТІ ЛЕЙКОЦИТІВ ТА РІВНЯ С-РЕАКТИВНОГО ПРОТЕЇНУ ЯК ПАТОГЕНЕТИЧНІ ЧИННИКИ ЗАПАЛЬНОГО ПРОЦЕСУ В ПАРОДОНТІ НА ТЛІ ВИКОРИСТАННЯ МЕТАЛЕВИХ КОРОНОК

Мета дослідження: дослідити зміни фагоцитарної активності лейкоцитів та рівня С-реактивного протеїну при експериментальному пародонтиті бактеріально-імунного генезу за умови використання різних типів металевих коронок.

Матеріали і методи. Дослідження проводили на клінічно здорових самцях білих щурів масою 150–200 г, яких утримували у віварії з дотриманням санітарно-гігієнічних норм та вимог належної лабораторної практики (GLP). Піддослідних тварин

Кафедра ортопедичної стоматології,
Тернопільський національний
медичний університет імені
І.Я. Горбачевського Міністерства
охорони здоров'я України,
Тернопіль, Україна

Орися Бандрівська
https://orcid.org/0000-0002-3274-1781
Кафедра ортопедичної стоматології,
Тернопільський національний
медичний університет імені
І.Я. Горбачевського Міністерства
охорони здоров'я України,
Тернопіль, Україна

Олександр Беденюк
https://orcid.org/0000-0002-9644-1809
Кафедра ортопедичної стоматології,
Тернопільський національний
медичний університет імені
І.Я. Горбачевського Міністерства
охорони здоров'я України,
Тернопіль, Україна

розподілили на чотири групи: I група – контрольна (n = 10); II група – тварини з пародонтитом (30-та доба експерименту, n = 8); III група – тварини з пародонтитом, яким встановлено штамповані коронки пародонтитом (30-та доба експерименту, n = 8); IV група – тварини з пародонтитом, яким встановлено суцільнолиті коронки пародонтитом (30-та доба експерименту, n = 8).

Бактеріально-імунний пародонтит моделювали шляхом ін'єкційного введення в тканини пародонту суміші мікроорганізмів *Staphylococcus aureus* та *Streptococcus hemolyticus*, суспендованих у яєчному білку. Для виготовлення незнімних конструкцій отримували відбитки з центральних різців нижньої щелепи піддослідних тварин, використовуючи силіконовий відбитковий матеріал «Speedex» та виготовляли коронки за стандартними методиками.

Для дослідження фагоцитарної активності лейкоцитів визначали шляхом підрахунку кількості нейтрофілів у мазках та виражали у вигляді фагоцитарного числа. Рівень С-реактивного протеїну (CRP) у сироватці крові визначали методом імуноферментного аналізу. Обробку даних здійснювали з використанням непараметричних статистичних методів у програмному забезпеченні STATISTICA 10.0 (StatSoft, США).

Результати та їх обговорення. Аналіз динаміки фагоцитарного числа гранулоцитів крові у тварин з пародонтитом за умов використання суцільнолитих коронок показав, що на 30-ту добу експерименту цей показник був значно вищим – у 1,48 раза, $p<0,001$, порівняно з контрольною групою. Також встановлено, що фагоцитарне число дещо перевищувало значення у тварин із запальним процесом без протезування у 1,07 раза, $p<0,05$. Водночас цей показник був нижчим у 1,07 раза, $p<0,01$, порівняно з аналогічними даними, отриманими у щурів зі штампованими коронками.

Вміст С-реактивного протеїну, на 30-ту добу досліду, в сироватці крові експериментальних тварин з пародонтитом бактеріально-імунного генезу, перевищував в 1,40 раза, $p<0,001$, показники, які були у тварин контрольної групи. У піддослідних тварин після проведеного протезування штампованими коронками вміст CRP був значно підвищеним порівняно із даними інтактної та групи з пародонтитом бактеріально-імунного генезу – 1,83 раза та 1,3 раза, відповідно, $p<0,001$.

У сироватці крові тварин з цільнолитими конструкціями спостерігалась аналогічна тенденція: вміст CRP істотно підвищився – у 2,14 раза $p<0,001$, порівняно з інтактними тваринами; в 1,53 раза, $p<0,001$, порівняно з групою із змодельованим пародонтитом на 30-ту добу без коронок та в 1,17 раза, $p<0,01$, у тварин із зафіксованими штампованими конструкціями.

Висновки. Отже, в результаті проведеного дослідження встановлено, що на 30-ту пародонтиту бактеріально-імунного генезу спостерігалася активація імунної відповіді із значним підвищенням фагоцитарної активності в експериментальних групах з використанням незнімних протезів, що вказує на посилення запальної реакції, ймовірно, через механічне подразнення або мікробну колонізацію навколо протезів. Перебіг експериментального пародонтиту супроводжувався, також, підвищенням рівня С-реактивного протеїну в сироватці крові, що

свідчить про наявність запального процесу, а показники отримані за умов використання незнімних конструкцій свідчать про більш несприятливий вплив цільнолитого протезування на дану ланку розвитку запального процесу в пародонтальному комплексі.

Ключові слова: незнімне протезування, штамповані коронки, суцільнолиті коронки, пародонт, запалення, пародонтит, фагоцитарна активність, С-реактивний протеїн.

***Автор, відповідальний за листування**: Орися Бандрівська, кафедра ортопедичної стоматології, Тернопільський національний медичний університет імені І. Я Горбачевського Міністерства охорони здоров'я України, м. Тернопіль, Україна, e-mail: bandrivska@tdmu.edu.ua*

INTRODUCTION

Inflammatory processes in periodontal tissues are accompanied by activation of the immune system, which can lead to an imbalance between protective and damaging mechanisms [1, 2]. One of the key factors in this process is changes in the phagocytic activity of leukocytes, which affect the efficiency of eliminating microorganisms and immune complexes [3].

The development and course of periodontitis is manifested by changes in the phagocytic activity of leukocytes (neutrophils, macrophages), which are the main cells of the immune response in the oral cavity [4, 5]. During the development of the inflammatory process in the periodontium, the number of neutrophils in the blood and gingival tissues increases, as the body tries to fight microorganisms, phagocytosis is activated – leukocytes absorb pathogens (bacteria, tissue debris), the release of reactive oxygen species increases, which help destroy pathogens, but can damage periodontal tissues [6]. However, in the case of chronic inflammation, phagocytic activity becomes hypoactive. This means that neutrophils and other phagocytes lose the ability to effectively cleanse tissues of bacteria and cellular detritus, which contributes to further progression of the disease [7].

On the surface of many bacteria, C-reactive protein forms compounds with phosphatidylcholine molecules, which are strong opsonins, i.e. antibodies and complement factors that enhance macrophage phagocytosis and also stimulate the process of digestion of microorganisms. C-reactive protein is a very sensitive indicator in the blood, which is one of the first to respond to tissue damage [8, 9].

However, despite the available data on the involvement of phagocytosis and C-reactive protein in the pathogenesis of periodontitis, the mechanisms of the immune response when using various types of fixed orthopedic structures, in particular metal crowns, which can act as an additional factor of irritation and stimulation of inflammation, remain poorly understood. This is what led to the need for a study aimed at clarifying changes in the phagocytic activity of leukocytes and the level of C-reactive protein in conditions of periodontitis modeling and the use of stamped and cast crowns.

The aim of the study: The aim of this work was to investigate changes in the phagocytic activity of leukocytes and the level of C-reactive protein in experimental periodontitis of bacterial-immune genesis under the condition of using different types of metal crowns.

MATERIAL AND METHODS

The study was conducted on clinically healthy male white rats weighing 150–200 g, which were kept in a vivarium in compliance with sanitary and hygienic standards and requirements of good laboratory practice (GLP). The experimental animals were selected by random sampling and divided into four groups: Group I – intact animals (n = 10); Group II – animals with periodontitis (30^{th} day of the experiment, n = 8); Group III – animals with periodontitis, which were installed stamped crowns with periodontitis (30^{th} day of the experiment, n = 8); Group IV – animals with periodontitis, which were installed cast crowns with periodontitis (30^{th} day of the experiment, n = 8).

To manufacture fixed structures, impressions were first obtained from the central incisors of the lower jaw using the silicone impression material "Speedex". Crowns were manufactured using standard techniques: stamped – by stamping using standard sleeves manufactured by the company "Medtechnika" (Ukraine) [10], and cast – by casting from the cobalt-chromium alloy "Argeloy N.P. Supreme" ("ARGEN", USA) [11]. Orthopedic structures were designed so that they did not cover the occlusal surfaces of the teeth and were simultaneously fixed 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, in particular lipoteichoic acids, peptidoglycan, and lipoproteins, served as triggers of the inflammatory process through toll-like receptors 2, which contributed to the recognition of pathogens and the launch of innate immunity mechanisms. To enhance the immune response, experimental rats were additionally 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 the pathological process [12]. On the 30th day of the experiment, the animals were euthanized by exsanguination under general anesthesia with sodium thiopental. After that, blood serum was collected to assess the C-reactive protein levels.

To study the phagocytic activity of leukocytes, blood was obtained from mechanically damaged vessels in the area of inflammation or, in the case of intact animals, from periodontal tissues near the lower central incisors using a Pasteur pipette, with the addition of 2% sodium citrate solution. Microbial cells were used as objects of phagocytosis. Cultures of *Staphylococcus aureus* and *Streptococcus hemolytic*, previously diluted in isotonic sodium chloride solution, were added to the same test tube. The ratio of blood to sodium citrate and cultures was 2:1:1. The contents of the test tube were mixed and placed in a thermostat at a temperature of 37 °C for 30 min. After that, smears were prepared and stained according to the Ziehl-Neelsen method. The number of neutrophils among 100 analyzed cells was counted in smears. The results were expressed as the phagocytic number, which characterizes the average number of microorganisms absorbed by one neutrophil (conditional units, conventional units), and the phagocytic index, which determines the percentage of phagocytes among the total number of neutrophils [13].

The level of C-reactive protein (CRP) in blood serum was determined by enzyme-linked immunosorbent assay according to the instructions of Monobind Inc. (USA) using the High Sensitivity CRP (hs-CRP) test system.

All experimental procedures were performed in accordance with the provisions of the “European Convention for the Protection of Vertebrate Animals [14] used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and the “General Ethical Principles for Experiments on Animals” (Kyiv, 2001). The study was approved by the Bioethics Commission of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No. 77 dated August 18, 2024).

Data processing was carried out using nonparametric statistical methods in the STATISTICA 10.0 software (StatSoft, USA). Analysis of variation series included the calculation of the arithmetic mean (M) and its standard error (m). To assess the significance of differences between independent quantitative variables that had a normal distribution, the Mann-Whitney U-test, which is based on nonparametric characteristics, was used. All statistical analyses were performed at a critical significance level of $p<0.05$.

RESULTS AND DISCUSSION

Analysis of the phagocytic index revealed that on the 30th day of the inflammatory process in the periodontal complex, an increase in phagocytic activity was observed by 1.06 times ($p<0.01$) compared to the corresponding indicator in the group without pathology, which is evidence of the activation of the immune response.

During the study of the phagocytic index on the 30th day of periodontitis development under the conditions of fixation of stamped crowns, its increase was found by 1.16 times ($p<0.001$) compared to the indicators of the intact group of animals. Also, the phagocytic index was higher by 1.10 times ($p<0.001$) compared to the group of rats that had periodontitis without the use of prosthetics (Table 1). This indicates that the presence of stamped crowns can enhance the inflammatory response and stimulate phagocytic activity, probably due to additional mechanical irritation or microbial colonization around the prostheses.

Comparative analysis of innate immunity indicators on the 30th day of experimental periodontitis with the use of cast fixed structures revealed an increase in the phagocytic index of granulocytes in peripheral blood by 1,09 times ($p<0,001$) compared to the intact group. In addition, this indicator was slightly higher (by 1,03 times, $p<0,05$) compared to the values on the 30th day of the inflammatory process without prosthetics, but lower by 1.07 times ($p<0,01$) than similar indicators in animals with stamped crowns (Fig. 1). The results obtained indicate that cast fixed structures cause moderate activation of phagocytic activity, but this effect is less pronounced compared to stamped crowns. Such a difference may be due to differences in the physical and mechanical characteristics of the materials, the level of their biocompatibility [15]. In particular, stamped crowns may cause greater mechanical stress or have a higher susceptibility to microbial colonization, which induces a more pronounced immune response [16].

Analyzing the phagocytic count as an indicator of granulocyte activity in the blood of experimental animals with bacterial-immune periodontitis, it was found that on the 30th day of the experiment this indicator was significantly higher (by 1,39 times; $p<0,001$) compared to the intact group (Table 1, Fig. 2).

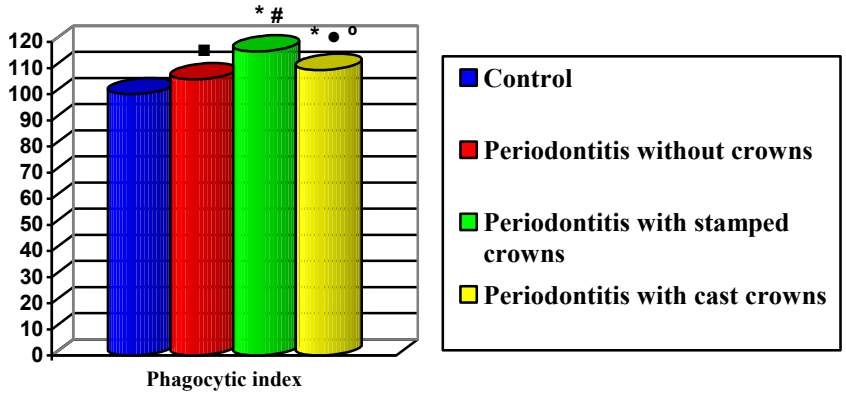

Figure 1 – Changes in the phagocytic index under conditions of experimental periodontitis development and the use of crowns (in % of control)

*Note. * – significance of differences relative to intact animals (p<0.001); ■ – significance of differences relative to intact animals (p<0.01); # – 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<0.05); ° – significance of differences relative to animals with periodontitis on the 30th day with stamped crowns (p<0.01)*

Table 1 – Indicators of phagocytic activity of blood granulocytes and C-reactive protein in white rats with experimental periodontitis and under the condition of using crowns (M±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
Phagocytic index (%)	80.80 ± 1.00	85.38 ± 0.65 p_1<0.01	94.00 ± 0.80 p_1<0.001; p_2<0.001	88.25 ± 0.62 p_1<0.001; p_2<0.05; p_3<0.01
Phagocytic number (c.u.)	6.10 ± 0.21	8.46 ± 0.18 p_1<0.001	9.68 ± 0.12 p_1<0.001; p_2<0.01	9.04 ± 0.14 p_1<0.001; p_2<0.05; p_3<0.01
C-reactive protein (mg/l)	0.35 ± 0.01	0.49 ± 0.01 p_1<0.001	0.64 ± 0.01 p_1<0.001; p_2<0.001	0,75 ± 0,02 p_1<0.001; p_2<0.001; p_3<0.01

Note: p_1 – differences relative to intact animals; p_2 – 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

The increase in the phagocytic count may be due to an increase in the number of activated neutrophils and macrophages, which are involved in protection against pathogens and removal of damaged cells [17].

On the 30th day of the experiment, the phagocytic number in animals with periodontitis using stamped crowns increased by 1.59 times (p<0,001) compared to the intact group. In addition, this indicator was significantly higher by 1.14 times (p<0,01) compared to the group of animals in which inflammation developed without the use of prosthetics.

Analysis of the dynamics of the phagocytic number of blood granulocytes in animals with periodontitis using cast crowns showed that on the 30th day of the experiment this indicator was significantly higher (by 1.48 times; p<0,001) compared to the intact group. It was also found that the phagocytic number slightly exceeded the value in animals with an inflammatory process without prosthetics (by 1,07 times; p<0,05). At the same time, this indicator was by 1.07 times lower (p<0,01) compared to similar data obtained in rats with stamped steel crowns. The results indicate that cast structures cause a moderate effect on the phagocytic number of peripheral blood granulocytes, compared to stamped crowns. At the same time, they still stimulate the immune response more strongly than in the absence of prosthetics, which may be associated with the adaptive reaction of the immune system to foreign material in the oral cavity [18, 19].

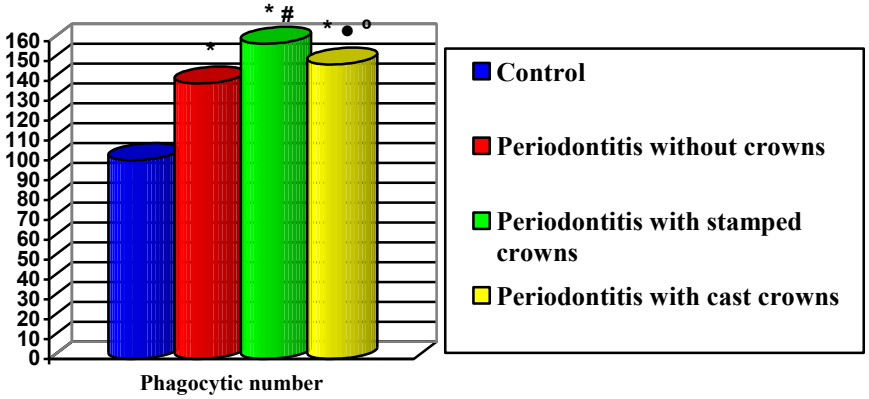

Figure 2 – Changes in phagocytic number under conditions of experimental periodontitis development and crown use (in % of control)

*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<0.01); ● – significance of differences relative to animals with periodontitis on the 30th day without prosthetics (p<0.05); ° – significance of differences relative to animals with periodontitis on the 30th day with stamped crowns (p<0.01)*

With prolonged use of fixed prosthetic structures, in particular stamped and cast crowns, against the background of periodontitis, a decrease in phagocytic activity may be observed, which is a consequence of the depletion of the immune system [20]. In particular, the ability of neutrophils to phagocytosis will decrease, which disrupts the main mechanism of protection against pathogenic microorganisms. An important factor is also the violation of chemotaxis – the process of directed movement of leukocytes to the site of inflammation, which becomes less effective due to changes in the microenvironment and the chronic nature of the inflammatory process [21, 22]. In addition, bacteria that colonize periodontal pockets and their layers on crowns are able to form a biofilm, which provides them with additional protection from phagocytes, reducing their accessibility to immune cells [23]. This protective barrier significantly complicates the elimination of pathogens, contributing to the chronicity of the inflammatory process.

Thus, the imbalance of phagocytic activity is a key pathogenetic factor in the development of chronic periodontitis, since it causes both excessive tissue destruction in the early stages and ineffective fight against infection in the chronic course of the disease.

Regarding the change in the content of C-reactive protein in the blood serum of experimental animals with periodontitis of bacterial-immune genesis, it should be noted that its content on the 30th day of the experiment significantly exceeded (by 1,40 times; p<0,001) the indicators that were in the animals of the intact group.

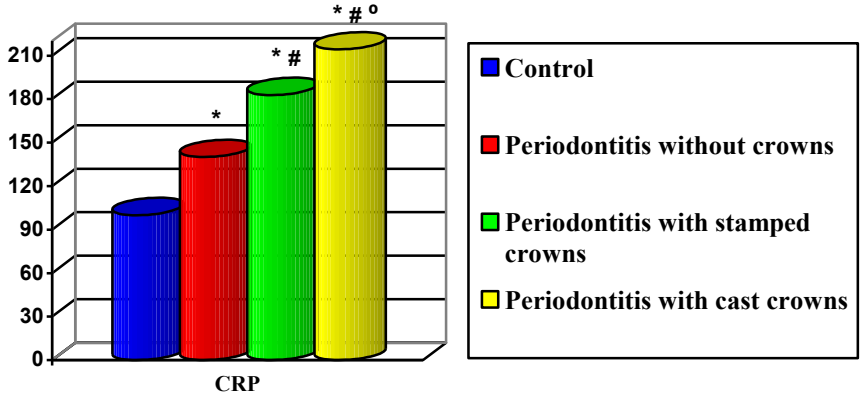

Figure 3 – Changes in the content of C-reactive protein in the blood serum of white rats under the conditions of experimental periodontitis and the use of crowns (in % of control)

*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<0.01)*

The studied content of C-reactive protein after prosthetics with stamped crowns was significantly increased compared to the data of the intact group (by 1,83 times; p<0,001). The results obtained show that it was also higher compared to the amount of protein in the blood serum of rats with periodontitis of bacterial-immune genesis without fixation of fixed stamped structures, namely by 1,31 times (p<0,001) (Table 1, Fig. 3).

In the blood serum of animals with periodontitis in combination with fixed cast prosthetics, the level of C-reactive protein also significantly increased, which was by 2.14 times higher (p<0,001) compared to intact animals and by 1,53 times (p<0,001) compared to the group with periodontitis on the 30th day without crowns.

The use of cast constructs led to an increase in the content of C-reactive protein in the blood serum of animals with experimental periodontitis, compared with rats with the indicated pathology, but with fixed stamped constructs (by 1,17 times; p<0,01). Detection or increase in the level of C-reactive protein in the blood serum indicates the presence of an inflammatory process, tissue alteration, as well as invasion by microorganisms, parasites or fungi [24].

CONCLUSIONS

Thus, as a result of the study, it was found that on the 30th day of periodontitis of bacterial-immune genesis, activation of the immune response was observed with a significant increase in phagocytic activity in the experimental groups using fixed dentures, which indicates an increase in the inflammatory reaction, probably due to mechanical irritation or microbial colonization around the dentures. The course of experimental periodontitis was also accompanied by an increase in the level of C-reactive protein in the blood serum, which indicates the presence of an inflammatory process, and the indicators obtained under the conditions of using fixed structures indicate a more adverse effect of integral prosthetics on this link in the development of the inflammatory process in the periodontal complex.

PROSPECTS FOR FUTURE RESEARCH

Further research should be aimed at developing biocompatible materials for fixed prosthetics to reduce the inflammatory response in periodontal tissues.

ETHICAL CONSIDERATIONS

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). The study was approved by the Bioethics Commission of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No. 77 dated August 18, 2024).

AUTHOR CONTRIBUTIONS

Oleh V. Marfiian [A, B, D]
Andrii Ye. Demkovych [E, F]
Orysia O. Bandrivska [E]
Oleksandr A. Bedenyuk [C]

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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.

FUNDING

None.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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INFORMATION ABOUT THE AUTHORS

Oleh V. Marfiian – post graduate student at Orthopedic Dentistry Department of I. Horbachevsky Ternopil National Medical University, Oleny Telihy, 7, Ternopil, Ukraine.
https://orcid.org/0009-0007-7782-8278
marfiian_o@gmail.com

Andrii Ye. Demkovych – Doctor of Medical Sciences, Professor of Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Oleny Telihy, 7, Ternopil, Ukraine.
https://orcid.org/0000-0001-9823-4283
demkovushae@tdmu.edu.ua

Orysia O. Bandrivska – Candidate of Medical Sciences, Associate Professor of Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Oleny Telihy, 7, Ternopil, Ukraine.
https://orcid.org/0000-0002-3274-1781
bandrivska@tdmu.edu.ua
+380679465584

Oleksandr A. Bedenyuk – Candidate of Medical Sciences, Associate Professor of Orthopedic Dentistry Department I. Horbachevsky Ternopil National Medical University, Oleny Telihy, 7, Ternopil, Ukraine.
https://orcid.org/0000-0002-9644-1809
bedenyukoa@tdmu.edu.ua

Received: 20.03.2025

Accepted for publication: 24.05.2025

Published: 23.06.2026