8. Krischer, JM, Albert, K, Pfaffenroth A. Mechanical ventilation-induced alterations of intracellular surfactant pool and blood-gas barrier in healthy and pre-injured lungs. Histochem Cell Biol 155, 183–202 (2021). https://doi.org/10.1007/s00418-020-01938-x.
9. Miserocchi G. The impact of heterogeneity of the air-blood barrier on control of lung extravascular water and alveolar gas exchange. Frontiers in Network Physiology. 2023. 3. 10.3389/fnetp.2023.1142245.
10. Nova Z, Skovierova H, Calkovska A. Alveolar-Capillary Membrane-Related Pulmonary Cells as a Target in Endotoxin-Induced Acute Lung Injury. Int J Mol Sci. 2019 Feb 15;20(4):831. doi: 10.3390/ijms20040831.
11. Sargon MF. Lungs and hypoxia: a review of the literature. Anatomy 2021;15(1):76–83.
12. Sturrock A, Woller D, Freeman A, Sanders K, Paine R 3rd. Consequences of Hypoxia for the Pulmonary Alveolar Epithelial Cell Innate Immune Response. J Immunol. 2018 Dec 1;201(11):3411–3420. doi: 10.4049/jimmunol.1701387.
13. Tedjasaputra V, Bouwsema MM, Stickland MK. Effect of aerobic fitness on capillary blood volume and diffusing membrane capacity responses to exercise. J Physiol. 2016 Aug 1;594(15):4359–70. doi: 10.1113/JP272037.
14. Tsagareli ZG, Gogiashvili LE, Topuria ZM, Dzhandieri KN. Morpho-functional estimation of blood-air barrier and lung surfactant in rats of different age. Georgian Med News. 2008 Jun;(159):47–52. PMID: 18633152.

Стаття надійшла 2.11.2022 р.

DOI 10 26724/2079-8334-2023-4-86-226-230
UDC 611.08-084+612.396.32:611.018.4-599.323.4

D.O. Sukhomeylo, O.E. Reyzvikh[1], S.A. Shnaider[1], M.T. Khrystova, S.V. Klenovska
Odesa National Medical University, Odesa, [1]State Establishment "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine", Odesa

EXPERIMENTAL SUBSTANTIATION OF PREVENTION OF BONE METABOLISM DISORDERS IN THE JAWS OF RATS UNDER EXPERIMENTAL PERIODONTITIS BECAUSE OF ALIMENTARY VITAMIN D DEFICIENCY

e-mail: sukhomeylod@gmail.com

The purpose of this study was to investigate the efficacy of prevention of remodeling disorders in the bone tissue of the alveolar process of the rat jaws with periodontitis against the background of alimentary deficiency of vitamin D. The experimental study was conducted on 36 white Wistar rats of both genders (1 month old, weight 100±5 g), which were divided into 3 groups. The degree of atrophy of the alveolar process was assessed. The activity of elastase, acid phosphatase, alkaline phosphatase, calcium and phosphorus content was determined in the homogenates of jaw bone tissue. The result of the analysis indicates the prevalence of osteoresorption processes over osteosynthesis. The proposed therapeutic and prophylactic complex showed a periodontal protective effect, which was formed due to its properties to increase the activity of alkaline phosphatase, calcium and phosphorus content against the background of a decrease in acid phosphatase and elastase in the bone tissue of the rat alveolar processes modeled with alimentary D-deficient periodontitis.

Key words: experiment, rats, vitamin D deficiency, periodontitis, biochemical parameters, bone metabolism.

Д.О. Сухомейло, О.Е. Рейзвіх, С.А. Шнайдер, М.Т. Христова, С.В. Кленовська

ЕКСПЕРИМЕНТАЛЬНЕ ОБГРУНТУВАННЯ ПРОФІЛАКТИКИ ПОРУШЕНЬ МЕТАБОЛІЗМУ КІСТКОВОЇ ТКАНИНИ ЩЕЛЕП ЩУРІВ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ПАРОДОНТИТІ НА ТЛІ АЛІМЕНТАРНОГО ДЕФІЦИТУ ВІТАМІНУ D

Метою цієї роботи було дослідження ефективності профілактики порушень ремоделювання у кістковій тканині альвеолярного відростка щелеп щурів з пародонтитом на тлі аліментарного дефіциту вітаміну D. Експериментальне дослідження було проведено на 36 білих щурах лінії Wistar обох полів (1 місяць, маса 100±5 г), яких поділили на 3 групи. Оцінювали ступінь атрофії альвеолярного відростка. У гомогенатах кісткової тканини щелеп визначали активність еластази, кислої фосфатази, лужної фосфатази, вміст кальцію та фосфору Результат аналізу вказує на превалювання процесів остеорезорбції над остеосинтезом. Запропонований лікувально-профілактичний комплекс виявив пародонтопротекторну дію, яка формувалась завдяки його властивостям підвищувати активність лужної фосфатази, вмісту кальцію та фосфору на тлі зниження кислої фосфатази та еластази в кістковій тканині альвеолярних відростків щурів, яким моделювали аліментарний D-дефіцитний пародонтит.

Ключові слова: експеримент, щури, дефіцит вітаміну D, пародонтит, біохімічні показники, метаболізм кісткової тканини.

The work is a fragment of the research project "Improving the diagnosis and treatment of diseases of the oral mucosa in people with chronic somatic diseases", state registration No. 0119U003571.

Micronutrient deficiency or "hidden hunger" is a global health problem that affects more than 2 billion people worldwide [11]. In recent decades, more and more attention has been paid to vitamin D and its relationship with various diseases. Vitamin D deficiency affects more than 80 % of children in developed countries, even in countries with sufficient sunlight [10]. Vitamin D plays a crucial role in the prevention of rickets associated with its deficiency, supports optimal bone health, muscle strength, and

immune function [8, 13]. Numerous studies have shown a correlation between vitamin D and periodontal tissue diseases [9, 12, 14]. The pleiotropic effect of vitamin D on periodontal tissues includes the influence on periodontal pathogens, regulation of the immune response and bone metabolism [6].

On the background of vitamin D insufficiency and deficiency, systemic changes in bone metabolism have been reported: parathyroid hormone secretion increases, and bone remodeling processes are enhanced, which are aimed at increasing bone formation and activating compensatory mechanisms [5].

Scientists are increasingly interested in conducting comprehensive studies to determine the role and participation of vitamin D in many physiological and pathological processes. Finding out new aspects of this vitamin's effect on bone metabolism and remodeling will help develop effective methods for the prevention and treatment of a number of dental diseases.

The purpose of the study was to investigate the effectiveness of preventing remodeling disorders in the bone tissue of the alveolar process of the jaws of rats with periodontitis against the background of vitamin D deficiency.

Materials and methods. The experimental studies were performed in accordance with the requirements of the Law of Ukraine No. 3447-IV of 21.02.2006 "On the Protection of Animals from Cruelty", the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes and the Procedure for conducting experiments and experiments on animals by scientific institutions in compliance with bioethical standards (Decree of the Ministry of Education and Science of Ukraine No. 249 of 01.03.2012). The work was carried out on the basis of the Department of Biochemistry of the SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS").

In the experimental studies, 36 white Wistar rats of both fields were used (two-month-old, weight 100±5 g).

To model D-deficient nutritional periodontitis, the daily diet of white laboratory rats consisted of wheat flour – 43 g, starch – 26 g, sugar – 15 g, skimmed cow's milk cheese – 15 g, table salt – 1 g, 0.02 % EDTA solution, vitamin A 48000 IU/kg of feed was diluted in 0.9 ml of unrefined sunflower oil and mixed with a daily portion of cheese [3]. Control animals were kept on a complete vivarium diet. 3 groups of 12 animals (6 of each sex) were formed: Group 1 – intact; Group 2 – reproduction of the model of D-deficient alimentary periodontitis (DP); Group 3 – reproduction of the model and application of the treatment and prevention complex (TPC). Table 1 shows the composition of the treatment and prevention complex used in the experimental study.

Table 1

Composition of the treatment and prevention complex

Used products	Active ingredients	Manufacturer	Mechanism of action
"Mumiye" gel, 1 time per day, 0.3 ml daily per os	infusion of goldenseal, echinacea infusion, sage infusion, mumijo (tablets), chlorhexidine 0.05 %.	Laboratory for the development and research of oral hygiene products of the SE "ISMFS NAMS" (Conclusion of the State Sanitary and Epidemiological Expertise No. 1378/16 dated 10.12.2019).	anti-inflammatory, adaptogenic, regenerating, osteotropic, anticoagulant properties, increases the rate of salivation, has antibacterial, hemostatic effect; is an immunomodulator
Dietary supplement "Zdorovye" "Strong teeth", 10 mg/100 g of rat daily per os	walnut leaves (207.6 mg); vitamin D3 (0.8 mg); vitamin mixture (A, E, C, B12, B2, nicotinamide, folic acid, etc.) (0.1 mg); magnesium oxide (16.5 mg), calcium carbonate (50 mg).	LLC PTC "Farmakom", Ukraine	anti-inflammatory, bactericidal effect; general strengthening and immunostimulating effect on the body. A source of calcium, magnesium and vitamin D
Aquadetrim vitamin D_3 aqueous solution 10 ml, 7.5-8.0 IU/100 g per rat per os	cholecalciferol; 1 ml (30 drops) of the solution contains: cholecalciferol 15000 IU (1 drop contains approximately 500 IU of vitamin D3);	Medana Pharma S. A., Poland	regulation of calcium and phosphate metabolism, promotes proper mineralization and skeletal growth; participates in the functioning of the immune system, affects the production of lymphokines, active anti-rheumatic factor, etc.

Animals were euthanized on day 60 of the experiment under thiopental anesthesia (20 mg/kg) by total bleeding from the heart. The lower jaws were separated, in which the degree of atrophy of the alveolar ridge was assessed [7]. Biochemical indicators of bone destruction (elastase and acid phosphatase activity)

and remodeling (alkaline phosphatase activity) of bone tissue, calcium and phosphorus content were determined in jaw bone homogenates (75 mg/ml of 0.05 M Tris buffer, pH 7.5) [1, 2].

In the statistical processing of the results obtained, the computer program STATISTICA 6.1 was used to assess their reliability and measurement errors [4].

Results of the study and their discussion. Fig. 1 shows the study results of the degree of atrophy of the alveolar process of the lower jaws of rats.

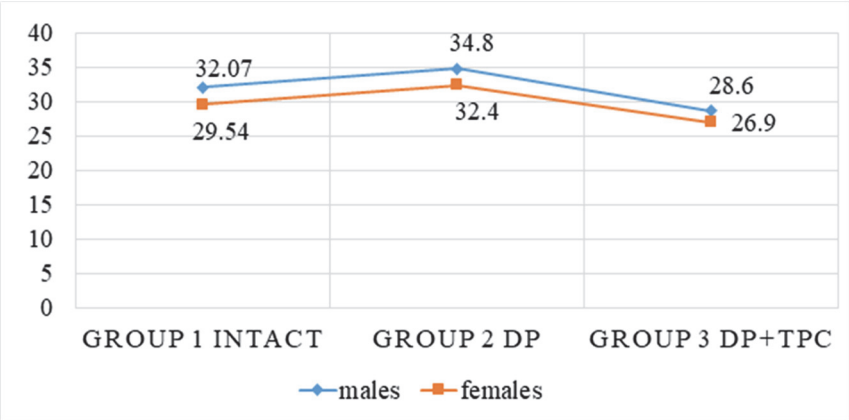

Fig. 1. Influence of TPC on the degree of atrophy of the alveolar process of the lower jaw of rats with periodontitis against the background of alimentary deficiency of vitamin D.

The studies have shown that nutritional deficiency of vitamin D in rats causes significant changes in periodontal tissues. In males this index increased by 8.5 % ($p_1<0.001$), in females – by 9.7 % ($p_1<0.001$). A probable increase in the degree of atrophy of the alveolar process of the jaws is a consequence of increased destructive and resorptive processes in the periodontal bone tissue.

The use of the therapeutic and prophylactic complex contributed to a significant decrease in the degree of atrophy of the alveolar process of the lower jaws of rats compared to group 2 (D-deficient nutritional periodontitis) by 17.8 % ($p_2<0.001$) in males and 17 % ($p_2<0.001$) in females.

The obtained data indicate the development of dystrophic processes in the bone tissue of the jaws of rats with periodontitis caused by nutritional deficiency of vitamin D and the positive effect of the treatment and prevention complex, which effectively inhibited atrophy of the alveolar process of rats.

The next step was to determine the markers of resorption and remodeling of bone tissue in the jaws of rats with periodontitis against the background of nutritional deficiency of vitamin D and after the use of TPC. A common enzyme of osteoclast lysosomes is acid phosphatase, which destroys bone hydroxyapatite at pH 4 to 5.

Nutritional deficiency of vitamin D contributed to an increase in the marker of osteoresorption – the activity of acid phosphatase, which is involved in the destruction of hydroxyapatite in bone tissue (Table 2). Thus, in the bone tissue of the jaws of males of group 2, the index was 22.6 % higher ($p_1<0.002$), and in females – 44.6 % ($p_1<0.001$) higher than in intact animals. The prophylactic use of TPC in animals of group 3 contributed to the inhibition of bone acid phosphatase, increased under conditions of periodontitis, by 26.8 % in females ($p_2<0.001$) and by 16.2 % in males ($p_2<0.002$). The obtained data indicate a decrease in the intensity of bone tissue destruction, stabilization of lysosomal membranes of experimental animals under the influence of TPC, which was more pronounced in females (Table 2).

Elastase is also involved in the destruction of the organic basis of bone. Our studies have shown that in the bone tissue of the jaws of females with periodontitis (group 2 DP), elastase activity increased by 22.8 % ($p_1<0.02$), and in the jaws of males – by 31.5 % ($p_1<0.001$). The results obtained indicate that vitamin D deficiency enhances the degradation of alveolar proteins. More pronounced destruction of both protein and inorganic bone tissue was found in the jaws of males. Therefore, atrophy of the alveolar process in alimentary D-deficient periodontitis is more pronounced in males. The use of the therapeutic and prophylactic complex allowed to reduce the level of elastase activity in the jaws of females by 14.5 % ($p_2<0.02$), and in the bone tissue of males – by 31 % ($p_2<0.001$).

The level of other studied parameters in the bone tissue of the jaws of animals of group 2 decreased: alkaline phosphatase activity in males – by 16.2 % ($p_1>0.1$), in females – by 18.8 % ($p_1<0.05$); calcium content in males – by 10.2 % ($p_1<0.02$), in females – by 8.6 % ($p_1>0.6$); phosphorus content in males – by 25.4 % ($p_1<0.02$), in females – by 17.7 % ($p_1<0.001$). The decrease in these bone tissue parameters explains the increase in atrophy of the alveolar process in rats.

It is known that bone alkaline phosphatase is a marker of functional activity of osteoblasts. The therapeutic and prophylactic complex had a positive effect on the state of mineral metabolism in the bone of the alveolar process under the influence of vitamin D deficiency. Thus, the activity of alkaline phosphatase in males of group 3 increased by 24.2 % ($p_2<0.02$) compared with the values of group 2, and

in females – by 19 % ($p_2<0.05$); calcium content in males – by 9.01 % ($p_2<0.02$), females – by 5.08 % ($p_2>0.4$); phosphorus content in males – by 45.6 % ($p_2<0.001$), females – by 26.7 % ($p_2<0.02$).

Table 2

Effect of treatment and prophylactic complex on the state of mineral metabolism in periodontal bone tissue of rats under conditions of periodontitis reproduction against the background of vitamin D deficiency

Animal groups		Indices				
		acid phosphatase activity, μ-kat/kg	elastase activity, μ-kat/kg	activity of alkaline phosphatase, μ-kat/kg	content calcium, mmol/kg	content phosphorus content, mmol/kg
Group 1 intact	Males n=6	6.63±0.21	16.0±0.48	49.79±2.72	4.69±0.18	1.38±0.08
	Females n=6	6.12±0.15 p>0.1	14.96±0.75 p>0.25	48.70±3.15 p>0.8	4.74±0.15 p>0.6	1.41±0.05 p>0.6
Group 2 DP	Males n=6	8.13±0.38 $p_1<0.002$	21.04±0.96 $p_1<0.001$	41.73±3.13 $p_1>0.1$	4.21±0.10 $p_1<0.02$	1.03±0.07 $p_1<0.02$
	Females n=6	8.85±0.35 p>0.25 $p_1<0.001$	18.37±0.72 p<0.02 $p_1<0.02$	39.55±2.94 p>0.6 $p_1<0.05$	4.33±0.21 p>0.6 $p_1>0.6$	1.16±0.10 p>0.3 $p_1<0.001$
Group 3 DP+TPC	Males n=6	6.81±0.24 $p_1>0.6$ $p_2<0.002$	14.52±0.56 $p_1<0.05$ $p_2<0.001$	51.83±2.88 $p_1>0.6$ $p_2<0.02$	4.59±0.12 $p_1>0.6$ $p_2<0.02$	1.50±0.05 $p_1>0.25$ $p_2<0.001$
	Females n=6	6.48±0.30 p>0.4 $p_1>0.3$ $p_2<0.001$	15.70±0.94 p>0.3 $p_1>0.5$ $p_2<0.02$	47.06±2.27 p>0.2 $p_1>0.7$ $p_2<0.05$	4.55±0.15 p>0.7 $p_1>0.4$ $p_2>0.4$	1.47±0.06 p>0.7 $p_1>0.8$ $p_2<0.02$

Note. p – is the index of significance of differences between males and females; p1 is the index of significance of differences with group 1 (intact); p2 is the index of significance of differences with group 2 (periodontitis)

The imbalance of mineralization processes, namely the activity of bone phosphatases, in D-deficient alimentary periodontitis in rats is demonstrated by the ratio of ALP/AP. In male rats of the intact group, this ratio was 7.5; in females – 7.9. In animals with nutritional deficiency of vitamin D, it decreased to 5.13 and 4.4 in males and females, respectively.

The results of the analysis of the values of the index of the ratio of ALP/AP, indicating the prevalence of osteoresorption over osteosynthesis, showed that in the experimental groups of animals the values of this index were reduced compared to the data of intact rats of both sexes of the first group: by 31.6 % in males and 44.3 % in females.

This study delves into the profound implications of vitamin D on the remodeling of jaw bone metabolism, particularly in the context of periodontal diseases. The TPC used in our study demonstrated significant efficacy, aligning well with the global research narrative on the role of vitamin D in bone health and disease prevention. The anti-inflammatory, adaptogenic, regenerating, osteotropic, immunostimulating, and bactericidal properties of the TPC were pivotal in rectifying the observed disorders of jaw bone remodelling and the state of its antioxidant and prooxidant system in our rat models. Post-prophylaxis, the parameters in rats with periodontitis aligned with normal values, indicating the TPC's potent antiresorption qualities. This echoes findings from other studies, such as those by Povoroznjuk et al. [6] and Jagelavičienė et al. [14], highlighting the multifaceted impact of vitamin D on periodontal health. Our study adds to the growing body of evidence on the correlation between vitamin D levels and periodontal tissue diseases [9, 12, 14], underscoring the pleiotropic effects of vitamin D, which range from influencing periodontal pathogens to regulating immune response and bone metabolism. This is particularly relevant given the alarming prevalence of vitamin D deficiency globally, affecting a significant portion of children even in sun-rich regions [10]. The systemic changes in bone metabolism in the backdrop of vitamin D insufficiency, as reported by Mazur and Novoshytskyy [5], resonate with our findings. The increased secretion of parathyroid hormone and the enhanced bone remodeling processes observed in our study are indicative of the body's compensatory mechanisms in response to vitamin D deficiency. Our results resonate with the works of other researchers, such as Yeroshenko et al. [15], who studied morphological and metric changes in the gastric gland in rats under the influence of a complex of nutritional supplements. These data emphasize the importance of understanding the influence of nutrition on oral health, confirming our assumption about the importance of micronutrients, including vitamin D, in the prevention and treatment of periodontal diseases. Looking forward, the relationship between somatic pathology, changes in the bone system, and dental status in children warrants deeper exploration. Our future research endeavors aim to develop effective prevention

methods for major dental diseases in children, tailored to serum vitamin D levels. This aligns with the broader global research efforts, such as those by Gayer and Smith [11], to understand and leverage micronutrient fortification for better health outcomes. In conclusion, our study not only corroborates global findings on the significance of vitamin D in treating periodontal diseases but also opens ways for targeted interventions and preventive strategies. The path to optimal oral health, especially in pediatric populations, appears to be closely intertwined with the management of vitamin D levels, necessitating an integrated approach to diagnosis and treatment.

Conclusions

1. According to the results of the studies, it can be concluded that nutritional factors, in particular vitamin D deficiency, directly affect bone metabolism.

2. The proposed therapeutic and prophylactic complex in the conditions of reproduction of periodontitis in rats against the background of nutritional deficiency of vitamin D showed periodontal protective effect.

3. The periodontoprotective effect of the used complex was formed due to its properties: to increase the activity of alkaline phosphatase, calcium and phosphorus content against the background of a decrease in acid phosphatase and elastase in the bone tissue of the alveolar processes of rats modeled with alimentary D-deficient periodontitis.

References

1. Goryachkovskiy AM. Klinicheskaya biokhimiya v laboratornoy diagnostike (spravochnoe posobie), Odessa; Ekologiya: 2005: 616 p. [in Russian]

2. Granchuk A, Granchuk G, Gudumak VS. Aktivnost metabolicheskikh protsessov v mandibulyarnykh kostnykh tkanyakh belykh krys pri ispolzovanii koordinatsionnykh soyedineniy tsinka (Eksperimentalnoye issledovaniye, etap 1). Mezhdunarodnyy nauchno-issledovatelskiy zhurnal. 2019;12-2(90): 196–200. doi: 10.23670/IRJ.2019.90.12.041 [in Russian]

3. Kozhemyakin YuM, Khromov OS, Filonenko MA, Sayfetdinova HA. Naukovo-praktychni rekomendatsiyi z utrymannya laboratornykh tvaryn ta roboty z nymy. Kyiv: Avitsenna; 2002. 156 p. [in Ukrainian]

4. Mayboroda RYe. Kompyuterna statystyka: pidruchnyk. Kyiv: VPTS "Kyyivskyi universytet" 2019. 497 p. [in Ukrainian]

5. Mazur IP, Novoshytskyy VYe. Stan tkanyn parodonta u patsiyentiv serednoho viku iz heneralizovanym parodontytom v zalezhnosti vid rivnya vitaminu D. Innovatsii v stomatolohiyi. 2016;3: 2–7 [in Ukrainian]

6. Povoroznyuk VV, Mazur IP, Novoshytskyy VYe. Vitamin D ta zakhvoryuvannya parodonta. Zhurnal "Bil. Suhloby. Khrebet". 2013;3(11). Available from: http://www.mif-ua.com/archive/article_print/37589 [in Ukrainian]

7. Tkachenko YeK, Nikolayeva AV, Novoselskaya NG. Vliyaniye preparatov rastitelnykh polifenolov i vitaminno-mineralnogo kompleksa na sostoyaniye mezhkletochnogo matriksa parodonta i slizistoy obolochki polosti rta krys pri gipoestrogenii. Vestnik stomatologii. 2014;1(86):16–20. [in Russian]

8. Al Khalifah R, Alsheikh R, Alnasser Y, Alsheikh R, Alhelali N, Naji A, et al. The impact of vitamin D food fortification and health outcomes in children: a systematic review and meta-regression. Syst Rev. 2020 Jun 16;9(1):144. doi: 10.1186/s13643-020-01360-3.

9. Bonnet C, Rabbani R, Moffatt MEK, Kelekis-Cholakis A, Schroth RJ. The Relation Between Periodontal Disease and Vitamin D. J Can Dent Assoc. 2019 Mar; 84: j4.

10. Cashman KD, Sheehy T, O'Neill CM. Is vitamin D deficiency a public health concern for low middle income countries? A systematic literature review. Eur J Nutr. 2019 Feb;58(1):433–453. doi: 10.1007/s00394-018-1607-3.

11. Gayer J, Smith G. Micronutrient fortification of food in Southeast Asia: recommendations from an expert workshop. Nutrients. 2015 Jan 19;7(1):646–58. doi: 10.3390/nu7010646.

12. Hu Z, Zhou F, Xu H. Circulating vitamin C and D concentrations and risk of dental caries and periodontitis: A Mendelian randomization study. J Clin Periodontol. 2022 Apr;49(4):335–344. doi: 10.1111/jcpe.13598.

13. Hujoel PP, Lingström P. Nutrition, dental caries and periodontal disease: a narrative review. J Clin Periodontol. 2017 Mar;44 Suppl 18:79–84. doi: 10.1111/jcpe.12672.

14. Jagelavičienė E, Vaitkevičienė I, Šilingaitė D, Šinkūnaitė E, Daugėlaitė G. The Relationship between Vitamin D and Periodontal Pathology. Medicina (Kaunas). 2018 Jun 12;54(3):45. doi: 10.3390/medicina54030045.

15. Yeroshenko GA, Yachmin AI, Shevchenko KV, Lysachenko OD, Riabushko OB, Sokolenko VM, et al. Morphological and metric changes of the glandular apparatus of the rat stomach fundus under the effect of a complex of food additives. World of Medicine and Biology. 2022;1(79);189–194. doi: 10.26724/2079-8334-2022-1-79-189-194

Стаття надійшла 8.11.2022 р.