UDC 616.311.2/.314.17-002-022.7:612.017.2:612.017.1:616.314-77

Type of fixed prosthetics affects cytokine status in experimental bacterial-immune periodontitis

O.V. Marfiian, A.Ye. Demkovych

I. Horbachevsky Ternopil National Medical University; e-mail: demkovushae@tdmu.edu.ua

In more than 10% of patients who use fixed dental prosthetic structures, gingivitis and stomatitis are observed. The aim of our research was to study cytokine status changes in rats with experimental bacterial-immune periodontitis and under the conditions of using stamped and solid-cast fixed prosthetic constructions Experimental bacterial-immune periodontitis was induced by injecting a mixture of microorganisms, diluted with egg protein, into the periodontal complex tissues. On the 30th day of the experiment, the blood serum levels of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) were determined using the Solid-phase enzyme immunoassay. In animals with periodontitis and solid-cast crowns, there was a significant increase in the production of pro-inflammatory cytokines (IL-1β and IL-6) and a decrease in the formation of anti-inflammatory cytokine IL-10, compared to the data in rats with the use of stamped constructions. The obtained results indicate a differential influence of prosthetics type on the periodontal inflammation development.

Key words: periodontitis; cytokines; interleukins; immune system; fixed prosthetics; stamped crowns; solid-cast crowns.

INTRODUCTION

In modern orthopedic dentistry, the success of treatment depends on not only the knowledge and skills of the doctor and dental technician, but also significantly on the proper selection and use of dental materials for fabricating fixed prosthetic structures [1].

The influence of orthopedic structures on the periodontal tissues and the oral cavity mucous membrane can manifest itself in the form of both local and general disorders: hyperemia, diffuse inflammation, erosions, ulcers, hyperplastic growth, galvanic syndrome, allergic reactions [2].

In more than 10% of patients who use fixed dental prosthetic structures, gingivitis and stomatitis caused by contact with metal ions are observed [3]. In particular, alloys that contain Ni^{2+}, Co^{2+} and Cr^{3+}, which are key components of dental prostheses and implants, can cause unwanted reactions in the oral cavity and body; this subsequently leads to intolerance of these materials [4]. Allergic reactions caused by metal ions have been extensively studied at the molecular level. Molecular immunological mechanisms responsible for the recognition of metal ions by T-lymphocytes have been experimentally established [5].

Metal components of structures fixed in the oral cavity are subject of biological degradation, which can affect factors regulating both specific and non-specific tissue protection. This can lead to suppression of local immunity [6]. One of the main pathogenesis factors is the weakening of effectiveness of interaction between the components of specific and non-specific resistance of the oral cavity local immunity, such as lysozyme activity, the presence of neutrophils in saliva and the immunoglobulins level [7].

Cytokines, which are humoral mediators that control and participate in many immunological and biochemical processes of the body, play a crucial role in the development of inflammatory

processes, including periodontitis [8]. Change in the concentration of cytokines in blood serum can be an indication of the development or completion of inflammatory processes and characterizes the immune response direction. Therefore, the immune system activation can be characterized by analyzing the level of pro- or anti-inflammatory cytokines synthesized by immunocompetent cells [9].

Despite extensive research revealing the molecular mechanisms of contact hypersensitivity, and confirming the high sensitizing effect of metal ions of fixed structures on the human body, the issue of further study of the impact of the relevant materials remains relevant [10].

The aim of our research was to study changes in cytokine status in experimental periodontitis of bacterial-immune genesis under the conditions of using stamped and solid-cast crowns.

METHODS

The research was conducted using clinically healthy white rats weighing 150-200 g, kept under usual vivarium conditions in accordance with sanitary standards and the principles of Good Laboratory Practice. The investigations were conducted in accordance with the general rules and regulations of "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and "General Ethical Principles of Animal Experimentation" (Kyiv, 2001). The Commission on Bioethics of I. Horbachevsky Ternopil National Medical University has not detected violations of moral and ethical norms during the research (protocol No. 77 from 18.04.2024).

Experimental animals were randomly divided into 4 groups: I – control (n = 10); II – experimental periodontitis (n = 8); III – experimental periodontitis with fixed stamped crowns (n = 8); IV – experimental periodontitis with fixed solid-cast crowns (n = 8).

For the manufacture of permanent constructions, impressions were first taken from the central incisors of the lower jaw with "Speedex" impression material. The crowns were made using generally accepted methods: stamping standard sleeves [11] and casting from a metal alloy [12]. The manufactured orthopedic structures were designed in such a way that they did not cover the teeth occlusal surfaces and were simultaneously fixed on both central incisors.

Experimental bacterial-immune periodontitis was induced by injecting a mixture of microorganisms diluted with egg protein into the test animals' periodontal tissue. To enhance the immune response, complete Freund's adjuvant was administered into a rat's paw.

To reproduce the periodontitis model, *Staphylococcus aureus* and *Streptococcus hemolytic* were involved in a dose of 4CFU (colony-forming units). According to the literature, staphylococci and streptococci occupy an important niche in the microbiota, which participates in the development of inflammatory processes in the periodontium. Preliminary studies have shown that most patients with chronic inflammatory diseases of the oral cavity, including periodontitis, have a mixed microflora consisting of streptococci and staphylococci. The structural components of the cell wall of Gram-positive bacteria include lipoteichoic acids, peptidoglycan, and lipoproteins, which act as triggers for the development of inflammation through toll-like receptors 2. These molecules are key in pathogen recognition and the initiation of the innate immune response [13]. This was the bacterial link in our developed model. That is why cultures of these microorganisms were chosen in the process of modeling periodontitis. Pathomorphologically, changes were observed in the soft periodontium tissues, characterized by an increased hydration of the main substance, cellular infiltration by phagocytes, microabscesses and abscesses formation, and cellular infiltration by phagocytes with destruction of the periosteal plate edges, which is especially visible when compared with the control [14].

To enhance the efficiency of inducing and sustaining chronic periodontitis, we administered repeated injections of a microbial-protein mixture with an adjuvant on the 14th day of the experiment [15]. On the 30th day, the experimental animals were euthanized by exsanguination under thiopental anesthesia. The blood serum was collected for further research, and the levels of interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and interleukin-10 (IL-10) were determined using the Solid-phase enzyme immunoassay method with a set of RayBio Rat Cytokine Antibody Array reagents ("RayBiotech", USA) [16].

The results were analyzed with STATISTICA 10.0 ("Statsoft", USA) software. The reliability of the differences was determined using the Mann-Whitney U test [17].

RESULTS AND DISCUSSION

Interleukins are of significant clinical and immunological interest. According to the mechanism of action, these polypeptides are conventionally divided into pro-inflammatory (inducing an inflammatory response) and anti-inflammatory (limiting the development of an inflammatory response), as well as regulators with their own effector functions (cytotoxic, antiviral, etc.) [18].

Our study showed that on the 30th day of the development of experimental periodontitis of bacterial-immune genesis, the level of IL-1β increased significantly by 2.33 times ($P < 0.001$) compared to the control group parameters (Table).

In the experiments, under the condition of experimental periodontitis with the use of solid-cast crowns, there was a significant increase in the blood serum IL-1β content compared to the control – by 3.78 times ($P < 0.001$). It should be noted that when comparing the indicators against the non-prosthetics group of animals with periodontitis in the same period, it was established that they increased by 1.62 times ($P < 0.001$), this indicates that their use enhanced the inflammatory process development.

The use of stamped crowns in our experimental study contributed to an increase in the blood serum level of pro-inflammatory interleukin-1β by 3.32 times ($P < 0.001$), relative to the control group. Compared to non-prosthetics animals with experimental periodontitis, this indicator was by 1.43 times higher ($P < 0.01$). It should be noted that the level of this cytokine was by 1.14 times lower ($P < 0.05$) compared to the group of rats with solid-cast crowns, this evidences a less negative effect of stamped crowns on the course of periodontal complex inflammatory process.

The analysis showed that the level of IL-6 in the experimental group also increased significantly on the 30th day of the development of the inflammatory process in the periodontal complex – by 1.27 times ($P < 0.01$) compared to the control. The interleukin-6 level was also increased after fixation of a stamped crown compared to the control data and the indicators of animals with an inflammatory process, namely by 1.53 times ($P < 0.001$) and by 1.20 times ($P < 0.01$), respectively. A similar tendency to increase was found when determining the blood serum content of IL-6 in animals with solid-cast crowns and periodontitis. It was established that the cytokine content exceeded its indicators in the control group by 1.84 times ($P < 0.001$). However, in comparison with non-prosthetics animals with periodontitis, the interleukin-6 concentration was by 1.44 times higher ($P < 0.001$), this indicates an increase in the activity of a non-specific link of the body's defense and the inflammatory process initiation. It should be noted that the obtained data proved that the level of IL-6 in the experimental group with periodontitis and prosthesis with solid-cast structures was reliably by 1.20 times higher ($P < 0.01$) compared to the group with inflammation and stamped crowns (Table).

Our research revealed that during the formation of experimental bacterial-immune periodontitis there was an increase in the blood serum level of pro-inflammatory cytokines, compared to the control.

The levels of cytokines (ng/l) in blood serum of rats with experimental periodontitis and if using different types of crowns M±m

Experimental group	IL-1β	IL-6	IL-10	IL-1β/IL-10
Control (n = 10)	5.40±0.51	10.24±0.53	20.37±0.76	0.27±0.02
Experimental periodontitis no prosthetics (n = 8)	12.58±0.55 P_1<0.001	13.04±0.59 P_1<0.01	11.61±0.49 P_1<0.001	1.01±0.07 P_1<0.001
stamped crowns (n = 8)	17.92±0.82 P_1<0.001; P_2<0.01	15.64±0.46 P_1<0.001; P_2<0.01	9.42±0.32 P_1<0.001; P_2<0.01	1.91±0.11 P_1<0.001; P_2<0.001
solid-cast crowns (n = 8)	20.42±1.18 P_1<0.001; P_2<0.001; P_3<0.05	18.79±0.59 P_1<0.001; P_2<0.001; P_3<0.01	7.70±0.29 P_1<0.001; P_2<0.001; P_3<0.01	2,75±0,13 P_1<0.001; P_2<0.001; P_3<0.01

P_1 – statistical significance relative to control; P_2 – statistical significance relative to experimental periodontitis animals with no crowns; P_3 – statistical significance relative to experimental periodontitis animals with stamped crowns.

The anti-inflammatory cytokine IL-10 plays an important biological role in the organism's reactivity. In particular, it suppresses the production of IL-1β, IL-6, TNF-α, superoxide and nitroxide radicals by activated monocytes. The blood serum concentration of IL-10 in animals with periodontitis was found to be significantly lower compared to the control, with a reduction by 1.76 times ($P < 0.001$). This indicates the presence and progression of periodontal complex inflammatory process during this period.

When analyzing the indicators of anti-inflammatory cytokines, it is important to note the decrease in these indicators when stamped crowns were used against the background of periodontitis. The blood serum level of anti-inflammatory cytokines was by 2.16 times lower than in control animals ($P < 0.001$) and by 1.23 times lower ($P < 0.01$) than in the group with inflammation in the periodontal complex.

The content of anti-inflammatory IL-10 significantly decreased in animals with solid-cast prostheses. The impact of this type of crown on the inflammatory process course is confirmed by a decrease in the content of interleukin-10 (by 2.65 times; $P < 0.001$) compared to intact animals. Notably, during the fixation of solid-cast crowns, the level of anti-inflammatory cytokines also decreased by 1.51 times ($P < 0.001$) compared to the group of bacterial-immune periodontitis rats without prosthetics. This decrease reflected an increase in the periodontal tissues inflammatory reaction. Comparing the indicators of anti-inflammatory cytokines when using prosthetics with different types of metal crowns, it was observed that the level of IL-10 was significantly lower with the fixation of solid-cast structures, specifically by 1.22 times ($P < 0.01$), compared to the group with stamped crowns.

The decrease in anti-inflammatory cytokines and increase in pro-inflammatory cytokines in the experimental animals' blood serum resulted in an imbalance in their ratio (IL-1β/IL-10). It was found that this ratio was by 3.74 times higher ($P < 0.001$) in rats with the experimental inflammation, compared to the control group. As a result of changes in the indicators of pro- and anti-inflammatory cytokines in the blood serum of animals after fixation of stamped crowns under conditions of simulated periodontitis, changes in their ratio (IL-1β / IL-10) occurred. Characterizing the obtained results, it should

be noted that it significantly exceeded (by 7.07 times; $P < 0.001$) the indicators in animals of the control group, this indicates the activation of the inflammatory process and disturbance of the dynamic balance in the cytokinegenesis system. As shown in Table, the ratio of pro-inflammatory and anti-inflammatory cytokines in prosthetics animals with stamped crowns also increased significantly, compared to the non-prosthetics periodontitis group (by 1.89 times; $P < 0.001$).When determining the ratio value of pro- and anti-inflammatory cytokines in animals with solid-cast fixed structures, it was found its increase by 10.19 times ($P < 0.001$), compared to the control. The use of this type of crowns led to an increase in the IL-1β/IL-10 ratio by 2.72 times ($P < 0.001$), compared to the data of the non-prosthetics animals with periodontitis (Table).The performed immunoenzymatic studies of the pro- and anti-inflammatory cytokines ratio showed a significant difference, namely, the IL-1β/IL-10 index was 1.44 times higher ($P < 0.01$) in animals with cast crowns compared to stamped ones. A dynamic increase in the IL-1β/IL-10 ratio indicates the progressive development of the inflammatory reaction in periodontal tissues.

Our results show that the synthesis of cytokines increases dramatically as a result of "tissue stress", that is, it is an inducible process. Cytokinogenesis is almost inactive in the absence of an inflammatory process and immune response [19]. Under the action of a bacterial agent, in particular, molecules of lipopolysaccharides, peptidoglycans and muramyldipeptides, which are part of the cell wall of gram-negative periodontopathogenic bacteria, activation of macrophages occurs, this increases the production of pro-inflammatory cytokines (IL-1β, IL-6). These cytokines, circulating in the blood, stimulate the subsequent secretion of proteins of the acute phase of the inflammatory reaction [20].

According to the modern achievements of clinical immunology, it can be argued that the blood cytokine profile is important for determining the general immunopathogenesis of many chronic diseases, including dental ones [21]. Recent studies have confirmed the importance of cytokines in the intercellular interaction that underlies the development of chronic inflammation of the periodontal complex, including the mechanisms of dystrophic-inflammatory lesions that can lead to osteoporosis and resorption of alveolar bone, which in turn can lead to impaired function or even loss teeth [22].

Therefore, the data obtained give reason to assert that the use of fixed metal orthopedic structures in animals during the period of exacerbation of experimental periodontitis of bacterial-immune genesis promotes an increase in cytokine spectrum indicators and activates the further development of the inflammatory process. These results may be promising in the plan of conducting further experimental studies in order to identify the impact of metal crowns on other indicators of inflammatory processes in the maxillofacial area, in particular, in bacterial-immune periodontitis.

We conclude that the application of various types of metal crowns in experimental periodontitis of bacterial-immune genesis led to an intensified inflammatory response in periodontal tissues. This was evidenced by an increased titer of pro-inflammatory cytokines and a reduction in the anti-inflammatory interleukin-10 in blood serum. In experimental animals with periodontitis and fixed solid-cast crowns, a significant increase in the production of pro-inflammatory cytokines (IL-1β and IL-6) and a decrease in the formation of the anti-inflammatory cytokine IL-10 were observed. This contrasts with the data from rats with simulated inflammation and stamped fixed structures, suggesting that solid-cast prosthetics may have a more detrimental impact on these aspects of the inflammatory process in the periodontal complex.

The authors of this study confirm that the research and publication of the results were

not associated with any conflicts regarding commercial or financial relations, relationships with organizations and/or individuals that may have been related to the study, and interrelations among the co-authors of the article.

О.В. Марфіян, А.Є. Демкович

ЗМІНИ ЦИТОКІНОВОГО СТАТУСУ ПРИ БАКТЕРІАЛЬНО-ІМУННОМУ ПАРОДОНТИТІ ПРИ ВИКОРИСТАННІ РІЗНИХ ТИПІВ НЕЗНІМНИХ ПРОТЕЗНИХ КОНСТРУКЦІЙ

Тернопільський національний медичний університет імені І.Я. Горбачевського МОЗ України;
e-mail: demkovushae@tdmu.edu.ua

Понад 10% пацієнтів, які використовують незнімні зубні протези, страждають на гінгівіт та стоматит. Метою нашої роботи було дослідити зміни цитокінового статусу у щурів при експериментальному пародонтиті бактеріально-імунного генезу за умов використання штампованих та суцільнолитих коронок. Пародонтит у дослідних тварин був індукований введенням суміші мікроорганізмів, розведеної яєчним протеїном, у тканини пародонтального комплексу. На 30-ту добу дослідження методом твердофазного імуноферментного аналізу визначали концентрацію у сироватці крові інтерлейкінів(ІЛ): ІЛ-1β, ІЛ-6 та ІЛ-10. У експериментальних тварин із пародонтитом та зафіксованими суцільнолитими коронками достовірно підвищувалися концентрації прозапальних цитокінів ІЛ-1β та ІЛ-6, а також зменшувався вміст протизапального ІЛ-10 порівняно зі значеннями у щурів з штампованими незнімними конструкціями. Отримані результати свідчать про різницю у впливі типу протезу на розвиток запального процесу в тканинах пародонта.

Ключові слова: пародонти; цитокіни; інтерлейкіни; імунна система; незнімне протезування; штамповані коронки; суцільнолиті коронки.

REFERENCES

1. Sokolovska VM, Tsvetkova NV. Displays of unbearableness of stomatological materials are in cavity of mouth (clinical case). Ukr Dent Almanac. 2022;4:48-52. Available from: http://repository.pdmu.edu.ua/handle/123456789/20543. [Ukrainian].
2. Sylenko YI, Perepelova TV, Khrebor MV, Sylenko BYu, Pysarenko OA. The reactions of lipid's free radical oxidation, hemocoagulant properties of oral fluid in patients with galvanic currents in the mouth. Wiad Lek. 2018;71(4):879-82. Available from: http://repository.pdmu.edu.ua/handle/123456789/14554?locale=en
3. Belikov OB, Roshchuk OI, Havaleshko VP, Karavan YR. Modern approaches to the diagnosis of the negative influence of fixed dentures on periodontal tissues and its prevention (literature review). Clin Dent. 2023;1:17-23. [Ukrainian].
4. Hasiuk P, Kindiy D, Vorobets A, Kindiy V, Demkovych A, Odzhubeiska O. Analysis of the advisability of using different types of base plastics by studying the needs of the population in removable prosthesis. Wiad Lek. 2022;75(12):3055-9.
5. Mittermüller P, Hiller KA, Schmalz G, Buchalla W. Five hundred patients reporting on adverse effects from dental materials: Frequencies, complaints, symptoms, allergies. Dent Mater. 2018;34(12):1756-68.
6. Bandrivsky Y, Bandrivska O, Bandrivska N, Bedenyuk O, Kuchyrka L, Zmarko I. Medication correction of the main clinical symptoms of generalized periodontitis in patients with different blood groups. Pharmacia. 2023;70(3):499-507.
7. Hajishengallis G. New developments in neutrophil biology and periodontitis. Periodontology 2000. 2020;82(1):78-92.
8. Aref Nezhad R, Motedayyen H, Roghani-Shahraki H. Do cytokines associate periodontitis with metabolic disorders? An overview of current documents. Endocrinol Metab Immun Dis Drug Target. 2022;22(7):778-86.
9. Plemmenos G, Evangeliou E, Polizogopoulos N, Chalazias A, Deligianni M, Piperi C. Central regulatory role of cytokines in periodontitis and targeting options. Curr Med Chem. 2021;28(15):3032-58.
10. Hasiuk P, Kindiy D, Radchuk V, Kindiy V, Demkovych A, Yarkoviy V. Biological compatibility of metal structures of dentures made from multiple melted alloys. Pol Merkuriusz Lek. 2022;50(296):114-7. Available from: http://repository.pdmu.edu.ua/bitstream/123456789/18107/1/Biological_compatibility_of_metal_structures.pdf
11. Agrawal H. Stainless steel crown: A review article. Ind J Forensic Med Toxicol. 2020;14(4):9929-32.
12. Haraguchi M, Towithelertkul C, Ali IE, Han X, Sumita YI. An indirect-direct technique with hot water for fabricating a cast metal crown under an existing removable partial denture. J Prosthet Dent. 2022;14:S0022-3913(22)00491-7.
13. AlQallaf H, Hamada Y, Blanchard S, Shin D, Gregory R, Srinivasan M. Differential profiles of soluble and cellular toll like receptor (TLR)-2 and 4 in chronic periodontitis. PLoS One. 2018;13(12):e0200231.
14. Demkovych AYe, Bondarenko YuI., inventor; I. Horbachevsky Ternopil National Medical University, assignee. A method of modeling periodontitis in laboratory animals (rats). Ukraine patent UA 82388, G 09 B 23/28 (2006.01) A 61 B 17/00. No. u201303000. 2013 Jul 25.
15. Demkovych A. Endogenous intoxication in development of experimental periodontitis of bacterial-immune genesis. Folia Medica. 2023;65(1):149-54.
16. Muñoz OB. Determination of proinflammatory and antiinflammatory cytokines by ELISA Technique. Method Mol Biol. 2023;2612:101-8.

17. Zhu X. Sample size calculation for Mann-Whitney U test with five methods. Int J Clin Trial. 2021;8(3):184-95.
18. Mashchenko IS, Gudaryan OO, Kucherenko TO. Clinical, immunological and metabolic features of accelerated and quickly progressing options of generalized periodontitis. Actual Dentistry. 2020;4:26-32. [Ukrainian].
19. Batra P, Das S, Patel P. Comparative evaluation of gingival crevicular fluid (GCF) levels of Interleukin-34 levels in periodontally healthy and in patients with chronic and aggressive periodontitis – A cross-sectional study. Saudi Dental J. 2019;31(3):316-21.
20. Plemmenos G, Evangeliou E, Polizogopoulos N, Chalazias A, Deligianni M, Piperi C. Central regulatory role of cytokines in periodontitis and targeting options. Curr Med Chem. 2021;28(15):3032-58.
21. Nagata M, English JD, Ono N, Ono W. Diverse stem cells for periodontal tissue formation and regeneration. Genesis. 2022;60(8-9):e23495.
22. Lysokon Y, Bandrivsky YL, Luchynskyi MA. Analysis of the results of treatment of destructive forms of apical periodontitis with osteotropic drugs in a short term. Wiad Lek. 2022;75(1):228-31.

Received 05.08.2024