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Submicroscopic changes of periodontal components under experimental periodontitis combined with hypothyroidism

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Endocrine disorders are an extremely urgent medical and social problem of modern society. Changes in the thyroid gland activity cause violations of the regulation of metabolism in periodontal tissues. Expressed clinical signs of periodontal tissues inflammation directly depend on the severity and duration of disorders of the functional state of the thyroid gland. The purpose of this study was to describe the ultrastructural changes of periodontal tissues under the conditions of experimental periodontitis in combination with hypothyroidism. To model periodontitis lipopolysaccharide was injected into the gum tissue of rats. The experimental thyroid gland hypofunction was simulated using intragastrical administration of mercazolyl. To confirm the state of hypothyroidism we determined the content of free thyroxine, free triiodothyronine and thyroid-stimulating hormone. Submicroscopic studies revealed profound destructive-degenerative changes in all components of the periodontium. Changes in periodontal tissues in experimental hypothyroidism are stereotyped: dystrophic, atrophic and destructive changes in parenchymal structures, mucinous edema of the stroma, mild reparative processes. Thus, it can be concluded that during experimental periodontitis in combination with hypothyroidism, there are violations of the functioning of all the components of the vessels of the periodontal microcirculatory channel. This is manifested by damage and deformation of the blood vessel wall with desquamation of the endothelium, edema, thinning of the basement membrane, stasis, thrombosis and spasm. Also, significant destruction and lysis of fibrous structures and components of the connective tissue amorphous substance in the lamina propria of the attached and free part of gums and periodontium are characteristic.

Key words: periodontitis; hypothyroidism; periodontium; ultrastructural changes.

INTRODUCTION

Among all dental pathologies, periodontal diseases occupy one of the leading places, taking into account their spread among the population and its negative consequences (loss of teeth and dysfunction of the dental system) [1, 2]. The severity of generalized periodontitis, which according to epidemiological studies affects from 80 to 90% of the world's population over the age of 40, frequent recurrences and complications lead to reduced quality of life of patients, significant socio-economic losses, impaired masticatory function and speech [3, 4].

Thyroid diseases are now firmly established in one of the leading places among endocrine pathology in terms of overall morbidity and prevalence in the world [5]. According to official WHO data, about 1.5 billion people suffer from thyroid diseases. Despite the success of medicine in the treatment of endocrine diseases, the number of patients is increasing every year [6]. Changes in the thyroid gland activity inevitably affect various body systems, including the functions and morphology of organs and tissues of the oral cavity. The thyroid gland makes an important contribution to the regulation of all types of metabolism, especially energy processes that affect the periodontal tissues [7].

Violation of hormonal regulation complicates the course of chronic generalized periodontitis, and a more severe course is characteristic of patients with hypothyroidism. The lower jaw is more sensitive to changes in thyroid hormone levels [8]. Studies show a decrease in the mineral density of the alveolar process in patients with hypothyroidism, which may be one of the pathogenetic mechanisms of chronic generalized periodontitis in this group of people [9]. Low activity of thyroid hormones in patients indirectly inhibits bone formation, causing a tendency to decrease calcitonin levels and moderately increasing the release of parathyroid hormone, which contributes to increased production of tumor necrosis factor-α (TNF-α), proinflammatory cytokines IL-6, IL-1 and reduce the function of osteoblasts [10].

There is evidence that metabolic disorders in periodontal tissues occur in patients with inflammatory and inflammatory-dystrophic periodontal diseases on the background of thyroid disease [11]. Modern research has proven the dualism of the effects of thyroid hormones on bone metabolism. Metabolic disorders lead to the fact that all patients with diffuse toxic goiter, even in the early stages of the disease, have inflammatory-dystrophic changes in the periodontium [12, 13].

Patients with periodontitis and hypothyroidism have a pathological change in metabolic processes, increased proteolytic activity of the blood and increased excretion of mineral components, especially calcium and phosphorus [14, 15]. Analysis of the research results shows that thyroid dysfunction negatively affects the course of chronic inflammatory periodontal disease.

Despite a large number of studies, the molecular mechanisms of thyroid hormones influence on bone metabolism in general and the condition of periodontal tissues in particular have not been fully studied. Research in this area can establish more sensitive diagnostic and prognostic criteria for the severity of periodontal disease in thyroid dysfunction and improve treatment and prevention systems to correct the manifestations of generalized periodontitis in this group of people.

The aim of our research was to study the ultrastructural changes of periodontal tissues under the conditions of experimental periodontitis combined with hypothyroidism.

METHODS

The experiments were carried out in compliance with the general rules and provisions of the "European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes" (Strasbourg, 1986) and the "Bioethical appraisal of preclinical and other scientific research conducted on animals" (Kyiv, 2006). The Commission on Bioethics of I. Horbachevsky Ternopil National Medical University did not detect violations of moral and ethical norms during the research (protocol No. 56 from 08.01.2020).

The experiments were performed on outbred adult white male rats weighing 180-200 g, which were kept in standard vivarium conditions and diet. To simulate the experimental hypofunction of the thyroid gland, animals (n = 12) were daily administered intragastrically with mercazolyl in 1% starch solution at a rate of 1 mg/day per 100 g of body weight for 21 days. To model periodontitis, starting from the 8th day of the experiment for 2 weeks every other day, animals were injected into the gum tissue with 40 μl (1 mg/ml) of lipopolysaccharide (LPS) E. Coli ("Sigma-Aldrich", USA). On the 22th day from the beginning of the experiment, rats were euthanized by bloodletting under conditions of thiopental-sodium anesthesia. To confirm the state of hypothyroidism, we determined the content of free thyroxine (FT4), free triiodothyronine (FT3) and thyroid-stimulating hormone (TSH) using enzyme-linked immunosorbent assay kits ("Vector-Best", RF).

Collection of material for electron microscopic studies was performed according to generally accepted methods [16]. Pieces of

organocomplexes (frontal group of teeth of both jaws: alveolar processes with a mucous membrane) were fixed in 2.5% solution of glutaraldehyde with an active reaction medium pH 7.2-7.4, prepared on phosphate buffer according to Millonig. Fixed material in 50-60 min transferred to a buffer solution and washed for 20-30 min. Postfixation was performed with 1% a solution of osmium tetroxide in Millonig buffer for 60 min, after which it was dehydrated in alcohols and acetone and poured into the mixture epoxy resins and araldite. Ultrathin sections made on an ultramicrotome LKB-3 were stained with 1% aqueous solution uranyl acetate, contrasted with lead citrate according to the Reynolds method and studied in an electron microscope PEM-125 K («SELMI», Ukraine).

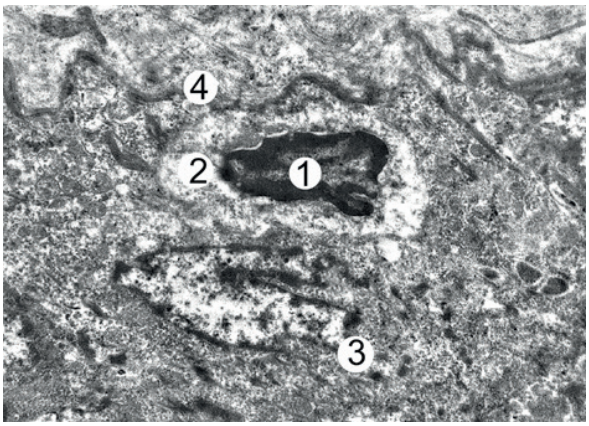

Figure 1 – Submicroscopic changes of the epithelium of the free part of the gums of rats with experimental periodontitis combined with hypothyroidism. x 10000. Notes: 1 – nucleus; 2 – cytoplasm of the pyknotically changed epitheliocyte of the basal layer; 3 – epitheliocyte of the spinous layer; 4 – basement membrane.

RESULTS AND DISCUSSION

Submicroscopic studies of the gums mucous membrane tissues of animals with lipopolysaccharide inflammation combined with hypothyroidism revealed profound destructive-degenerative changes in all periodontium components. Karyopyknosis and karyorexis were detected in some basal layer cells of the epithelial plate of gums area, and separate fragments of nuclei were present. There were few tonofilaments in the cytoplasm of epithelial cells. They were indistinctly contoured, merged into homogeneous bundles, which indicated the initial violation of the process of epithelial differentiation. The mitochondria were vacuolated with destroyed crystas and an electron-light matrix. Disturbances of intercellular connections were revealed, single altered desmosomes were observed, cell plasmolemas were indistinct, homogeneous (Fig. 1).

In the cytoplasm of keratinocytes of the granular layer, there were few osmophilic inclusions of keratohyalin, as well as impaired formation of cornified protein keratin. Destructive-apoptotic changes have been established in the epitheliocytes of the epithelial layer of the gingival sulcus.

Significant cell changes have been found in the attached part of the gums epithelial plate. Some epitheliocytes had signs of apoptosis and contained osmophilic fragments of the nucleus (micronuclei). In the cytoplasm of such cells, electron-bright unstructured regions with lysosomes were observed. Necrotic processes led to the destruction of organelles, fragmentation and lysis of tonofilaments (Fig. 2).

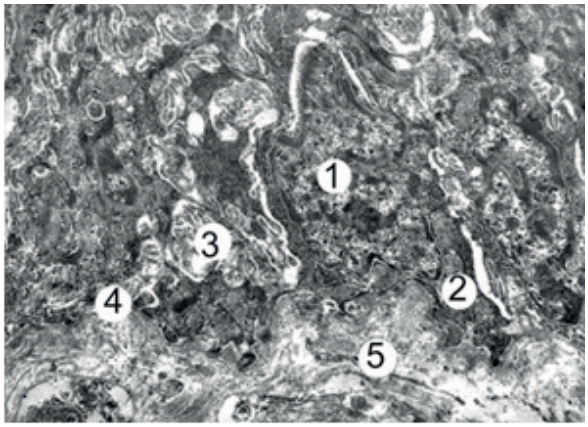

Figure 2 – Submicroscopic changes of the epithelium of the attached part of the gums of rats with experimental periodontitis combined with hypothyroidism. x 9000. Notes: 1 – nucleus; 2 – cytoplasm of the epitheliocyte of the basal layer; 3 – expanded intercellular spaces; 4 – basement membrane; 5 – loose connective tissue of the lamina propria.

Electron microscopic studies of the animals' periodontium revealed significant changes in its structural components. Collagen fibers in the bundles were partially destroyed and fragmented, their orderly arrangement was disturbed. Significant edema of the amorphous component of the intercellular substance of the connective tissue was manifested by electron-bright, unstructured areas between the collagen fibers. The submicroscopic organization of fibroblasts and fibrocytes was significantly changed.

The nuclei of many cells had fuzzy contours of the karyolema, the karyoplasm contained significant areas of heterochromatin.

The plasmolema, especially fibrocytes, was indistinctly contoured. In the cytoplasm there were many damaged organelles, unevenly thickened and fragmented tubules of the endoplasmic reticulum, and tanks of the Golgi complex. The mitochondria were swollen, with a light matrix and destroyed crystals, there were primary and secondary lysosomes (Fig. 3).

Electron microscopic examination of the gums mucous membrane also revealed profound changes in the microcirculatory tract vessels. The lumens of the blood capillaries were filled mainly with erythrocytes, in some there was a sludge effect. The nuclei of endothelial cells change significantly, due to deep intussusception the karyolemma of the nucleus had an irregular shape. Pyknosis and karyorexis of part of the nuclei have also been established. Heterochromatin occupies a significant area in their karyoplasm. The cytoplasm of endothelial cells formed thickened and narrow areas; they had few organelles and pinocytic vesicles. The basement membrane was inhomogeneous and indistinctly contoured. Coarse bundles of collagen fibers were observed in the perivascular spaces, this reflected the development of sclerotic changes (Fig. 4).

In the opinion of many scientists, thyroid hormones play an important role among the factors influencing the state of the dental system. The joint long course of thyroid dysfunction and dental diseases has a mutually aggravating effect on the immune system and leads to the formation of a vicious circle. This leads to low efficiency of treatment in this group of patients [17, 18].

The effect of impaired thyroid function on the health of the oral cavity is due to violation of mineral metabolism, redox processes and immunoresistance in such patients. Scientific publications of a number of authors testify to

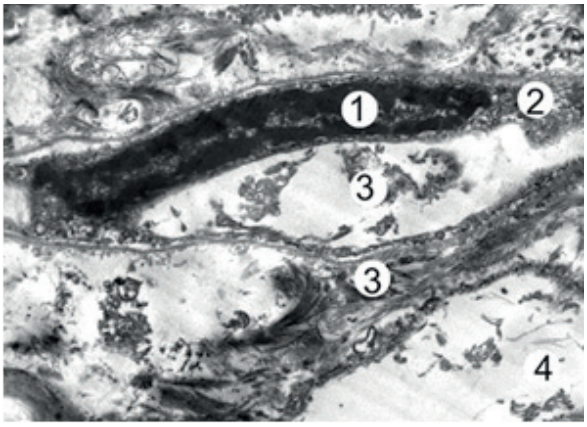

Figure 3 – Ultrastructural changes in the periodontium of the rat tooth with experimental periodontitis combined with hypothyroidism. x 11000. Notes: 1 – osmophilic nucleus; 2 – fibroblast cytoplasm; 3 – fragmented bundles of collagen fibers; 4 – amorphous connective tissue.

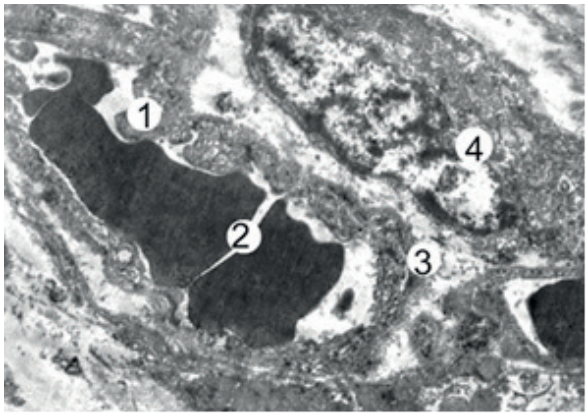

Figure 4 – Ultrastructural changes in the mucous membrane of the rat periodontium with experimental periodontitis combined with hypothyroidism. x 12000. Notes: 1 – endothelial cell cytoplasm; 2 – capillary lumen with erythrocytes; 3 – homogeneous, fuzzy basement membrane; 4 – perivascular connective tissue; 5 – fibroblast in connective tissue.

the impact of disruption of thyroid hormone production on the loss of bone tissue, a decrease in its mineral density and the occurrence of osteoporosis [19, 20]. Nevertheless, it is difficult to assess this effect in dynamics, since patients with reduced thyroid function are immediately prescribed replacement treatment with the use of hormonal drugs.

Chronic generalized periodontitis refers to pathology with systemic etiology and pathogenesis. When the thyroid function is impaired, changes occur in the morphology and functions of the oral cavity organs. There is a sharp inhibition in the periodontal tissues of the redox process; diffuse damage to histohematic barriers with a sharp violation of their permeability; development of edema of surrounding tissues; tissue hypoxia; decreased perfusion. All this leads to the development of a chronic sluggish inflammatory process, with a pronounced decrease in calcium metabolism in the bone tissue of the alveolar process.

In addition, a violation of the microelement composition is observed not only in the hard tissues of the teeth, but also in saliva. It is noted that a decrease in thyroid function leads to a decrease in the amount of phosphorus and an increase in the calcium content in the oral fluid. As a result, there is a decrease in the mineralizing potential of saliva and an increase in the viscosity of saliva in patients with hypothyroidism, which leads to violations of the oral cavity self-cleaning and an increase of hygiene indices. Also, in patients with hypothyroidism, the values of interleukin-6 and tumor necrosis factor - alpha in saliva and blood serum increase; pH of saliva decreases.

Violation of oral hygiene in conjunction with a decrease possibility of local immunity steadily leads to inflammatory periodontal diseases. Therefore, it is no coincidence that many authors attribute thyroid disease to risk factors for the onset and further progression of periodontal disease [21, 22]. This is evidenced by data on a higher prevalence of periodontitis with hypothyroidism. Some authors have identified the features of the genesis of periodontal diseases with a decrease in thyroid function [23]. They consist in a progressive impairment of redox processes, diffuse damage to the histohematic barriers with a sharp violation of their permeability, the development of edema of the surrounding tissues, an increase in tissue hypoxia, and a decrease in perfusion. Periodontal diseases occurring against the background of hypothyroidism are characterized by the development of a chronic sluggish inflammatory process and a significant decrease in calcium metabolism in the bone tissue of the alveolar process. In patients with postoperative hypothyroidism, a clinical picture of active inflammation in periodontal tissues was found; it expressed by bleeding gums, the formation of deep periodontal pockets with serous-purulent exudate and was characterized by a recurrent course. From the initial stages of development, the pathological process in periodontal tissues acquired a tendency to a chronic prolonged course, developing against the background of dry mouth, increased saliva viscosity, and was also accompanied by intense deposition of tartar, an increase in hygiene index values and the need for comprehensive dental care.

Disturbances in periodontal tissues in experimental hypothyroidism are stereotyped: dystrophic, atrophic and destructive changes in parenchymal structures, mucinous edema of the stroma, mild reparative processes are probably due to a decrease in the level of basal metabolism, slowing oxidation-reduction processes “metabolic depression” and, consequently, tissue hypoxia. Impaired growth and differentiation of cells and tissues result in a slowdown in physiological regeneration. Excess of glycosaminoglycans changes the colloidal structure of tissues, enhances their hydrophilicity, which leads to swelling and loosening of collagen fibers. In this case, chromotropic substances are released from bonds with proteins and accumulate mainly in the intercellular substance with the subsequent

replacement of collagen fibers by mucoid masses. As a result, mucinous edema occurs intermediate compression cell degeneration, morphologic alterations, necrosis and atrophy. These changes are in the epithelium and lamina propria mucosa, a layer of odontoblasts in the pulp of the tooth, periodontal tissues. The results of our study are confirmed by the work of a number of scientists [24, 25].

Chronic generalized periodontitis is characterized by a prolonged latent course with clinical manifestations in the form of a combination of generalized recession of the gums and severe loss of clinical attachment. Bone tissue is a dynamic structure that is constantly updated and is controlled by a large number of systemic and local factors, among which thyroid hormones play an important role.

In conclusion, submicroscopic studies of periodontal components under lipopolysaccharide inflammation combined with hypothyroidism revealed profound violations of the ultrastructure of epitheliocytes, fibroblastic and leukocyte cells, which showed apoptosis and necrosis signs. The fibrous structures and components of the amorphous substance of the connective tissue of the own plate of the attached and free part of the gums and especially the periodontium underwent significant destruction and lysis. Insufficient functioning of the vessels of the microcirculatory tract in all components of periodontium was manifested by damage and deformation of the wall with endothelial desquamation, edema, thinning of the basement membrane, stasis, thrombosis and spasm.

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, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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СУБМІКРОСКОПІЧНІ ЗМІНИ КОМПОНЕНТІВ ПАРОДОНТА ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ПАРОДОНТИТІ В ПОЄДНАННІ З ГІПОТИРЕОЗОМ

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Зміни у діяльності щитоподібної залози спричиняють порушення регуляції метаболізму у тканинах пародонта, що є надзвичайно актуальною медико-соціальною проблемою. Вираженість клінічних ознак запалення тканин пародонта залежить від функціонального стану та тривалості альтерацій щитоподібної залози. Метою нашого дослідження було вивчення ультраструктурних змін тканин пародонта білих щурів за умов експериментального пародонтиту в поєднанні з гіпотиреозом. Для моделювання пародонтиту щурам вводили в тканину ясен ліпополісахарид. Експериментальну гіпофункцію щитоподібної залози відтворювали внутрішньошлунковим введенням мерказолілу. Для підтвердження стану гіпотиреозу визначали вміст вільного тироксину, вільного трийодтироніну та тиреотропного гормону. При субмікроскопічному дослідженні виявлено глибокі деструктивно-дегенеративні порушення всіх компонентів пародонта. Зміни тканин пародонта при експериментальному гіпотиреозі стереотипні: дистрофічні, атрофічні та деструктивні ушкодження паренхіматозних структур, муцинозний набряк строми, помірні репаративні процеси. Таким чином можна зробити висновок, що при експериментальному пародонтиті в поєднанні з гіпотиреозом спостерігається порушення функціонування всіх компонентів судин мікроциркуляторного русла пародонта, що проявляється пошкодженням і деформацією стінки з десквамацією ендотелію, набряком, витонченням базальної мембрани, стазом, тромбозом, спазмом. Також характерним є значне руйнування та лізис фіброзних структур і компонентів аморфної речовини сполучної тканини власної пластинки та вільної частини ясен та пародонта.
Ключові слова: пародонтит; гіпотиреоз; пародонт; ультраструктурні зміни.

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