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Research Article

# Quercetin effects on adaptive immune response in experimental periodontitis of bacterial-immune genesis

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## Abstract

In the article was studied the effects of flavonol quercetin on indices of adaptive immune response in experimental animals on the 14th day of the experimental bacterial-immune periodontitis development. Indices of immune protection were determined by the relative number of lymphocytes with CD3+, CD4+, CD8+, CD19+, CD16+ and immunoregulatory index (CD4+ / CD8+) in intact animals and on the 14th day of inflammatory process development in periodontal tissues as well as the therapeutic effects of flavonol quercetin. As a result of the study, characterized changes associated with the activity of both the cell-mediated and humoral-immune response were found, both in the development of experimental periodontitis, and apply of flavonol. In particular, there was an increase in the animal's blood relative amount of CD8+, CD16+ cells on the 14th day, and content of CD3+, CD4+, CD19+ was decreased. In this case, the immunoregulatory index (CD4+ / CD8+) as an important index of immunological activity was decreased. The apply of flavonol quercetin in the period development of bacterial-immune periodontitis animals functional activity of the T-cell line of the immune system was increased, as evidenced percentage increase of B- and T-cells due to T-helper cells decrease as well as T-killers content during this period of inflammatory reaction in the periodontal complex, in comparison with animals, which were not treated.

## Keywords

Periodontitis, immune system, T-lymphocytes, B-lymphocytes, inflammation, quercetin

## Introduction

Inflammatory diseases of periodontal tissues are widespread in the world. The etiology and pathogenesis of periodontal diseases are complicated, insufficiently elucidated and one of the important problems of theoretical and practical medicine (Demkovych et al. 2019). The main role in this case play infectious factors and the failure mechanisms of immune protection (local cellular nonspecific and general adaptive) to form an adequate character of the development and course of pathological process in the oral cavity (Hasiuk et al. 2021). That defines the effectiveness of therapeutic and preventive measures (Plessas 2019). At present, the development and progression of periodontal inflammatory disease is considered not so much as local inflammation of peritoneal tissues, but also as the

reaction of the whole organism to the bacterial invasion of periodontal tissues. It is known that the development of periodontitis is a result of an imbalance between the microflora of the oral cavity, its barrier characteristics and immune protection of the organism (Thumbigere-Math et al. 2014). It should also take into account the adequate activity of the inflammatory cells and adequacy of the resistance mechanisms (Cekici et al. 2014). For the development of inflammation in the periodontal complex of one bacterial factor is not enough, there should be constellation of pathogenetic factors. Inflammatory diseases of periodontal complex, as a rule, chronicity, with periodic remissions, that considers necessity of timely and repeated treatment course (Silva et al. 2014).

Research of membrane receptors of lymphocytes at different stages of their development, differentiation, activation, cellular proliferation, and apoptosis is a modern informative, objective and accessible method which is used with scientific, diagnostic, therapeutic and prognostic purpose (Guzman et al. 2014). Therefore, the research of this problem should be based on a comprehensive study of the basic mechanisms that promote transition of acute inflammation into chronic form, with further definition of pathogenetically grounded therapeutic measures that available effectively influence on the processes of oral cavity sanogenesis and complete regeneration of periodontium (Silva et al. 2015).

Treatment of periodontitis does not require ordinary approaches to their decisions. In this regard, the effectiveness of plant origin substances, which are related to polyphenolic compounds that are able to modulate immune response as well as inflammatory process in the organism, have not been studied.

Quercetin is referred to flavonols with antioxidant, anti-ischemic, membrane-stabilizing and immunomodulatory properties (D'Andrea 2015; Miles et al. 2014). It has a great restorative potential and anti-inflammatory, anabolic, anti-apoptotic properties (Demkovych et al. 2021). The antioxidant activity of the drug is related to its ability to reduce the formation of active forms of oxygen, suppress lipid peroxidation and accumulation of toxic products of lipoperoxidation, and stimulate catalase and superoxide dismutase activity. Anti-inflammatory and anti-allergic effects are also related to the ability of quercetin to suppress the synthesis of leukotrienes. Flavonol suppresses the activity of hyaluronidase and thus affects vascular permeability, as well as is able to increase the production of the immune cells (phagocytes, T-lymphocytes, B-lymphocytes) and reduce manifestations of secondary immunosuppression (Stechyshyn et al. 2021).

Objective. To determine effects of the flavonol quercetin to adaptive immunity in respect of the experimental bacterial-immune periodontitis development.

## Materials and methods

The study was conducted on white, clinically healthy male rats weighing 150–200 g under vivarium conditions. The animals were in a standard diet balanced with basic nutrition elements, with free access to water. Experiments were carried out in compliance with the general rules and regulations of the "European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments" (Kyiv, 2001). Experimental animals were divided into three groups: the first group – intact animals (n = 10); the second group – animals with experimental periodontitis on the 14$^{th}$ day of the study (n = 8), and third group – animals with experimental periodontitis on the 14$^{th}$ day of the study, which was treated by quercetin (n = 8).

Experimental bacterial-immune periodontitis in experimental animals was caused by the introduction into the tissues of the periodontal complex of a mixture of microorganisms diluted with egg protein (Demkovych 2019). In order to enhance the immune response, the complete adjuvant of Freund was introduced into the rat's paw at the same time. The experimental animals of the third group received intramuscular injections of water soluble drug quercetin (Corvitin®, PJSC SIC "Borshchahivskiy Chemical Pharmaceutical Plant", Kyiv) at a dose of 100 mg / kg of animal mass within 7 days (from the 7$^{th}$ to the 14$^{th}$ day). On the 14$^{th}$ day of experimental animals were exsanguinated under thiopental anesthesia. The indices of cellular immunity were determined by the method based on the interaction of monoclonal antibodies labeled with fluorescence label, with surface antigens of lymphocytes (Groff et al. 2019). Determination of T-and B lymphocyte subpolations was performed using Rat ELISA Kits ("NeoScientific" and "MyBioSource", USA). Samples were analyzed by the Beckman Coulter (USA) Epics-XL flow cytometry. The relative numbers of CD3+ (common T-lymphocytes), CD4+ (T-helper), CD8+ (cytotoxic cells, T-killers), CD19+ (B-lymphocytes), CD16+ (natural killers, NK-cells) and immunoregulatory index (CD4+ / CD8+). Statistical processing of digital data was carried out using the software STATISTICA 10.0 ("Statsoft", USA) using the variational and statistical methods for evaluating the obtained data. For all indicators, the average arithmetic mean (M), its variance and mean errors (m), sample size (n) were calculated. The reliability of the difference in values between independent quantitative values was determined with normal distribution by U-criterion Mann-Whitney (Orlov 2015).

## Results

It has been established that development of experimental bacterial-immune periodontitis occurs in accordance with changes in the cell-mediated and humoral levels of adaptive immune defense. Analysis of the immune status in rat's blood with periodontitis showed that quercetin is able to change the activity of the cellular –mediated immune response (Table 1).

The therapeutic use of this flavonol caused increase of common mature T-lymphocytes (CD3+) by 1.43 times ($p<0.01$), as compared with the animals group that were not treated by this drug (Fig. 1). However, there was a decrease in the titers of CD3+ cells in the rats with treatment in relation to similar indices of the control animals group (by 1.19 times; $p<0.01$).

**Table 1.** Indices of adaptive immunity response in the experimental animals with periodontitis and treatment by quercetin (M ± m).

| Experiment conditions and indices | Control, intact animals | Animals with experimental periodontitis | |
|---|---|---|---|
| | | Without treatment | After treatment by quercetin |
| Experiment duration (days) | – | 14 | 14 |
| Number of the animals | 10 | 8 | 8 |
| CD3+, % | 45.94±0.73 | 27.07±0.58 $p_1$<0.01 | 38.65±0.61 $p_1$<0.01; $p_2$<0.01 |
| CD4+, % | 33.32±0.56 | 21.73±0.48 $p_1$<0.01 | 30.11±0.81 $p_1$<0.05; $p_2$<0.01 |
| CD8+, % | 15.70±0.74 | 25.46±0.45 $p_1$<0.01 | 20.79±0.93 $p_1$<0.01; $p_2$<0.01 |
| CD19+, % | 11.65±0.39 | 8.23±0.27 $p_1$<0.01 | 10.14±0.27 $p_1$<0.05; $p_2$<0.01 |
| CD16+, % | 9.63±0.40 | 13.62±0.19 $p_1$<0.01 | 10.79±0.26 $p_1$<0.05; $p_2$<0.01 |

**Notes**: $p_1$ – significant of differences in relation to intact animals; $p_2$ – significant of differences in relation to animals with experimental bacterial-immune periodontitis on the 14th day of the research without treatment.

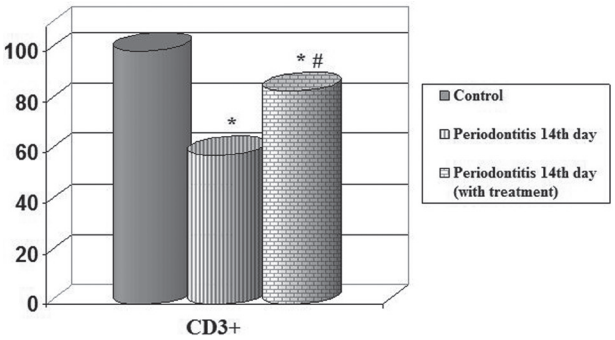


**Figure 1.** Dynamics of the content of common mature T-lymphocytes in the rat's blood on the 14th day of the research in conditions of experimental bacterial-immune periodontitis development and treatment by quercetin (% of control). Notes: * – significant of differences in relation to the intact animals (p<0.01); # – significant of differences in relation to the animals with periodontitis on the 14th day of the experiment without treatment (p<0.01).

**Table 2.** Immunoregulatory index in the experimental animals with periodontitis and treatment by quercetin (M ± m).

| Experiment conditions and indices | Control, intact animals | Animals with experimental periodontitis | |
|---|---|---|---|
| | | Without treatment | After treatment by quercetin |
| Experiment duration (days) | – | 14 | 14 |
| Number of the animals | 10 | 8 | 8 |
| CD4+ / CD8+ | 2.17±0.11 | 0.85±0.03 $p_1$<0.01 | 1.44±0.12 $p_1$<0.01; $p_2$<0.01 |

**Notes**: $p_1$ – significant of differences in relation to intact animals; $p_2$ – significant of differences in relation to animals with experimental bacterial-immune periodontitis on the 14th day of the research without treatment.

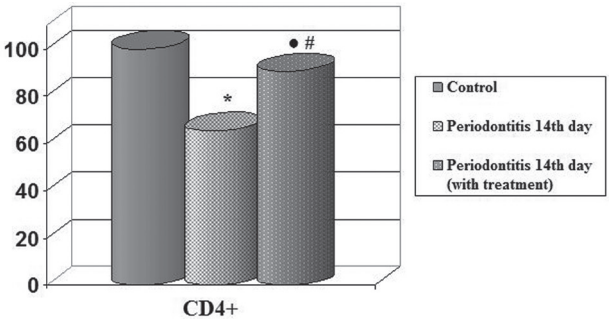


**Figure 2.** Dynamics of the content of T-helpers in the rat's blood on the 14th day of the research in conditions of experimental bacterial-immune periodontitis development and treatment by quercetin (% of control). Notes. * – significant of differences in relation to the intact animals (p<0.01); ● – significant of differences in relation to the intact animals (p<0.05); # – significant of differences in relation to the animals with periodontitis on the 14th day of the experiment without treatment (p<0.01).

In a result of the experimental bacterial-immune periodontitis development after quercetin treatment during 7 days was observed on the 14th day significant increase in the blood of the T-helper cell content (CD4+), in comparison with the same term in animals without administration of the drug (by 1.39 times; p<0.01). At the same time, the content of T-helper cells in the blood of the studied group remained low (Fig. 2), as compared to the control values, and was lower (by 1.11 times; p<0.05).

The introduction of flavonol antioxidant resulted significant changes of the T-effectors (CD8+) content that perform both killer and suppressor functions in the blood of experimental animals with bacterial-immune periodontitis. Quercetin effectively changed the course of the inflammatory process in the periodontal tissues, but as well reduced level of CD8+ in the blood (by 1.23 times; p<0.01) in relation to animals with experimental periodontitis on the 14th day without treatment (Table 1; Fig. 3). At the same time, this index did not reach the values of the control animal group and was higher by 1.32 times (p<0.01).

There was significant increase of the immunoregulatory index (CD4+ / CD8+) in the experimental animals treated by quercetin, as compared with the animals on the same research term but without drug administration (by 1.69 times, p<0.01) (Table 2).

At the same time, this indices of the studied animal group remained at a rather low level (Fig. 4), in comparison with control values, and were lower by 1.51 times (p<0.01).

Regarding the influence of this flavonol on the parameters of general B-lymphocytes (CD19+) in the blood of experimental animals with periodontitis it should be noted that quercetin increased their content (by 1.23 times, p<0.01), as compared with animals on the 14th day, which were not treated by quercetin (Table 1; Fig. 5). However, in a result comparing of the immune index were observed on the 14th day of the experimental periodontitis after administration of quercetin that it remained lower in relative to the control animal group (by 1.15 times, p<0.05).

As it was established in our previous studies, on the 14th day of the inflammatory process development in the periodontal complex, the natural killers (NK-cells, CD16 +) content in the blood was increased by 41.43% (p<0.01), as compared with control animal group. The use of flavonol quercetin with the immunomodulatory effect resulted to decrease of the NK-cells content (by 1.26 times; p<0.01) in blood, with respect to the same parameters of the animal group that were not treated (Fig. 6). However, they were revealed , nevertheless, higher than of the control animal group (by 1.12 times; p<0.05).

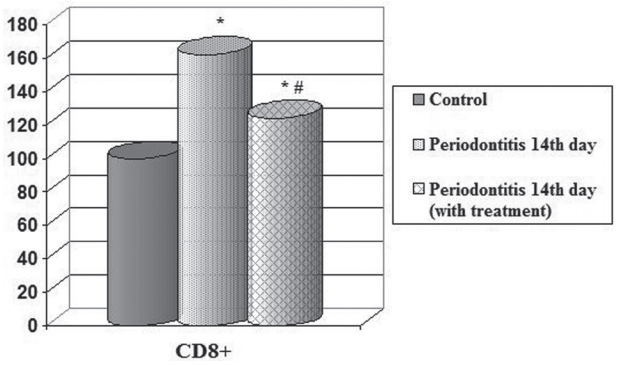


**Figure 3.** Dynamics of the content of T-effectors in the rat's blood on the 14th day of the research in conditions of experimental bacterial-immune periodontitis development and treatment by quercetin (% of control). Notes. * – significant of differences in relation to the intact animals ($p<0.01$); # – significant of differences in relation to the animals with periodontitis on the 14th day of the experiment without treatment ($p<0.01$).

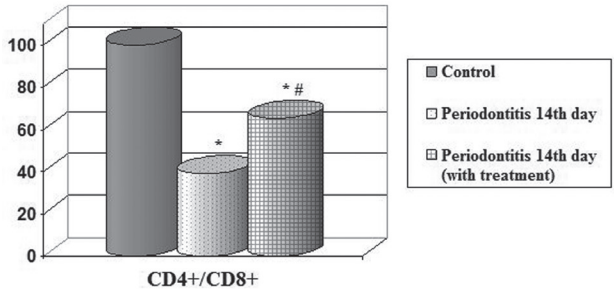


**Figure 4.** Dynamics of immunoregulatory index on the 14th day of the research in conditions of experimental bacterial-immune periodontitis development and treatment by quercetin (% of control). Notes. * – significant of differences in relation to the intact animals ($p<0.01$); # – significant of differences in relation to the animals with periodontitis on the 14th day of the experiment without treatment ($p<0.01$).

## Discussion

An analysis of the changes peculiarity in the cellular level of adaptive immunity for the experimental bacterial-immune periodontitis has been carried out, which will contribute to deepening the essential understanding of the inflammatory process development and search of pathogenetic treatment (Thumbigere-Math et al. 2014). In particular, ascertain of chracterized disorders of immunological processes will permit to determine the role of one of important parts of the structural damage of the periodontal complex which lead to formation of inflammatory process of different severity (Wang et al. 2013). The cellular immunity is a type of immune response which no involves participation of complement system and antibodies. The cellular link of innate immunity or cellular unspecific factors of protection include monocytes/macrophages, dendritic cells, mast cells, Nk-cells, polymorphonuclear leukocytes. The adaptive immunity or specific factors of protection include T-lymphocytes and B-lymphocytes, that is cell-mediated of adaptive immune response (T-cells) and humoral immune response (B-cells, antibodies) (Zhu et al. 2013). Peculiarities of the T-cell response of adaptive immunity in experimental bacterial-immune periodontium helped to deepen the understanding of the essence of disorders of immunological processes, their role in the mechanisms of development of this inflammatory process and the formation of a protective reaction of the organism, on the one hand, and pathological periodontal complex, on the other hand, which determines the severity and prognostic effect of the inflammatory process. T-cell adaptive immunity is a type of immune response in which there is no involvement of the complement system and circulating antibodies. Two types of cells belong to cellular innate factors of protection: phagocytes and natural killers.

The general assessment of the immune status of rats with experimental bacterial-immune inflammation in the tissues of the periodontal complex showed that the degree and direction of changes in all its parts were different. It is known that T-lymphocytes, which recognize antigens with the participation of cellular receptors, play a key role in immune responses.

Regarding the changes in the content of T-effectors, which perform the functions of both killers and suppressors (CD8+), their number in the blood of animals under these experimental conditions increased. It is these cells that suppress the activity of cellular and humoral immunity, influencing the mechanisms of intercellular interaction. Their presence in the blood in the acute phase of the inflammatory process indicates the activation of immunological reactivity in response to antigenic stimulation. The consequence of a decrease in the content of T-helpers and an increase in the content of T-suppressors in the blood of experimental animals were changes in the immunoregulatory index (CD4+ / CD8+). The obtained results indicate the appearance of an immunosuppressive state in rats under the condition of bacterial-immune periodontitis, which developed in the late period of the inflammatory reaction due to both T-helpers and cytotoxic T-suppressors / killers. Quantitative and qualitative changes in lymphocytes and monocytes occur within certain limits, which allows to maintain "cellular homeostasis", but at the same time respond sharply to any adverse environmental influences.

The antioxidant activity of the drug is related to its ability to suppress lipid peroxidation, reduce the concentration of free radicals and toxic peroxidation products, and activates catalase and superoxide dismutase activity (D'Andrea G 2015). Anti-inflammatory and antiallergic effects are also related to the ability of quercetin to suppress calcium ATPase and the synthesis of leukotrienes. This flavonoid is able to suppress the activity of hyaluronidase, increase content of immune system cells (phagocytes, T- and B-lymphocytes) in the blood, in a result manifestations of secondary immunosuppression are decreased (D'Andrea G 2015). The determination of the

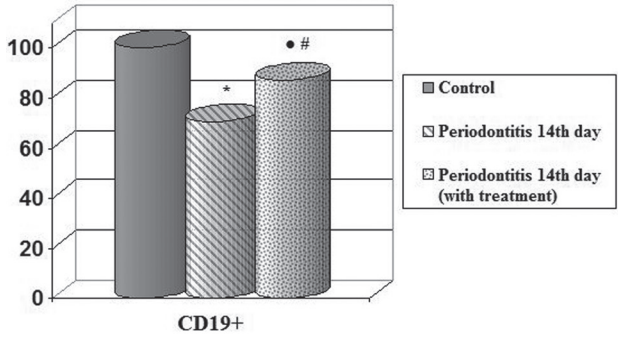


**Figure 5.** Dynamics of the common B-lymphocytes content in the rat's blood on the 14th day of the research in conditions of experimental bacterial-immune periodontitis and treatment by quercetin (% of control). Notes. * – significant of differences in relation to the intact animals ($p<0.01$); ● – significant of differences in relation to the intact animals ($p<0.05$); # – significant of differences in relation to the animals with periodontitis on the 14th day of the experiment without treatment ($p<0.01$).

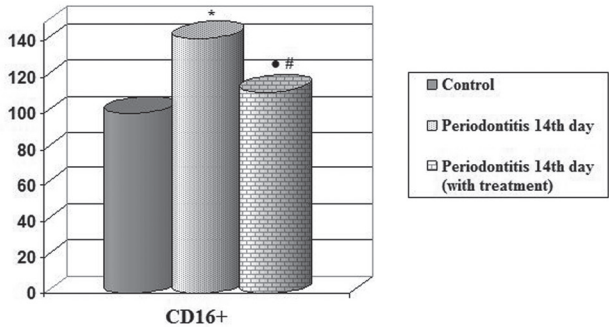


**Figure 6.** Dynamics of the natural killers content in the rat's blood on the 14th day of the research in conditions of experimental bacterial-immune periodontitis development and treatment by quercetin (% of control). Notes. * – significant of differences in relation to the intact animals ($p<0.01$); ● – significant of differences in relation to the intact animals ($p<0.05$); # – significant of differences in relation to the animals with periodontitis on the 14th day of the experiment without treatment ($p<0.01$).

pathomorphological characteristic of the ultrastructural changes will permit establish the damaging mechanisms of the periodontal structural complex, the lawfullness of the inflammatory process formation and possibility of their correction (Demkovych et al. 2017; Miles et al. 2014).

Therapeutic use of this flavonol led to an increase in the content of all mature T-lymphocytes (CD3+), an increase in the content of the helper fraction of the cellular immune system (CD4+) in the blood, a decrease in CD8 +, an increase in the immunoregulatory index (CD4+ / CD8+) in animals without drug administration. Regarding the effect on the content of B-lymphocytes (CD19+) in the blood of experimental animals with periodontitis in this model of the inflammatory process, it should be noted that quercetin increased their content compared with animals on the 14th day who did not receive the drug. Therefore, the use of flavonol quercetin contributes to the normalization of a number of indicators of humoral immune protection and cytokine spectrum.

## Conclusion

Development of a destructive inflammatory process in the periodontal complex due to bacterial and immune factors is accompanied by decrease in the blood of active T-lymphocytes, T-helpers, B-lymphocytes content as well as increase of the T-killer and NK-cells.

The application of the flavonol quercetin for the bacterial-immune periodontitis development promotes the normalization of the cellular adaptive immunity indices and reversion of the inflammatory process in the periodontal complex.

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## References

Cekici A, Kantarci A, Hasturk H, Van Dyke TE (2014) Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000 64(1): 57–80. https://doi.org/10.1111/prd.12002

D'Andrea G (2015) Quercetin: A flavonol with multifaceted therapeutic applications? Fitoterapia 106: 256–271. https://doi.org/10.1016/j.fitote.2015.09.018

Demkovych A (2019) Effects of flavonol quercetin on activity of lipid peroxide oxidation in experimental bacterial-immune periodontitis. Interv Medicine and Applied Science 11(1): 55–59. https://doi.org/10.1556/1646.10.2018.48

Demkovych A, Bondarenko Yu, Hasiuk P (2019) Effects of quercetin on antioxidant potential in the experimental periodontitis development. Interventional Medicine and Applied Science 11(1): 60–64. https://doi.org/10.1556/1646.11.2019.06

Demkovych A, Shcherba V, Yaremchuk O, Stoikevych H, Machogan V, Luchynskyi V (2021) Effects of flavonol quercetin on syndrome of endogenous intoxication in experimental periodontitis. Pharmacia 68(3): 627–632. https://doi.org/10.3897/pharmacia.68.e67341

Groff BD, Kinman AWL, Woodroof JF, Pompano RR (2019) Immunofluorescence staining of live lymph node tissue slices. J Immunol Methods 464: 119–125. https://doi.org/10.1016/j.jim.2018.10.010

Guzman YA, Sakellari D, Arsenakis M, Floudas CA (2014) Proteomics for the discovery of biomarkers and diagnosis of periodontitis: a critical review. Expert Rev Proteomics 11(1): 31–41. https://doi.org/10.1586/14789450.2014.864953

Hasiuk PA, Vorobets AB, Demkovych AYe, Tkachenko IM, Klitynska OV, Rosolovska SO, Pyasetska LV (2021) Features of occlusal correlations of molars in the dental clinic. Wiadomosci Lekarskie 74(5): 1130–1133. https://doi.org/10.36740/WLek202105115

Miles SL, McFarland M, Niles RM (2014) Molecular and physiological actions of quercetin: Need for clinical trials to assess its benefits in human disease. Nutr Rev 72: 720–734. https://doi.org/10.1111/nure.12152

Orlov AI (2015) Structure of nonparametric statistics (generalizing article). Industrial laboratory. Diagnostics of materials 81(7): 62–72.

Plessas A (2014) Nonsurgical periodontal treatment: review of the evidence. Oral Health Dent Manag 13(1): 71–80.

Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, Hernandez M, Gamonal J (2015) Host response mechanisms in periodontal diseases. J Appl Oral Sci 23(3): 329–355. https://doi.org/10.1590/1678-775720140259

Stechyshyn I, Pavliuk B, Chornij N, Kritsak M, Prokopovych O (2021) Pol Merkur Lekarski 49(291): 238–241.

Thumbigere-Math V, Michalowicz BS, Hodges JS, Tsai ML, Swenson KK, Rockwell L, Gopalakrishnan R (2014) Periodontal disease as a risk factor for bisphosphonate-related osteonecrosis of the jaw. J Periodontol 85(2): 226–233. https://doi.org/10.1902/jop.2013.130017

Wang XE, Xu L, Meng HX, Lu D, Chen ZB, Lu RF (2013) Long-term clinical and hematologic effects of non-surgical treatment on aggressive periodontitis. Zhonghua Kou Qiang 48(8): 467–471.

Zhu C, Yang J, Sun J, Shi J, Gou J, Li A (2013) Induction of immune response and prevention of alveolar bone loss with recombinant Porphyromonas gingivalis peptidylarginine deiminase. Arch Oral Biol 58(12): 1777–1783. https://doi.org/10.1016/j.archoralbio.2013.09.006