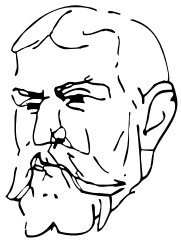
Wiadomości Lekarskie

Czasopismo Polskiego Towarzystwa Lekarskiego



Pamięci
dra Władysława
Biegańskiego

TOM LXXII, 2019, Nr 5 cz I, maj

Rok założenia 1928

Aluna Publishing

Wiadomości Lekarskie is abstracted and indexed in: PubMed/Medline, EBSCO, SCOPUS, Index Copernicus, Polish Medical Library (GBL), Polish Ministry of Science and Higher Education.

Zasady prenumeraty miesięcznika Wiadomości Lekarskie na rok 2019

Zamówienia na prenumeratę przyjmuje Wydawnictwo Aluna:

– e-mailem: prenumerata@wydawnictwo-aluna.pl
– listownie na adres:

Wydawnictwo Aluna
ul. Z.M. Przesmyckiego 29, 05-510 Konstancin-Jeziorna

Prosimy o dokonywanie wpłat na numer rachunku Wydawnictwa:
Credit Agricole Bank Polska S. A.: 82 1940 1076 3010 7407 0000 0000

Cena prenumeraty dwunastu kolejnych numerów: 240 zł/rok (w tym 5% VAT)

Cena prenumeraty zagranicznej: 120 euro/rok.
Cena pojedynczego numeru – 30 zł (w tym 5% VAT) + koszt przesyłki.
Przed dokonaniem wpłaty prosimy o złożenie zamówienia.

SPIS TREŚCI

PRACE ORYGINALNE / ORIGINAL ARTICLES

PRACA ORYGINALNA
ORIGINAL ARTICLE

THE EFFECT OF LOW FREQUENCY ELECTROMAGNETIC RADIATION ON THE MORPHOLOGY OF DENTAL AND PERIODONTAL TISSUES (EXPERIMENTAL INVESTIGATION)

Iryna V. Markovskaya
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

Introduction: Low intensity electromagnetic effects possess a high biological activity, reduce the adaptive reserves of the body, impair immunity, adversely affect the functional state of the organs and body systems.

The aim of the study was to identify in the experiment the effect of low-frequency electromagnetic radiation on the morphological state of the dental and periodontal tissue.

Materials and methods: The experiment was conducted on WAG rats weighing 180–200 g, during which two groups were formed: group 1 (control group) included 12 WAG rats, which were not performed any manipulations; the rats of group 2 (investigation group) (n=12) for 30 days were exposed to a 70 kHz low-frequency alternating electric field (5th frequency range) daily from 9.00 to 12.00. To simulate a low-frequency 70 kHz alternating electric field, certified experimental equipment was used. The study material was the upper jaw tissue. Histological and histochemical staining methods were used. Morphometric study was conducted.

Results: Complex morphological study on the experimental material allowed identifying the damaging effect of low-frequency electromagnetic radiation on the structural components of tooth and periodontal tissues.

Conclusions: Our findings suggest that the workers who are exposed to occupational low-frequency electromagnetic radiation should be included in the risk group for developing diseases of the dentomandibular system in order to carry out timely therapeutic and preventive measures.

KEY WORDS: morphology, dental and periodontal tissues, low frequency electromagnetic radiation, experiment

Wiad Lek 2019, 72, 5 cz. I, 773-778

INTRODUCTION

The evolutionary development of science and technology has allowed creation and use of the latest developments in order to obtain the products necessary for life and meet the growing needs of the mankind. This increased the comfort of living in a technocratic society, but at the same time gave rise to many factors affecting the health, one of which is electromagnetic radiation [1]. At present, electromagnetic radiation is being actively introduced into various spheres of human activity: industry, medicine, agriculture, etc. [2].

The interaction of electromagnetic radiation with the human body depends on the intensity of electromagnetic radiation, the time of exposure, the frequency, the greatest point of application. Electromagnetic waves are known to be low frequency (from 3 to 300 kHz), medium frequency (from 300 kHz to 3 MHz) and high frequency (from 3 to 30 MHz) [3], with electromagnetic waves of low frequencies having the greatest negative impact on human health.

Low intensity electromagnetic effects possess a high biological activity, reduce the adaptive reserves of the body, impair immunity, adversely affect the functional state of the organs and body systems [4], with the most sensitive being the nervous, immune, endocrine, cardiovascular and reproductive systems.

The literature analysis revealed isolated clinical studies showing the negative effect of low-frequency electromagnetic radiation on the dentomandibular system, the results of which in most cases are inconsistent; complex morphological studies on the effect of the above factor on tooth and periodontal tissue have not been revealed, which is relevant, given the high prevalence and incidence of dentomandibular diseases in the population [5].

THE AIM

The aim of the study was to identify in the experiment the effect of low-frequency electromagnetic radiation on the morphological state of the dental and periodontal tissue.

MATERIALS AND METHODS

The experiment was conducted on WAG rats weighing 180–200 g, during which two groups were formed: group 1 (control group) included 12 WAG rats, which were not performed any manipulations; the rats of group 2 (investigation group) (n=12) for 30 days were exposed to a 70 kHz low-frequency alternating electric field (5^{th} frequency range) daily from 9.00 to 12.00. To simulate a low-frequency 70 kHz alternating electric field, certified experimental equipment was used.

When the animals were removed from the experiment, it was the upper jaw that was dissected, given that in humans

this jaw is more susceptible to the damage compared to the lower jaw due to the peculiarities of the blood supply and worse cleaning from the food debris. The material was fixed in 10% neutral formalin, decalcified, and then the pieces of hard and soft tissues of the upper jaw about 0.4-0.6 cm thick were dissected longitudinally through the center of the upper central incisor, which were dehydrated using standard alcohol treatment and embedded in paraffin. 5-6 micron thick slices were made. The specimens stained with hematoxylin and eosin were used for general assessment of the state of hard and soft tissues of the upper jaw. Van Gieson staining with picrofuchsin was used to identify and assess the degree of development of collagen fibers in the studied tissues. To assess the content of nucleic acids in the nuclei of cells, Einarson staining with gallocyanin-chromic alum for total nucleic acids was used. MacManus-Hotchkiss PAS reaction (control with amylase) was used to revealed neutral glycosaminoglycans. The microscopic specimens were studied using Olympus BX-41 microscope (Japan).

Morphometry was carried out on Olympus BX-41 microscope (Japan) using Olympus DP-soft version 3.1 software, during which the thickness of enamel, dentin, predentin, ameloblast and odontoblast density were determined. To identify the density of odontoblasts in each specimen, the area occupied by these cells (in mm^2) was determined, the number of cells on it was counted, and the ratio of the number of ameloblasts and odontoblasts to the corresponding area was calculated. On the specimens stained with gallocyanin-chromic alum for total nucleic acids, optical density of nuclei of the basal gum epithelial cells, ameloblasts and odontoblasts was determined.

Statistical processing of the data was performed using non-parametric (Mann-Whitney) methods. The results were considered significant at $p<0.05$. Statistical processing of the results obtained was performed using Excel and Statistic Soft 6.0.

RESULTS AND DISCUSSION

Microscopically, in group 2, the periodontal tissue of the maxillary incisor was represented by the gum, periodontium, bone tissue of the dental alveolus, and cementum. The gum was covered with a multi-layered flat keratinizing epithelium, which contained 16-18 rows of cells differentiated into basal, spinous, granular and horny layers. The basal layer cells were rounded with a weakly eosinophilic cytoplasm and a moderately basophilic nucleus, the mitosis figures were few. Compared with group 1, the spinous layer cells were enlarged and contained a large rounded basophilic nucleus. Pyknotic or weakly basophilic nuclei surrounded by a narrow rim of cytoplasm were found in individual cells and their small groups in the basal and spinous layers. In some cells or their groups, the nuclei were defined as "shadows", the intercellular connections between them were broken (fig. 1). The horny layer was loose, voluminous, with foci of thickening and loci of horny scales with rod-shaped basophilic nuclei (foci of parakeratosis). The optical density of the basal cell nuclei was 0.162±0.014 relative units of optical density, which was significantly ($p<0.05$) less compared to the indicator of group 1 (0.235±0.017 relative units of optical density). The basement membrane of the epithelium contained the areas of thickening and thinning, was not visualized focally. The intensity of the PAS reaction was greatest in the areas of parakeratosis.

The papillary layer of the lamina propria was somewhat thickened due to elongation of the papillae, widening of the optically empty spaces between the collagen fibers. The collagen fibers were weakly fuchsinophilic. There were foci of swelling and dissociation of yellow connective tissue fibers. The capillaries were lined with endotheliocytes with a swollen nucleus lying on a moderately PAS-positive membrane. Some of the capillaries had signs of stasis, some were in a collapsed state and had a slit-like lumen. Perivascular spaces were somewhat enlarged, containing small focal accumulations of macrophages and lymphocytes.

The bundles of collagen fibers of the reticular layer at van Gieson staining, were moderately fuchsinophilic with loci of homogenization, dissociation and reduction of fuchsinophilia. A moderate number of fibroblastic differon cells with weakly basophilic cytoplasm and a rounded bright nucleus was visualized between the fibers; their content was somewhat reduced compared with group 1. The arterioles showed signs of irregular spasm, endothelial cells desquamation and proliferation loci. The venules of the reticular layer were unevenly expanded, plethoric, and the endotheliocytes lining them were flattened and contained an elongated basophilic nucleus. At PAS reaction, the vascular basement membrane was irregularly thickened, sometimes dissociated. The enlarged perivascular spaces demonstrated a few small focal lymphohistiocytic infiltrates (fig. 2).

In the slit-like periodontal space, collagen fibers of dense periodontal connective tissue were visualized. The direction of the bundles varied from radial in the zone of cemento-enamel attachment, to tangential in the zone of the lateral surfaces of cementum and vertical in the zone of the apical foramen. The bundles of collagen fibers were thick, moderately fuchsinophilic, with foci of swelling, dissociation and reduced fuchsinophilia. Periodontal arteries with uneven lumens, the endothelium lining them focally had signs of karyopyknosis and desquamation, proliferation loci. The venous vessels were lined with flattened endothelium with a moderately basophilic extended nucleus and a weakly basophilic cytoplasm; their lumens were unevenly widened, overfilled with blood. PAS reaction demonstrated that the vascular basement membrane had areas of thickening and thinning, in some places with cleavage loci (fig. 3). Fibroblasts with a rounded or oval, moderately basophilic nucleus and spindle-shaped fibroblasts prevailed among the cellular elements of the periodontium, there were macrophages, lymphocytes, plasma cells and tissue basophiles. In some specimens they formed small perivascular clusters.

In the area of the anatomical neck of the tooth, the epithelium of the gum attachment was replaced by a layer of differentiated active ameloblasts, which towards the root of

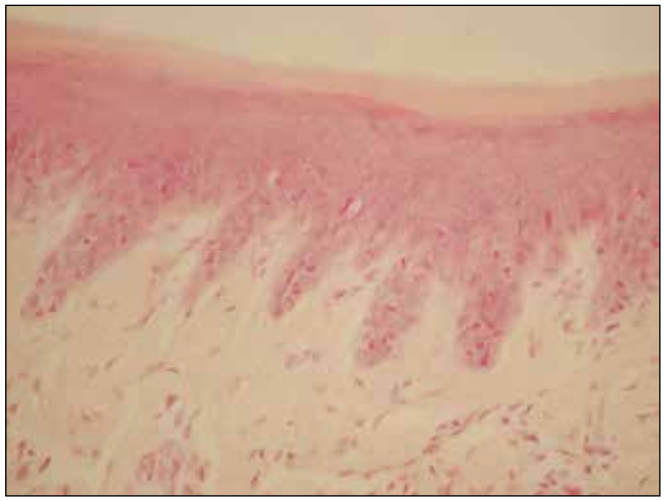


Fig. 1. Gum epithelium. Karyopyknosis in the cells of the basal and spinous layers. "Shadow" cells in the basal layer. Group 2. Stained with hematoxylin and eosin, × 400

Fig. 2. Swelling of the gingival lamina propria, weakly and moderately fuchsinophilic collagen fibers of the gum. Plethora of venous vessels, small-focus perivascular lymphohistiocytic infiltrates. Group 2. Van Gieson staining with picrofuchsin, × 400

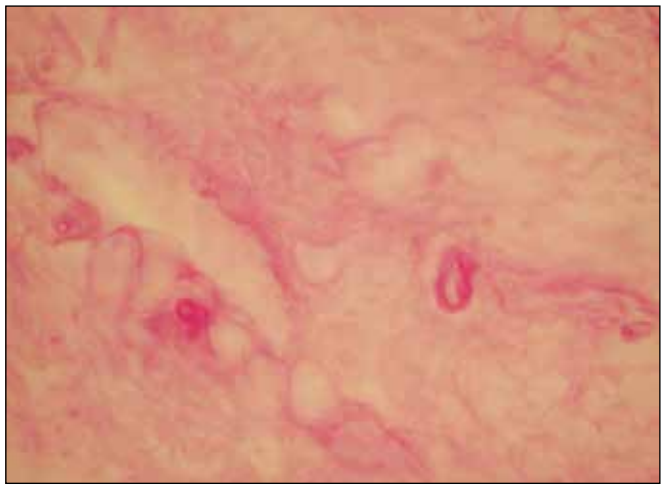


Fig. 3. Thickening and dissociation of the basement membrane of the periodontal vessels. Group 2. PAS-reaction with saliva amylase control, × 400

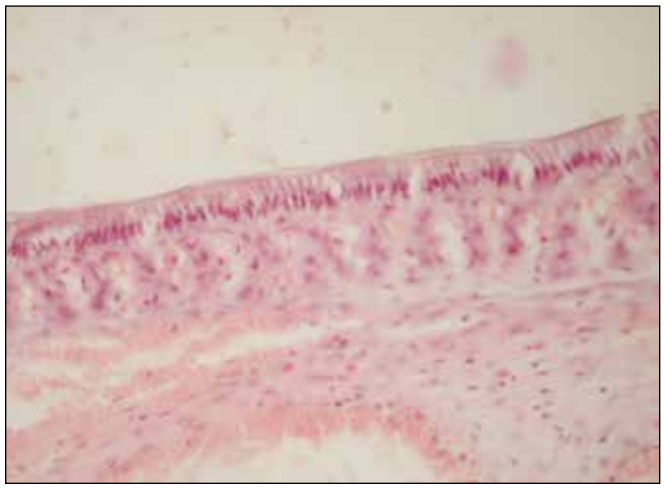


Fig. 4. Dystrophic and necrobiotic changes of ameloblasts. Group 2. Stained with hematoxylin and eosin, × 400

the tooth acquired a more elongated shape. The ameloblasts were located parallel to each other, their cytoplasm was slightly eosinophilic, the nucleus was elongated, moderately took the basic dyes. In comparison with group 1, in this group numerous cells or their groups were in a state of hydropic or balloon degeneration. The boundaries between such groups of ameloblasts were blurred, the cytoplasm contained vacuoles or was completely filled with a clear liquid, the nuclei showed the signs of karyopychnosis and karyolysis (fig. 4). The optical density of the nuclei of ameloblasts was 0.118±0.011 relative units of optical density, which was significantly ($p<0.05$) less as compared to the same indicator of group 1 (0.164±0.016 relative units of optical density). In the groups of cementoblasts visualized on the border with the cementum, cytoplasmic eosinophilia decreased, in some cells dystrophic and necrobiotic changes were determined. PAS-positive basement membrane of ameloblasts and cementoblasts was uneven.

The bone plate of the dental alveoli wall, surrounding the tooth root, consisted, as in group 1, of the system of osteons; however, in this group, pyknotic wrinkling of the nuclei was observed in individual cells with an increase in their basophilia. Thin connective tissue fibers of the bone plate, penetrating from the periodontium, contained loci of fuchsinophilia reduction at van Gieson staining. In the bone trabeculae of the spongy bone of the dental alveoli, the nuclei of osteocytes were smaller and hyperchromic compared with group 1. At van Gieson staining, the collagen fibers of the organic bone beam base were unevenly fuchsinophilic, and in the areas where their fuchsinophilia decreased, the fibers looked slightly swollen. The cells between the bone trabeculae contained bone marrow and blood vessels. Compared with group 1, the number of hematopoietic cells decreased in the bone marrow, and the fatty tissue content increased (fig. 5).

The decrease in the number of hematopoietic cells testified to the inhibitory effect of electromagnetic radiation

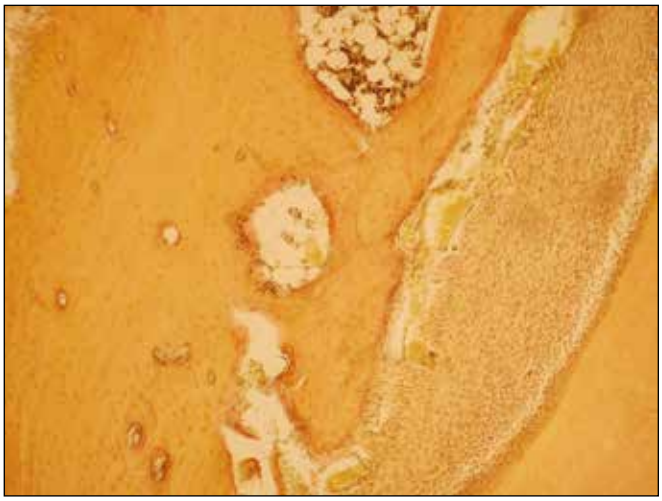


Fig. 5. Unevenly pronounced fuchsinophilia of periodontal collagen fibers and bone trabeculae. Bone marrow hypoplasia. Group 2. Van Gieson staining with picrofuchsin, ×100.

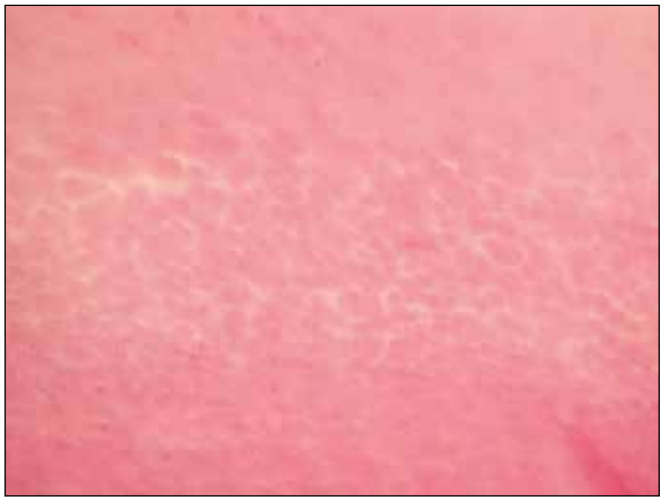


Fig. 6. Uneven arrangement of small demineralization foci in dentin. Group 2. Stained with hematoxylin and eosin, ×400.

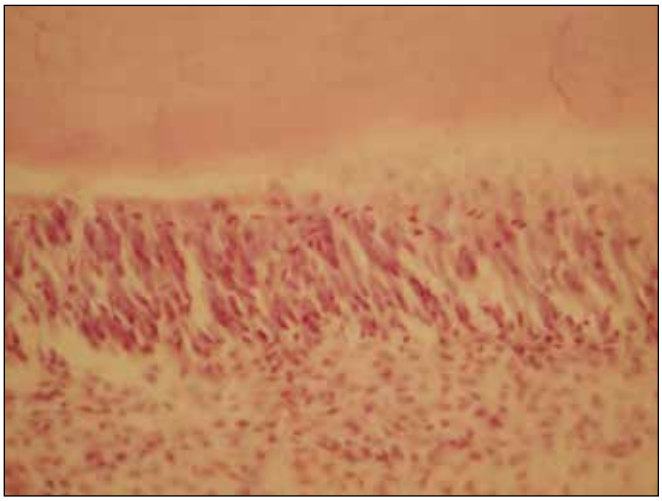


Fig. 7. Dystrophic and necrobiotic changes in odontoblasts. Group 2. Stained with hematoxylin and eosin, ×400

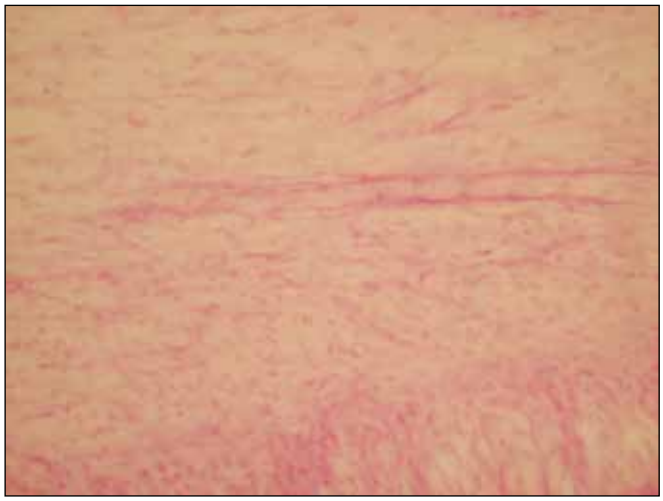


Fig. 8. Irregular vascular basement membrane with dissociation sites in the dental pulp. Group 2. PAS-reaction with saliva amylase control, ×400.

on hematopoietic processes, which has been noted in other investigations [6].

The described necrotic changes in periodontal tissues are possibly due to the fact that radiation leads to ionization of the molecules and atoms, formation of free radicals and peroxide compounds, which in turn violate the biochemical processes, cell functions and contribute to necrosis development [7].

The hard tooth tissues were represented by cementum, enamel, dentin and predentin. Van Gieson staining demonstrated that, the collagen fibers of the main substance of the cementum were moderately fuchsinophilic; there were foci of mucoid swelling with fuchsinophilia reduction, swelling and dissociation of collagen fibers, expansion of the spaces between them. Demineralization sites were uneven.

A weakly eosinophilic layer of enamel began to be visualized under a layer of narrowing cementum in the area of the cementoenamel border and covered the vestibular surface of the incisor crown. Enamel hypomineralization sites were unevenly located, Gunter-Schreger lines in them were weakened, Retzius lines were widened. In the area of the anatomical neck of the tooth, the average value of the enamel thickness in this group was 8.57±0.25 μm, which was significantly less ($p<0.05$) compared with the indicator of group 1 (10.59±0.12 μm).

Dentinoenamel border was focally blurred. The layers the dentine and predentin were somewhat narrowed. Morphometrically, the average thickness of the dentin in the group was 166.67±0.72 μm, which was significantly ($p<0.05$) lower compared with the indicator of group 1 (173.67±0.77 μm). The thickness of the predentin layer in group 2 (28.19±0.42 μm) was significantly ($p<0.05$) lower compared with group 1 (33.68±0.35 μm). Dentin and predentin had areas of uneven location of dentinal tubules, expanded spaces between them. Van Gieson staining shoed the main substance with the signs of mucoid swelling in these areas, the collagen fibers were swollen, fibrous, weakly fuchsinophilic. The boundary between dentin and preden-

tin was defined as a corrugated line and was somewhat blurred in the areas of the mucoid swelling of the main substance. Predominantly small demineralization foci were visualized in the dentin, which were poorly stained by the main dyes or were not stained, were located unevenly (fig. 6). The area of demineralized foci averaged 136.71±6.67 μm², which was significantly ($p<0.05$) lower compared with the indicator of group 1 (251.45±14.97 μm²).

It has been noted that electromagnetic radiation leads to necrosis of hard tooth tissues due to direct influence of this factor on protein structures of enamel and dentin. Besides, electromagnetic radiation violates the function of the salivary glands, up to xerostomia, which in turn can lead to violation of remineralization [7]. Moreover, not only decrease in the production of saliva, but also a change in its structural and mineralizing properties was revealed [8].

Pronounced carious changes in workers who were subjected to electromagnetic radiation while performing their duties have been described [8].

In the pulp of the incisor, the odontoblast layer was narrowed; its average thickness in the group was 47.53±1.29 μm, which was significantly ($p<0.05$) less than in group 1 (61.71±0.86 μm). The cytoplasm of odontoblasts was poorly basophilic, the nucleus was moderately or poorly demonstrated by the basic dyes. In groups of cells, the protoplasm contained vacuoles with cytoplasmic fluid, the nucleus was in the state of karyopyknosis or karyolysis and shifted towards the dentinal processes (fig. 7). The optical density of odontoblast nuclei in the average was 0.159±0.014 relative. Units of optical density, which was significantly ($p<0.05$) less in comparison with the indicator of group 1 (0.201±0.004 relative units of optical density). The intercellular spaces of the odontoblast layer were enlarged, optically empty. Some capillaries had signs of stasis, were lined with endothelium with a swollen nucleus. Some capillaries were collapsed, their lumens were slit-like. The vascular basement membrane was uneven at PAS reaction. Morphometrically, the density of odontoblasts was 6167.41± 316.48 pcs/mm², which was significantly ($p<0.05$) less than the corresponding indicator of group 1 (7261.93±272.36 pcs/mm²).

In the intermediate layer of the pulp, there was an uneven narrowing of the outer zone, decreased content of cellular elements in the inner zone. Increased content of fibroblasts and unevenly fuchsinophilic collagen fibers was observed in the stroma of the pulp nucleus. Collagen fibers were focally swollen, slightly fibrous and slightly fuchsinophilic. The spaces between the fibers were somewhat expanded due to accumulation of edematous fluid. The vessels were unevenly filled with blood. PAS reaction demonstrated that the vascular basement membrane had areas of thickening and splitting; it was not visualized focally (fig. 8). In the endothelial lining, there were loci of cells with a pyknotic nucleus and signs of desquamation, as well as groups of proliferating cells with a swollen basophilic nucleus. Some capillaries had signs of stasis, some were collapsed with a slit-like lumen. Small focal hemorrhages were perivascularly visualized.

Thus, a complex morphological study on the experimental material allowed identifying the damaging effect of low-frequency electromagnetic radiation on the structural components of tooth and periodontal tissues.

CONCLUSIONS

1. In the multilayered flat epithelium of the gingival mucosa, pronounced necrobiotic changes in the epitheliocytes of the basal and spinous layers developed, proliferative activity in the basal epithelium was inhibited, cell differentiation with focal parakeratosis was disturbed; these changes were accompanied by reduction in the optical density of the basal cell nuclei. Moderate hemodynamic disturbances, focal dystrophic changes of the connective tissue and mild cellular perivascular lymphohistiocytosis infiltration appeared in the gingival lamina propria mucosa and periodontium.
2. In the bone component of the periodontal, signs of focal alterative changes in osteocytes and reduction in collagenization of the bone trabeculae organic matrix were found. In ameloblasts, widespread dystrophic and necrobiotic changes developed, which was accompanied by reduction in the optical density of their nuclei. The revealed alterative changes of ameloblasts caused development of hypoplasia and impaired mineralization of the enamel, which was confirmed by reduction in its thickness.
3. In the pulp of the tooth, dyscirculatory changes were detected, which were accompanied by focal mucoid swelling of the stromal pulp nucleus, appearance of fibroblast hyperplasia loci with focal enhancement of stromal collagenization. In odontoblasts, signs of hydropic degeneration and necrobiosis appeared, inhibition of their proliferative activity was noted, which was confirmed by reduction in the thickness of the odontoblast layer and their density, decrease in the optical density of the cell nucleus.
4. The identified pathological changes in the structural components of the pulp caused development of focal dystrophic changes in dentin and predentin, disrupting the process of their mineralization, which was confirmed by narrowing of the predentin and dentin zones, reduction in the area of dentin demineralization and their uneven location.
5. Our findings suggest that the workers who are exposed to occupational low-frequency electromagnetic radiation should be included in the risk group for developing diseases of the dentomandibular system in order to carry out timely therapeutic and preventive measures.

REFERENCES

1. Cherny A.P., Nikiforov V.V., Rodkin D.I. et al. Sovremennoe sostojanie issledovanij vlijanija jelektromagnitnyh izluchenij na organizm cheloveka [The current state of research on the influence of electromagnetic radiation on the human body]. Engineering and Educational Technologies. 2013; 2:112-124. (Ru)
2. Smirnov A.I. Vlijanie jelektromagnitnyh polej nizkoj chastoty na rost sejancev sosny obyknovennoj [Influence of low frequency electromagnetic fields on the growth of pine seedlings]. Forestry Bulletin. 2014; 4:52-56. (Ru)

3. Odinayev F.I., Odinayev Sh.F., Shafiev Sh.I. et al. Jelektromagnitnye izluchenija i zdorove cheloveka [Electromagnetic radiation and human health]. Tomsk State University Journal Vestnik. 2015; 20(6):1714-1717. (Ru)
4. Schepina T.P., Nekrasova D.A., Egorkina S.B. Vlijanie vrashhajushhego jelektricheskogo polja nizkoj chastoty na reproduktivnyj potencial jeksperimentalnyh zhivotnyh [Influence of a rotating low-frequency electric field on the reproductive potential of experimental animals]. Population health and life environment. 2014; 8(257): 53-55. (Ru)
5. Bugorkova I.A. Rasprostranennost stomatologicheskih zabolevanij u narkozavisimyh bolnyh dlja razrabotki programmy po snizheniju riskov [The prevalence of dental diseases in drug-addicted patients for developing a risk reduction program]. Ukraine. Health of the nation. 2015; 2(34): 28-31. (Ru)
6. Solovyov V.S., Zhevnovskaya A.N., Gashev S.N. et al. Vlijanie jelektromagnitnogo izluchenija promyshlennoj chastoty na gematologicheskie pokazateli perifericheskoj krovi gryzunov [The influence of electromagnetic radiation of industrial frequency on the hematological parameters of the peripheral blood in rodents]. Principles of the ecology. 2016; 2: 84-90. (Ru)
7. Hadzhibragimov M.S. Vlijanie jelektromagnitnogo izluchenija kompjuterov na tverdye tkani zuba [The effect of electromagnetic radiation of computers on tooth hard tissues]. Bulletin of medical internet conferences. 2018; 8: 4: 144-145. (Ru)
8. Leontyeva E.Yu., Bykovskaya T.Yu., Ivanov A.S. Vlijanie neionizirujushhego izluchenija na sostojanie tkanej polosti rta: obzor literatury [Influence of non-ionizing radiation on the state of the tissues of the oral cavity: a review of the literature]. Chief doctor. 2018; 64: 6-9. (Ru)

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

CORRESPONDING AUTHOR
Iryna V. Markovskaya
Kharkiv National Medical University
Pobedy Avenue 78a, apartment 77, 61204 Kharkiv, Ukraine
tel: +380501699763
e-mail: msmyroshnychenko@ukr.net

Received: 15.03.2019
Accepted: 26.04.2019