Levitsky A. P., Selivanskaya I. O., Tishchenko T. L., Stupak O. P. Mineralizing activity of periodontal bone tissue of rats with experimental diabetes. Journal of Education, Health and Sport. 2023;42(1):115-123. eISSN 2391-8306. DOI http://dx.doi.org/10.12775/JEHS.2023.42.01.010
https://apcz.umk.pl/JEHS/article/view/45435
https://zenodo.org/record/8200432

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 1, 2021. No. 32343. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical Culture Sciences (Field of Medical sciences and health sciences); Health Sciences (Field of Medical Sciences and Health Sciences).

Punkty Ministerialne z 2019 - aktualny rok 40 punktów. Załącznik do komunikatu Ministra Edukacji i Nauki z dnia 1 grudnia 2021 r. Lp. 32343. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe:Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).

Received: 10.07.2023. Revised: 19.07.2023. Accepted: .31.07.2023.

UDC 616.71+616.203+579.26

MINERALIZING ACTIVITY OF PERIODONTAL BONE TISSUE OF RATS WITH EXPERIMENTAL DIABETES

A. P. Levitsky[1], I. O. Selivanskaya[2], T. L. Tishchenko[2], O. P. Stupak[3]

[1]Odessa National Academy of Food Technologies

[2]Odessa National Medical University

[3]Ukrainian Medical and Dental Academy, Poltava

Abstract

Background. To determine the effect of diabetes on the state of periodontal bone tissue.

Methods. In rats, diabetes mellitus of the first type (DM1) was reproduced with the help of alloxan and diabetes mellitus of the second type (DM2) with the help of protamine. The activity of alkaline (ALP) and acid (ACP) phosphatases, calcium content, protein and elastase activity were determined in periodontal bone tissue. The level of biochemical markers of inflammation and protective systems was determined in the blood serum and in the gums. The mineralizing activity of bone tissue was determined by the ALP/ACP ratio.

Results. It was established that the mineralizing activity of periodontal bones is significantly higher in males and increases with age. The reproduction of DM1 and DM2 in rats caused a significant decrease in the mineralizing activity of periodontal bone tissue and insignificant changes in the level of calcium and protein. In rats with diabetes, an

inflammatory process develops in the gums (increase in the level of elastase) against the background of a decrease in the level of markers of protective systems (catalase and lysozyme).

Conclusion. A decrease in the level of mineralizing activity of periodontal bone tissue under conditions of diabetes can be the reason for the development of diabetic periodontitis.

Keywords: diabetes mellitus; periodontitis; bone tissue; mineralizing activity.

Introduction

Dental pathology is a frequent complication of diabetes [1, 2]. The most important are periodontal lesions, namely, diabetic periodontitis [3].

As is known, the most important factor in the pathogenesis of periodontitis is the atrophy of the periodontal bone tissue, which causes the appearance of periodontal pockets in which the periodontal-pathogenic microflora is concentrated [4-6].

Atrophy of bone tissue is the result of an imbalance in the processes of osteogenesis and osteolysis [7].

Osteogenesis is carried out due to the action of osteoblasts and osteocytes, and osteolysis occurs under the influence of osteoclasts [7, 8].

One of the biochemical markers of osteoblasts is the enzyme alkaline phosphatase, and the biochemical marker of osteoclasts is acid phosphatase [9].

On this basis, we proposed a method for determining the mineralizing activity of bone tissue based on the ratio of alkaline and acid phosphatase activities [10].

The purpose of this work was to determine the mineralizing activity of the alveolar part of the periodontal bone tissue of rats in which type 1 diabetes (DM1) and type 2 diabetes (DM2) were reproduced.

Materials and research methods

Biological methods were carried out on white Wistar rats of different sexes and ages. Experimental diabetes mellitus type 1 was reproduced using the drug alloxan (100 mg/kg, once intraperitoneally, 2 weeks) [11]. Diabetes mellitus type 2 was reproduced with the help of protamine sulfate (18 mg/kg, intramuscularly, 2 weeks) [12].

After euthanasia of animals under thiopental anesthesia (20 mg/kg) by total bleeding from the heart, the alveolar part of the lower jaw was isolated, in the homogenate of which the activity of alkaline phosphatase (ALP) was determined by the hydrolysis of p-nitrophenyl phosphate pH 10.5 and the activity of acid phosphatase by hydrolysis of this substrate pH 4.8 [13].

The ratio of ALP/ACP activity was used to calculate the mineralizing indices bone tissue [10].

The content of calcium [13], protein [13] and elastase activity [14] were also determined in the bone tissue homogenate.

The content of glucose, malondialdehyde (MDA), activity of elastase, catalase, urease and lysozyme was determined in blood serum by various methods [14, 15]. Based on the ratio of elastase activity and MDA content, the antioxidant-prooxidant index of API was calculated [14].

All the above biochemical parameters (except glucose) were also determined in the homogenate of the gums of rats.

Statistical processing of the results was carried out by generally accepted methods [16].

Results and discussion

Table 1 presents the results of determination of glucose content in blood serum, the level of which significantly increases in rats with diabetes mellitus 1 (by 52 %) and with diabetes mellitus 2 (by 21,5 %).

Table 1. Biochemical indicators of blood serum of rats with experimental diabetes of the first type (DM1) and the second type (DM2)

Biochemical indicators	DM1, ♂ 3 months		DM2, ♀ 10 months	
	control	experiment	control	experiment
Glucose, mmol/l	7,5±0,5	11,4±0,5 p<0,01	6,05±0,19	7,35±0,38 p<0,05
Elastase, mk-cat/l	161,3±8,5	210,5±10,2 p<0,01	120±3	149±11 p<0,05
MDA, mmol/l	1,39±0,07	1,70±0,05 p<0,05	0,96±0,03	1,09±0,08 p>0,05
Catalase, mcat/l	0,41±0,01	0,36±0,01 p<0,05	0,43±0,02	0,35±0,02 p<0,05
Urease, mk-cat/l	2,07±0,55	2,37±0,34 p>0,3	2,1±0,6	2,8±0,8 p>0,3
Lysozyme, unit/l	105±3	89±3 p<0,05	84±8	59±5 p<0,05
API	2,95±0,18	2,12±0,19 p<0,05	4,48±0,21	3,21±0,28 p<0,05

The activity of elastase also increases significantly, which indicates the development of systemic inflammation [14], but the level of markers of protective systems decreases significantly: the activity of the antioxidant enzyme catalase and the antimicrobial enzyme lysozyme.

The API index also decreases significantly, which indicates a violation of the balance of antioxidant and pro-oxidant factors.

Table 2 presents the results of the determination of biochemical markers in the gum homogenate. These data show that the activity of elastase significantly increases in the gums of diabetic rats: by 18.3 % (DM1) and 55.5 % (DM2). Urease activity also increases significantly: by 91.6 % (DM1) and by 69.4 % (DM2). These data indicate the growth of microbial insemination of the gums and the development of inflammation in them under the conditions of diabetes.

Table 2. Biochemical indicators of the gums of rats with experimental diabetes of the first type (DM1) and the second type (DM2)

Biochemical indicators	DM1, ♂ 3 months		DM2, ♀ 10 months	
	control	experiment	control	experiment
Elastase, mk-cat/kg	34,5±1,5	40,8±1,9 p<0,05	23,8±1,3	37,0±1,3 p<0,01
MDA, mmol/kg	18,9±1,6	22,9±1,5 p>0,05	8,97±0,79	12,45±0,57 p<0,05
Catalase, mcat/kg	6,26±0,31	6,77±0,34 p>0,1	7,60±0,20	6,68±0,29 p<0,05
Urease, mk-cat/kg	0,95±0,24	1,92±0,22 p<0,05	0,49±0,11	0,83±0,10 p<0,05
Lysozyme, unit/kg	196±14	167±16 p>0,05	132±9	101±4 p<0,05
API	3,31±0,20	2,96±0,19 p>0,05	8,47±0,40	5,38±0,31 p<0,01

In fig. 1 shows how the mineralizing activity of the periodontal bone tissue of rats changes depending on age and sex. It can be seen that with age the mineralizing activity of bone tissue increases, and it is much greater in males Perhaps it is this circumstance that determines the significantly greater number of bone fractures in elderly women compared to men [17].

Table 3 presents the results of determining biochemical indicators of periodontal bone tissue in rats under conditions of experimental diabetes. It can be seen that in all cases the activity of acid phosphatase increases significantly in males with DM1 by 44.4 %, in males with DM2 by 40.4 %, and in females with DM2 by 60 %. The activity of alkaline phosphatase significantly decreases under the conditions of 1DM in males: by 40.1 % and in females by 23.7 %. In females with DM2, the activity of alkaline phosphatase decreases by 38 %, but $p>0.05$.

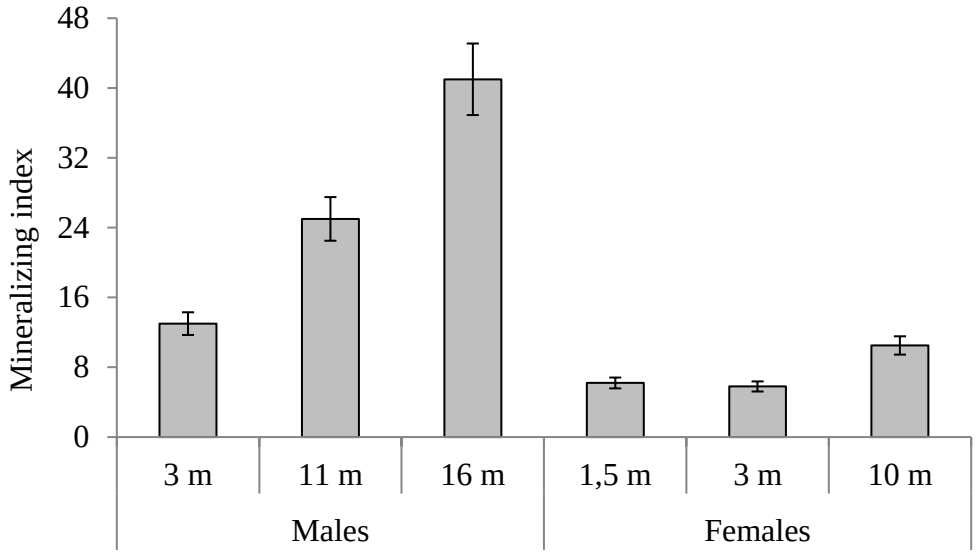

Fig. 1. Mineralizing index of periodontal bone tissue of rats of different ages and sexes

Table 3. Biochemical indicators of periodontal bone tissue of rats with experimental diabetes mellitus type 1 (DM1) and type 2 (DM2)

Biochemical indicators	Diabetes mellitus 1 type (DM1)				DM2	
	Males 3 month		Females 15 month		Females 10 month	
	control	experiment	control	experiment	control	experiment
Alkaline phosphatase, m-cat/kg	93,0±17,2	55,7±10,0 p<0,05	89,9±13,2	68,6±15,5 p>0,2	43,1±13,2	26,7±6,3 p>0,05
Acid phosphatase, m-cat/kg	7,2±0,8	10,4±0,6 p<0,05	14,6±1,4	21,5±1,7 p<0,05	4,00±0,23	6,4±0,23 p<0,01
Calcium, mmol/kg	1,55±0,14	1,39±0,09 p>0,3	2,91±0,14	2,79±0,18 p>0,3	2,03±0,17	2,25±0,10 p>0,05
Protein, gr/kg	24,6±2,4	20,7±1,5 p>0,05	–	–	14,8±1,5	17,7±1,3 p>0,05
Elastase, m-cat/kg	14,5±1,7	14,0±1,8 p>0,5	–	–	9,0±0,9	12,2±0,7 p<0,05

It is important to emphasize that, unlike males, the activity of another biochemical marker of osteoclasts, the enzyme elastase, significantly increases by 35.6 % in females with DM2.

The content of calcium and protein in the bone tissue of the periodontium in rats under conditions of diabetes does not change significantly.

In fig. 2 shows the level of mineralizing activity of the periodontium in rats with diabetes. It can be seen that in all cases, the mineralizing activity is significantly reduced in rats with diabetes: by 61.9 % in males with diabetes mellitus 1, by 48.4 % in females with

diabetes mellitus 1, and by 61.3 % in females with diabetes mellitus 2.

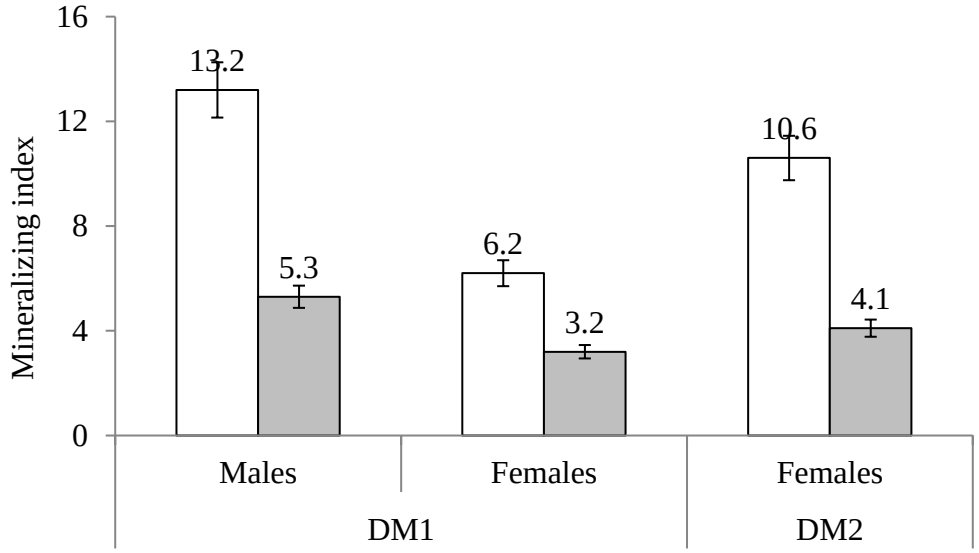

Fig. 2. Mineralizing index of periodontal bone tissue in rats with type 1 diabetes (DM1) and type 2 diabetes (DM2)

It has been established that lipid metabolism disorders play an important role in the pathogenesis of diabetogenic complications [18], especially against the background of dysbiotic syndrome [19].

It is possible that a decrease in the level of mineralizing activity of periodontal bone tissue may be a consequence of a deficiency of ω-3 polyunsaturated fatty acids, which stimulate osteogenesis through the activation of the membrane enzyme Ca-ATP-ase [20].

Published works showing the therapeutic and preventive role of ω-3 polyunsaturated fatty acid preparations in patients with periodontitis [21, 22].

Further research will allow to expand the search for effective means to stimulate the mineralizing activity of periodontal bone tissue, which can significantly increase the effectiveness of periodontitis prevention.

Conclusion

1. The mineralizing activity of bone tissue can be determined by the ratio of activities of alkaline and acid phosphatases.

2. The level of mineralizing activity is much higher in male rats, and it increases with the age of the animals.

3. In rats with experimental diabetes of the first and second type, the mineralizing activity of periodontal bone tissue is significantly reduced.

4. The content of calcium and protein in the bone tissue of the periodontium of rats

with diabetes does not change significantly.

5. In rats with diabetes, the level of markers of inflammation (elastase and urease) increases in the gums and blood serum, and the level of indicators of the protective systems (catalase and lysozyme) decreases.

Author Contributions

The authors agree on equal distribution of partial participation.

Funding

This research received no external funding.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All information is publicly available and data regarding this particular patient can be obtained upon request from corresponding senior author.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors declare that there are no conflicts of interest.

References

1. Mashchenko IS. Paradontal diseases – Dneproprtrovsk: KOLO, 2003: 272. (in Russian)

2. Bouchard P, Carra MC, Boillot A [and others]. Risk factors in periodontology: a conceptual framework. Journal of Clinical Periodontology. 2017;44(2): 125-131.

3. Iacopino AM. Diabetic periodontitis: possible lipid-induced defect in tissue repair through alteration of macrophage phenotype and function. Oral Dis. 1995;1(4):214-229.

4. Buslo AM, Sylenko JuI. Approaches to pathogenetic treatment of generalized periodontitis (literature review). Ukrainian dental almanac. 2001;5:75-77. (in Ukrainian)

5. Gereljuk VI, Nejko NV, Pavljuk TD. Osteotropic therapy in periodontology. Guidelines. – Ivano-Frankivs'k, 2000:23. (in Ukrainian)

6. Hernandez CJ, Guss JD, Luna M, Goldring SR. Links Between the Microbiome and Bone. Journal of Bone and Mineral Research. 2016;31(9):1638-1646.

7. Florencio-Silva R, Sasso GR, Sasso-Cerri E [and others]. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. BioMed Research International.

2015:421746. https://doi.org/10.1155/2015/421746

8. Thong BKS, Ima-Nirwana S, Chin K-Y. Proton Pump Inhibitors and Fracture Risk: A Review of Current Evidence and Mechanisms Involved. Int J Environ Res Public Health. 2019;16(9):1571. doi: 10.3390/ijerph16091571.

9. Levitsky AP, Pupin TI, Selivanskaya IO [and others]. Reduction of mineralizing activity of periodontal bone tissue in rats with consumption of ordinary sunflower oil. Journal of Education, Health and Sport. 2022;12(12):323-330. DOI http://dx.doi.org/10.12775/JEHS.2022.12.12.049

10. Levitsky AP, Makarenko OA, Khodakov IV, Zelenina YuV. The enzymatic method of the estimation of the state of osseous tissue. Odessa Medical Journal. 2006;3:17-21. (in Ukrainian)

11. Stefanov OV. The preclinical investigations of medicines. – Kiev: DFC, Avicena, 2001:527. (in Ukrainian)

12. Levitsky AP, Tsisel's'kiy YuV, Tsisel's'ka OYu [and others]. The method of simulation of II type diabetes mellitus. Patent of Ukraine 85843. IPC (2013.01) A61B 5/00, A61B 5/00. Publ.: 10.12.2013. Bul. № 23. (in Ukrainian)

13. Levitsky AP, Makarenko OA, Denga OV [and others].The experimental methods of the study of osteogenesis stimulators. Guidelines. – Kiev: GFK, 2005:50. (in Russian)

14. Levitsky AP, Makarenko OA, Demyanenko SA. Methods of experimental dentistry (teaching aid). Simferopol, Tarpan, 2018: 78. (in Russian)

15. Goryachkovskiy AM. The clinical biochemistry in laboratorial diagnostics] [3rd ed.]. − Odessa, Ekologiya, 2005:616. (in Russian)

16. Truhacheva NV. Mathematical Statistics in biomedical research using application package Statistica. – Moskva: GJeOTAR-Media, 2012:379. (in Russian)

17. Wright NC, Looker AC, Saag KG [and others]. The Recent Prevalence of Osteoporosis and Low Bone Mass in the United States Based on Bone Mineral Density at the Femoral Neck or Lumbar Spine. Journal of Bone and Mineral Research. 2014;29(11):2520-2526.

18. Tian L, Yu X. Lipid metabolism disorders and bone dysfunction - interrelated and mutually regulated (Review). Molecular Medicine Reports. 2015;12:783-794.

19. Das M, Cronin O, Keohane DM [and others]. Gut microbiota alterations associated with reduced bone mineral density in older adults. Rheumatology. 2019;58(12): 2295-2304.

20. Sepúlveda MR, Berrocal-Carrillo M, Gasset M, Mata AM. The Plasma Membrane Ca2+-ATPase Isoform 4 Is Localized in Lipid Rafts of Cerebellum Synaptic Plasma

Membranes. J Biol Chem. 2006;281:447-453.

21. Chee B, Park B, Fitzsimmons T [and others]. Omega-3 fatty acids as an adjunct for periodontal therapy-a review. Clin Oral Investig. 2016;20(5):879-94. doi: 10.1007/s00784-016-1750-2.

22. Kruse AB, Kowalski CD, Leuthold S [and others]. What is the impact of the adjunctive use of omega-3 fatty acids in the treatment of periodontitis? A systematic review and meta-analysis. Lipids in Health and Disease 2020 19:100.