ORIGINAL ARTICLE

MORPHOLOGICAL CHANGES OF PERIODONTAL COMPONENTS UNDER EXPERIMENTAL LIPOPOLYSACCHARIDE PERIODONTITIS COMBINED WITH HYPERTHYROIDISM

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ABSTRACT

The aim: Investigate structural changes in the tissues of the periodontal complex under the condition of experimental lipopolysaccharide periodontitis combined with hyperthyroidism.

Materials and methods: The studies were performed on adult white male rats, which simulated periodontitis combined with hyperthyroidism. Periodontal tissues were subjected to morphological examination on the 22nd day of the experiment. Collection of material for microscopic examinations was performed according to the generally accepted method; histological specimens were studied using a light optical microscope.

Results: Morphological studies of the components of the periodontal complex of experimental animals with experimental periodontitis established the reorganization of its structural elements. Damage to the epithelium in the area of attachment of the circular ligament and erosive-ulcerative changes of the gums led to a deepening of the gingival sulcus with the formation of a deep periodontal pocket. Intense hyperkeratosis was observed in the area of the bottom of the periodontal pocket. In the own plate of the mucous membrane of the gums – significant edema, collagen fibers were disorganized, defragmented. There were pronounced destructive-degenerative and inflammatory changes of the epithelial and own plates of all areas of the gums and periodontium, damage to the nuclei and cytoplasm of keratinocytes, fibroblasts, and leukocytes.

Conclusions: Experimental periodontitis combined with hyperthyroidism is accompanied by pronounced signs of destructive and inflammatory changes in the soft and dense tissues of the periodontal complex, as well as disruption of stromal-vascular interactions, which progress from reversible to irreversible disruption of periodontal connective tissue.

KEY WORDS: periodontitis, hyperthyroidism, periodontal complex, morphological changes, connective tissue

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INTRODUCTION

Periodontal diseases are the second most common dental diseases in the world and tend to be widespread regardless of the age, sex and place of residence of patients. According to the WHO, intact periodontitis occurs in only 10% of examined patients, periodontitis of moderate severity – in 25-45%, severe – in 5-20% of cases, with the prevalence of periodontal disease in the world in the age group 30- 50 years is 94.3% [1].

The phenomena of dysbiosis and inflammation play an important role in the pathogenesis of periodontitis [2]. Microorganisms secrete biologically active substances, toxins and enzymes (proteases, chondroitin sulfatase, hyaluronidase), which have highly toxic, allergenic and necrotic properties, which leads to inflammatory and destructive processes [3-5]. At the same time, the intensity of the inflammatory reaction is largely determined by the ability of the macroorganism to resist the influence of pathogenic microflora [6, 7]. The rate of destruction of periodontal tissues in the disease depends on the anatomical and physiological characteristics of the periodontium. The problem of morphogenesis of chronic generalized periodontitis remains unsolved today. In clinical conditions, it is impossible to obtain fragments of all periodontal tissues for examination at different stages of the disease. In experimental conditions is limited information about the state of the periodontium in the reproduction of this inflammatory disease [8].

THE AIM

Investigate structural changes in the tissues of the periodontal complex under the condition of experimental lipopolysaccharide periodontitis combined with hyperthyroidism.

MATERIALS AND METHODS

The experiments were performed on outbred adult white male rats weighing 180-200 g, which were kept on a standard diet of vivarium. The Commission on Bioethics of I. Horbachevsky Ternopil National Medical University (protocol № 56 from 08.01.2020) violations of moral and ethical norms during the research work were not detected. To model periodontitis in animals for 2 weeks every other day was injected into the gum tissue of 40 microliters (1 mg / ml) of lipopolysaccharide (LPS) *E. Coli* ("Sigma-Aldrich", USA) (n = 12) [9]. To simulate the experimental hyperfunction of

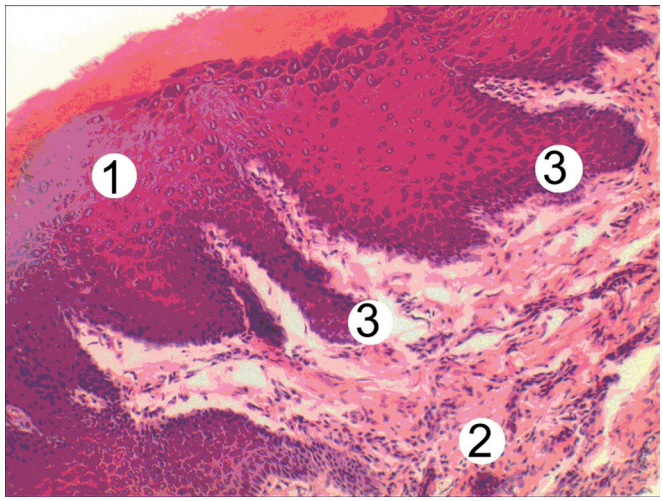

Fig. 1. Rat gums with experimental periodontitis combined with hyperthyroidism. Staining with hematoxylin and eosin. x100.
Notes: 1 – epithelial plate of the gums;
2 – histoleukocytic infiltration;
3 – acanthic strands.

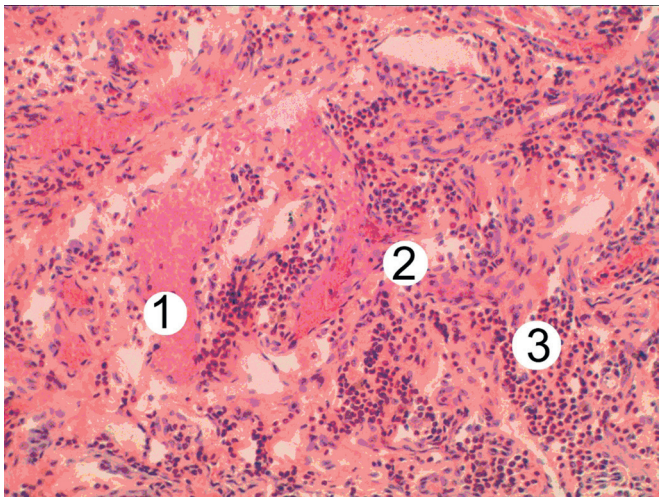

Fig. 2. Microscopic changes of microcirculatory vessels of periodontal complex with experimental periodontitis combined with hyperthyroidism. Staining with hematoxylin and eosin. x200
Notes: 1 – blood supply and stasis in the vessels; 2 – angiomatosis;
3 – hemorrhage; 4 – histoleukocytic infiltration.

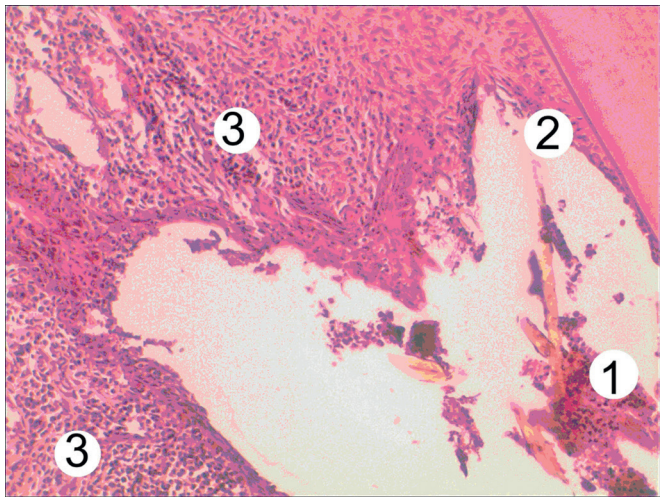

Fig. 3. Microscopic changes of rat periodontium with experimental periodontitis combined with hyperthyroidism. Staining with hematoxylin and eosin. x200.
Notes: 1 – necrotic masses in the periodontal pocket;
2 – destruction of the epithelial plate in the area of attachment;
3 – leukocyte infiltration of its own plate.

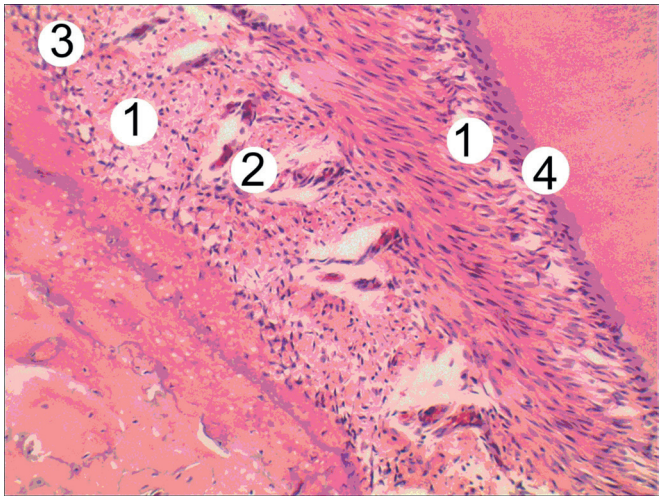

Fig. 4. Microscopic changes of in the bone tissue of the alveolar process with experimental periodontitis combined with hyperthyroidism. Staining with hematoxylin and eosin. x100.
Notes: 1 – hyperplasia and fibrosis of the periodontium;
2 – angiomatosis and sagging of the lumen of blood vessels,
3 –osteoblasts hyperplasia, 4 – cementum.

the thyroid gland, the animals were daily injected intragastrically with L-thyroxine in 1% starch solution at a rate of 10 μg / day per 100 g of body weight for 21 days. Starting from the 8th day of the experiment, rats were injected with LPS in the gum tissue for 2 weeks. Euthanasia of rats was performed by bloodletting under conditions of thiopental-sodium anesthesia on the 22nd day from the beginning of the experiment. All manipulations with experimental animals were carried out in accordance with the rules in accordance with the "European Convention for the protection of vertebrate animals used for research and other scientific purposes" [10]. To confirm the state of hyperthyroidism, the content of free thyroxine (FT4), free triiodothyronine (FT3) and thyroid-stimulating hormone (TSH) was determined by enzyme-linked immunosorbent assay using "Vector-Best" kits (Russia).

Histological examinations were performed on the basis of the morphological sector of the Interdepartmental educational and research laboratory of the I. Horbachevsky Ternopil National Medical University. Collection of material for microscopic examinations was performed according to conventional methods [11]. Organocomplexes were removed (frontal group of teeth of both jaws: alveolar processes with mucous membrane), fixed in 10% formalin solution, histological sections were prepared after previously performed decalcification, stained with hematoxylin-eosin [12]. Histological specimens were studied

using a MIKROmed SEO SCAN optical microscope and photodocuted using a Vision CCD Camera with an image output system from histological specimens.

RESULTS

Histological examination of periodontal tissues of rats with lipopolysaccharide inflammation combined with hyperthyroidism revealed significant changes in the structural components of the periodontal complex. In particular, destructive changes were observed in the epithelial plate of the mucous membrane of the gums of all departments and there were areas of hyperkeratosis. In the free area of the gums, areas of hyperkeratosis with enhanced desquamation and local ulcerative-necrotic changes of the epithelial plate were determined. The phenomena of acanthosis were also observed – epithelial cords were formed, which were deeply immersed in their own plate of the mucous membrane, and the connective tissue papillae were smoothed, thickened, rounded (Fig. 1). The basal layer of the epithelium was poorly contoured, which indicates the stratification and destruction of the basement membrane. Migrating, intraepithelial lymphocytes were identified throughout the epithelial layer, singly or in groups, indicating changes in the inflammatory nature.

Damage to the epithelium in the area of attachment, remodeling of the circular ligament and erosive-ulcerative changes of the gums led to a deepening of the gingival sulcus with increased inflammatory changes and the formation of a deep periodontal pocket. There was intense hyperkeratosis with exfoliation of the scales of the stratum corneum, detrital masses of keratin and fibrin of the epithelial plate in the area of the bottom of the periodontal pocket. Significant edema was observed in the lamina propria of the gingival mucosa, collagen fibers were disorganized, defragmented, often formed thick bundles, areas of lysis and thinning were revealed. Also in the area of the attached part of the gums were identified areas of ingrowth of connective tissue of its own plate into the epithelium – acanthosis. During this period of the experiment, disorders in the vessels of the hemomicrocirculatory tract were characterized by stasis and sludge effects or neglect of some microvessels, multiple agiomatosis. Violations of the integrity of the vessel wall and focal hemorrhages were detected (Fig. 2).

Changes of destructive-degenerative nature were observed in the deep layers of the periodontium. Periodontitis was characterized by hyperplasia, edema and stratification of collagen fibers, fragmentation, destruction of the fibrous structures of the circular ligament, which together with vascular and inflammatory disorders led to the expansion and deepening of the periodontal pocket (Fig. 3).

Diffuse leukohistiocytic infiltration was detected in the bony and white root layers. Detachment of fibrous structures of the periodontium from the periosteum of the alveolar process was observed, especially in the bony layer. Hemodynamic abnormalities in the periodontium were also characterized by stasis, sludge, collapse, and diapedetic hemorrhage. Changes in the bone tissue of the dental alveoli were manifested by lacunar resorption, with a decrease in the height of the alveolar membrane. Areas of growth of bone tissue into connective tissue were detected, which indicated damage to the organization and metabolic processes in the bone tissue of the alveolar process. Osteofibrosis of the Haversian canals was detected zonally. In the cement of the apex of the tooth root in the primary and secondary layers, the phenomena of hypercementosis were detected (Fig. 4).

DISCUSSION

The rate of destruction of periodontal tissues in inflammatory diseases depends on the anatomical and physiological features of the periodontium. Despite the large number of studies, the problem of morphogenesis of chronic generalized periodontitis remains unresolved today, because in clinical conditions it is impossible to obtain fragments of all periodontal tissues for study at different stages of the disease [13, 14]. Lipopolysaccharide inflammation of the periodontium in rats is accompanied by an increase in the level of endogenous intoxication, as indicated by an increase in the content of medium weight molecules [4]. Imbalance of thyroid hormones exacerbates endogenous intoxication in lipopolysaccharide periodontitis, especially in hyperthyroidism, which is further accompanied by connective tissue catabolism, as evidenced by a probable increase in collagenolytic activity, increased content of free oxyproline, growth of free oxyproline, growth increasing the content of markers of the breakdown of proteoglycans – glycosaminoglycans in the serum [15]. Thyroid dysfunction exacerbates the destruction of connective tissue in lipopolysaccharide inflammation of the periodontium in both hyperthyroidism and hypothyroidism.

Many researchers note that hyperplasia of the thyroid gland affects the formation and mineralization of tooth hard tissues, metabolic processes in periodontal tissues, morpho-functional state of salivary glands [16, 17]. It should be noted that in periodontitis there are violations of bone metabolism by increasing both the processes of resorption and bone formation. Thyroid dysfunction exacerbates disorders of bone metabolism in terms of lipopolysaccharide inflammation of the periodontium: hyperthyroidism is characterized by a simultaneous increase in the processes of resorption and bone formation [18]. The progression of destructive-degenerative processes leads to a weakening of the tone of microvessels with a violation of the ultrastructure of the wall and is accompanied by local endothelial dysfunction. That is why one of the pathogenetic factors in the development of periodontitis is a violation of microcirculation in periodontal tissues. Hemomicrocirculatory disorders cause the development of metabolic disorders in the periodontium, dystrophic and degenerative changes, initiate inflammatory reactions [19]. These factors lead to damage to the vascular wall, an increase in the number of dormant capillaries, parietal prolapse of thrombotic masses, disruption of transport

systems in the walls of blood vessels, and others. An important place in the pathogenesis of microcirculation disorders is occupied by LPS, which leads to disintegration and destabilization of the bilipid layer of endothelial cell membranes, disrupting their functional activity, causing electrical imbalance [20]. Oxidative stress causes damage to the vascular wall and endothelial dysfunction [21]. Under the condition of hyperthyroidism there is a direct stimulation of bone cells due to the high concentration of thyroid hormones, there is hypercalcemia.

The results of our studies are consistent with the authors, who found that thyroid dysfunction complicates the course of chronic generalized periodontitis [22, 23]. It should be noted that in experimental hypo- and hyperthyroidism there is a dependence of the severity of the lesion in periodontal tissues on the state of nonspecific resistance. Morphological studies of the periodontal complex in animals with thyroid dysfunction showed earlier and more pronounced pathological inflammatory-destructive changes with periodontitis relative to the control group of animals without this concomitant pathology [24].

CONCLUSIONS

Morphological studies of periodontal components under experimental lipopolysaccharide periodontitis combined with hyperthyroidism revealed signs of destructive and inflammatory changes in the soft and dense tissues of the periodontal complex, manifested by the formation of deep periodontal pockets with perforation into deeper tissues, and disturbance of microcirculation vascular interactions that progress from reversible to irreversible disorganization of periodontal connective tissue. There is a remodeling of dense periodontal tissues, which is manifested by the destruction and development of the resorptive process in bone tissue.

REFERENCES

1. Pavlenkova E., Pavlenko S., Tkachenko I., Sidorova A. Clinical and laboratory study of the use of the drug based on the antiseptic Troklosene for the treatment of patients with chronic generalized periodontitis II and III severity. Wiad Lek. 2018;71(4):855-860.
2. Lenartova M., Tesinska B., Janatova T. et al. The Oral Microbiome in Periodontal Health. Front Cell Infect Microbiol. 2021;11:629723.
3. Demkovych A. Effects of flavonol quercetin on activity of lipid peroxide oxidation in experimental bacterial-immune periodontitis. Interv Medicine and Applied Science. 2019;11(1):55-59.
4. Demkovych A., Shcherba V., Yaremchuk O. et al. Effects of flavonol quercetin on syndrome of endogenous intoxication in experimental periodontitis. Pharmacia. 2021;68(3):627-632.
5. Demkovych A., Bondarenko Yu., Hasiuk P. Effects of quercetin on antioxidant potential in the experimental periodontitis development. Interventional Medicine and Applied Science. 2019;11(1):60-64.
6. Slots J. Periodontitis: facts, fallacies and the future. Periodontol. 2000. 2017;75(1):7-23.
7. Krynytska I., Marushchak M., Svan O. et al. The indices of endogenous intoxication in rats with carrageenan solution consumption. Georgian Med News. 2018;279:196-200.
8. Avetisyan A., Markaryan M., Rokaya D. et al. Characteristics of Periodontal Tissues in Prosthetic Treatment with Fixed Dental Prostheses. Molecules. 2021;26(5):1331.
9. Keong J.Y., Low L.W., Chong J.M. et al. Effect of lipopolysaccharide on cell proliferation and vascular endothelial growth factor secretion of periodontal ligament stem cells. Saudi Dent J. 2020;32(3):148-154.
10. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Council of Europe. Strasbourg. 1986;123:152.
11. Hasiuk P., Hasiuk N., Kindiy D. et al. Characteristics of cellular composition of periodontal pockets. Interventional Medicine & Applied Science. 2016;8(4):172-177.
12. Hasiuk P.A., Vorobets A.B., Demkovych A.Ye. et al. Features of occlusal correlations of molars in the dental clinic. Wiad Lek. 2021;74(5):1130-1133.
13. Yi G., Ma Y., Chen Y. et al. A Review of the Functions of Matrix Vesicles in Periodontal Tissues. Stem Cells Dev. 2021;30(4):165-176.
14. Rahangdale S.I., Galgali S.R. Periodontal status of hypothyroid patients on thyroxine replacement therapy: A comparative cross-sectional study. J Indian Soc Periodontol. 2018;22(6):535-540.
15. Demkovych A., Bondarenko Yu.I., Fastovets O.O. et al. State of collagenolysis in experimental periodontitis of bacterial-immune genesis and its correction with flavonol. Medicni Perspektivi. 2021;26(2):26–32.
16. Greabu M., Giampieri F., Imre M.M. et al. Autophagy, One of the Main Steps in Periodontitis Pathogenesis and Evolution. Molecules. 2020;25(18):4338.
17. Redman R.S., Bayley N.C., Nylén E.S. Salivary and serum biomarkers of inflammation in a man with metastatic medullary thyroid carcinoma and hyperreactive gingiva: a fourteen year odyssey. Biotech Histochem. 2019;94(6):389-397.
18. Williams G.R., Bassett J.H.D. Thyroid diseases and bone health. J Endocrinol Invest. 2018;41(1):99-109.
19. Kolesnikova L.R., Darenskaya M.A., Kolesnikova L.I. et al. Morphofunctional State of the Periodontal Tissues in Humans and Rats with Arterial Hypertension. Bull Exp Biol Med. 2020;169(6):831-835.
20. Hasiuk P., Malko N., Vorobets A. et al. The intensity of chronic catarrhal gingivitis in children depending on the age. Wiadomości Lekarskie. 2020; 73(5): 846-849.
21. Demkovych A., Bondarenko Yu., Hasiuk P.A. Oxidative modification of proteins in the process of experimental periodontitis development. Interventional Medicine and Applied Science. 2017;(9)4:218-221.
22. Koman A., Näsman P., Discacciati A. et al. Increased risk for tooth extraction in primary hyperparathyroidism and hypercalcemia: a population study. Clin Oral Investig. 2020;24(8):2755-2761.
23. Aldulaijan H.A., Cohen R.E., Stellrecht E.M. et al. Relationship between hypothyroidism and periodontitis: A scoping review. Clin Exp Dent Res. 2020;6(1):147-157.
24. Bhankhar R.R., Hungund S., Kambalyal P. et al. Effect of nonsurgical periodontal therapy on thyroid stimulating hormone in hypothyroid patients with periodontal diseases. Indian J Dent Res. 2017;28(1):16-21.

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Conflict of interest:
The Authors declare no conflict of interest.

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