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CHANGES IN THE ORAL MUCOSA UNDER THE INFLUENCE OF LOW-FREQUENCY ELECTROMAGNETIC RADIATION

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The study was devoted to analyze the condition of the oral mucosa under the influence of low-frequency (70 kHz) industrial frequency electromagnetic radiation in the clinical setting (111 individuals – workers of the Kharkiv Tractor Plant) and in the experimental part (36 laboratory rats of the WAG-populational). The results of the clinical and experimental examination confirm the negative impact of prolonged professional exposure to electromagnetic radiation both on the oral mucosa of patients and laboratory animals, which requires implementation of therapeutic and preventive measures.

Key words: oral mucosa, low-frequency electromagnetic radiation, occupational exposure, oral lichen planus, recurrent aphthous stomatitis.

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ЗМІНИ СЛИЗОВОЇ ОБОЛОНКИ ПОРОЖНИНИ РОТА ПІД ВПЛИВОМ НИЗЬКОЧАСТОТНОГО ЕЛЕКТРОМАГНІТНОГО ВИПРОМІНЮВАННЯ

Дослідження присвячене аналізу змін слизової оболонки під впливом низькочастотного електромагнітного випромінювання промислової частоти (70 Гц) в клініці (111 осіб – працівників Харківського тракторного заводу) та в експерименті (36 лабораторних щурів популяції WAG). Проведені дослідження дозволили підтвердити негативні наслідки впливу низькочастотного електромагнітного випромінювання на стан слизової оболонки порожнини рота обраного контингенту пацієнтів та лабораторних тварин, що потребує проведення лікувально-профілактичних заходів.

Ключові слова: слизова оболонка порожнини рота, низькочастотне електромагнітне випромінювання, професійний вплив, червоний плоский лишай, хронічний рецидивуючий афтозний стоматит.

The work is a fragment of the research project "Development and implementation of modern algorithms for diagnostic, treatment and prevention of dental diseases in patients of different age periods", state registration No. 0121U110922.

The health status of the Ukrainian population, unfortunately, shows a steady trend towards deterioration [1]. In recent years, exacerbated by the prolonged COVID-19 pandemic and Russian armed aggression, the Ukrainian population has been experiencing an intensification of chronic stress in all areas of life: social, occupational, and familial. In this context, the additional effect of adverse environmental factors, including the influence of electromagnetic fields (EMF), acquires a more significant negative

nature. The WHO officially recognized "global electromagnetic environmental pollution" in 1995 and included the issue of electromagnetic pollution in the environment as one of humanity's priority problems. It is also noteworthy that the level of this pollution grows by 10-15 times every ten years [3].

There are numerous sources of electromagnetic radiation (EMR) surrounding us, including electrical wiring, fluorescent lamps, various hardware, electric transportation, radio and television antennas, phones including their base stations, and wireless technology like Wi-Fi access points, routers, smartphones, tablets, and Bluetooth devices. Electromagnetic radiation exposure is also prevalent in professional activities [9, 12].

Current evidence strongly suggests that prolonged exposure to certain EMFs can cause both acute and chronic physiological dysfunctions in humans and is a risk factor for developing diseases of the endocrine, nervous, and cardiovascular systems, as well as malignant neoplasms, allergic and skin diseases, digestive disorders, metabolic disturbances, immune and reproductive system issues, and psychological disorders. EMF of any frequency, including industrial frequencies, has high biological activity. Research results indicate that this activity can accumulate under conditions of prolonged multi-year exposure, leading to distant consequences in almost all systems of the human body [5, 7, 15]. However, some studies still prove a significant negative impact of EMR on biological systems even with short-term exposure [11, 13].

Despite the wide frequency spectrum of EMR, its negative consequences at the cellular level are similar, involving changes in intracellular ion concentration, the rate of synthesis of various biomolecules, and cell proliferation. Among the most profound effects of EMR exposure are disturbances in reproductive capacity, gene expression, DNA damage, and even cell necrosis [13].

Thus, there is considerable evidence available today of the negative impact of EMR on both humans and experimental animals. However, in the field of dentistry, such information is less extensive, mostly related to the therapeutic effects of EMR, and many issues remain unexplored, particularly regarding changes in the oral mucosa. Therefore, studying the mechanisms of the adverse effect of non-ionizing radiation on systemic and dental health corresponds to the current problems of modern medicine.

The purpose of the study was to analyze the condition of the mucous membrane in the clinical setting and experimentally under the influence of low-frequency (70 kHz) industrial frequency electromagnetic radiation.

Materials and methods. For the clinical component of the study, 65 individuals (including 44 males (67.7 %) and 21 females (32.3 %)) aged between 26 and 60 years were studied, they were divided into two age groups according to WHO guidelines. The younger group consisted of 35 individuals (25 males (71.4 %) and 10 females (28.6 %)). The middle-aged group was represented by 30 individuals (22 males (73.3 %) and 8 females (26.7 %)). All participants in the Main Group were workers of the "Kharkiv Tractor Factory" press-welding shop (PWS), exposed to low-frequency (70 kHz) electromagnetic radiation of industrial frequency. Their work experience in the PWS ranged from 5 to 15 years. The Comparison Group consisted of 46 healthy individuals – science and technology workers of the same factory, aged from 25 to 60 years, with a gender distribution corresponding to the Main Group, who were not directly exposed to electromagnetic radiation.

In addition to analyzing the condition of hard tooth tissues and periodontal tissues, a thorough objective examination of the mucous membrane of the vestibule, cheeks, palate, tongue, and the vermillion border of the lips was performed. This included assessments of color, moisture, integrity, and the presence of lesions. According to WHO recommendations, the analysis of the obtained data took into account the work experience in the production and age of the workers.

The experimental part of the study was conducted on 36 laboratory (sexually mature) rats of the Wistar Albino Glaxo population from the experimental clinic of Kharkiv National Medical University, weighing 180–200g. All animals were healthy, kept in standard conditions, namely: constant temperature (22±2º C), air humidity 50±5 %, 12-hour day/night regime, balanced diet, metal cages of sufficient area.

The Main Experimental Group (n=18) was formed through random selection. For 30 working days, from 9 am to 12 pm, these rats were subjected to low-frequency (70 kHz) EMF exposure using certified experimental equipment. The Gontrol Group (n=18) differed only in the absence of EMR exposure. Daily, animals of both groups were examined and assessed for general condition, appearance, behavior, amount of consumed food and water, and body weight gain. On the 30th day of the study, the rats were euthanized by cervical dislocation.

For the morphological examination, buccal tissue samples from both the Control and Main Experimental Group rats were used. Accepted histological methods were employed to study the morphological characteristics of the oral mucosa alterations. Initially, the obtained material was fixed in

10 % neutral formalin, decalcified, after which soft tissues of 0.4–0.6cm thickness were removed, subjected to dehydration using standard alcohol procession and embedded in paraffin. Finally, sections of 5–6μm thickness were made.

Several staining techniques were used: hematoxylin and eosin for general morphological structure assessment, Van Gieson's picrofuchsin for collagen fibers, Einarson's alcian blue for total nucleic acids, and McManus-Hotchkiss PAS reaction for neutral glycosaminoglycans. Histological sections have been analyzed using a conventional light (Axiostar, Zeiss) microscopy.

The morphometric parameters, such as the optical density of nuclei and cytoplasm of basal epitheliocytes, were objectively determined. Computer images of the microscopic preparations were used to determine these morphometric parameters. For assessing the morphofunctional state of endotheliocytes, the optical density during staining by Einarson's method was calculated. The optical density levels of nuclei and cytoplasm of endotheliocytes were determined using a raster graphics editor. Optical density (D) was calculated using the formula D=-lg Ip/Ib, where Ib is the intensity of the background light flow (i.e., areas without tissue), and Ib is the intensity of the light flow of the studied area.

Confidence intervals (CI) in groups were calculated on the assumption of binomial distribution of a random variable with probability of error $p<0.05$.

The Ethics and Bioethics Committee of the Kharkiv National Medical University at its meetings (Protocol No. 8 dated 12.10.2019; Protocol No. 8 dated 11.12.2023) considered and approved the protocols of experimental and clinical studies.

Results of the study and their discussion. According to the data from the dental examination of the workers in the Main and Comparison groups, apart from the pathology of the hard tooth tissue and the periodontium, 53 individuals (81.5 %) were diagnosed with a range of diseases of the oral mucosa. Specifically, in the Main Group (workers of the PWS), signs of meteorological cheilitis were most frequently observed (25 individuals, 38.5 %), followed by recurrent aphthous stomatitis (21 individuals, 32.3 %) and the typical form of oral lichen planus (7 individuals, 10.7 %) (Fig.1).

Only 12 individuals (18.5 %) in the Main Group did not have pathological changes of the oral mucosa. In the past history in 17 individuals (26.2 %) of the examined, there were episodes of laboratory-confirmed oral candidiasis. Regarding age and gender characteristics, meteorological cheilitis was predominantly diagnosed in middle-aged individuals (60.0 %) and males (76.0 %); Recurrent aphthous stomatitis – predominantly in young individuals (85.7 %) of both genders; oral lichen planus – only in middle-aged women (100.0 %).

In the Comparison Group, the state of the oral mucosa was as follows: in 80.5 % of individuals did not have pathological changes, while in 7 individuals meteorological cheilitis (15.2 %) and in 2 individuals recurrent aphthous stomatitis (4.3 %) were diagnosed. Manifestations of oral lichen planus in the Comparison Group were not recorded (Fig. 2).

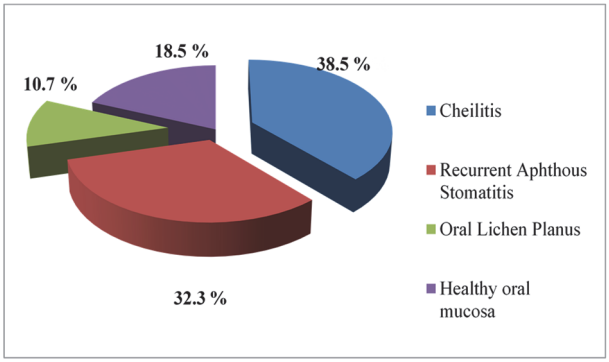


Fig. 1. Prevalence of the oral mucosa and the red lip border lesions in the Main Group individuals (%).

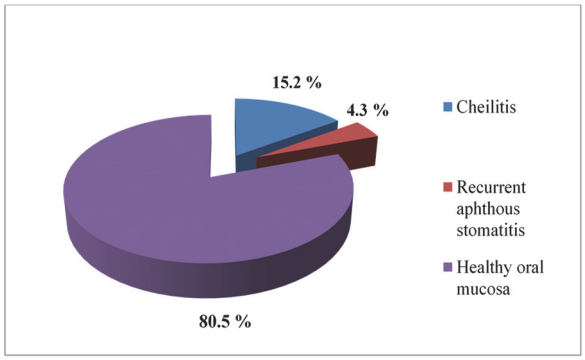


Fig. 2. Prevalence of the oral mucosa and the red lip border lesions in the Comparison Group individuals (%).

The dynamics of the prevalence of the oral mucosa lesions in the workers of the PWS (Main Group) depending on the length of service was also studied, which is presented in Fig. 3.

The results are as follows: manifestations of cheilitis in workers of the PWS with up to 5 years of service length were diagnosed in 3 individuals (12.0 % (CI 4.0 %–20.0 %, $p<0.05$)) of all cases diagnosed with meteorological cheilitis), the number of cases increased by 2.7 times in workers with a length of service of 5–10 years (8 individuals, 32.0 % (CI 16.0 %–44.0 %, $p<0.05$)), and increased by 1.8 times – in workers with more than 10 years of experience (14 individuals, 56.0 % (CI 40.0 %–68.0 %, $p<0.05$)).

The presence of aphthae on the oral mucosa was diagnosed in 4 individuals (19.1 % (CI 4.8 %–28.6 %, p<0.05)) of workers of the Main Group whose length of service did not exceed 5 years. If the length of service was within 5–10 years, then the frequency of detection of these lesions increased by 1.5 times (7 individuals, 33.3 %, (CI 19.1 %–47.6 %, p<0.05)), and if the length of service exceeded 10 years – then frequency of detection of these lesions increased by additional 1.7 times (10 individuals, 47.6 %, (CI 28.6 %–61.9 %, p<0.05)). The percentage of individuals with identified foci of Oral Lichen Planus on the oral mucosa with up to 5 years of length of service was 14.3 % (1 individual, CI 0.0 %–28.6 %, p<0.05), and then the number of diagnosed cases doubled (2 individuals, 28.6 %, (CI 0.0 %–42.9 %, p<0.05)) both in the individuals with a length of service of 5–10 years and in individuals where the service was longer than 10 years (4 individuals, 57.1 %, (CI 28.6 %–71.4 %, p<0.05)).

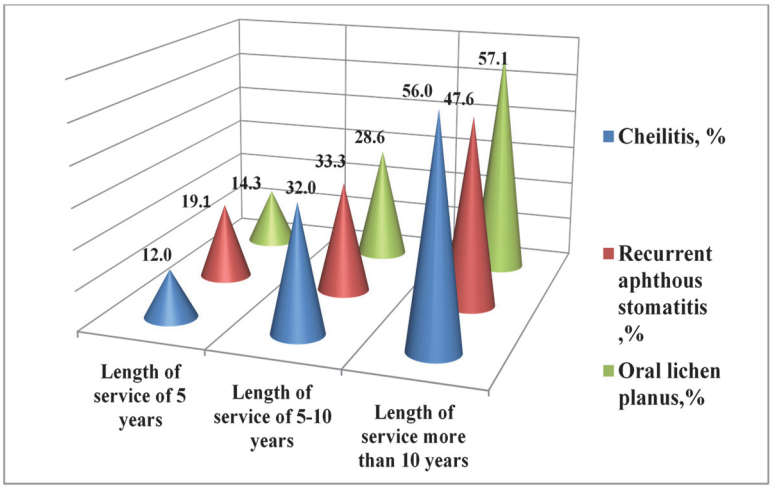


Fig. 3. Dynamics of the prevalence of the oral mucosa and the red lip border lesions in the Main Group individuals depending on the length of service, %

Overall, the increase in prevalence depending on the length of service in the Comparison Group (service up to 5 years and more than 10 years) in the case of all three nosological forms was statistically significant (p<0.05).

Taking into account the obtained data, we considered it necessary to conduct an experimental study to determine the impact of EMR on the oral mucosa of rats.

In the study of the mucous membrane's histological sections of the experimental animals in the Control Group, it was determined that the buccal mucosa is covered with multi-layered squamous keratinized epithelium and consisted of 18–20 rows of cells, differentiated into stratum basale, stratum spinosum, stratum granulosum and stratum corneum. The basement membrane of the buccal mucous epithelium was thin, continuous, and moderately PAS-positive. The stratum basale was represented by epitheliocytes of prismatic shape with a round or oval moderately basophilic nucleus and weakly basophilic cytoplasm. In some cells, mitotic figures were present. Towards the stratum spinosum and stratum granulosum layer, the cells became denser. The lamina propria of the mucous membrane also corresponded to normal structure, namely: two layers – reticular and papillary, represented by moderately fuchsinophilic thin collagen fibers, among which fibroblasts with moderately basophilic nuclei were visualized. The blood filling of capillary-type vessels was moderate. Mainly perivascularly, occasional macrophages and lymphocytes were observed. In the reticular layer, bundles of collagen fibers were arranged without a certain orientation. Compared to the papillary layer, the collagen fibers were slightly thickened and more intensely stained with fuchsin. Among the collagen fibers, fibroblasts, elements of the vascular bed (arterioles and venules) are located, whose lumen was filled with a moderate number of erythrocytes. Around the vessels, there was a small number of lymphocytes and macrophages, both located separately and in clusters.

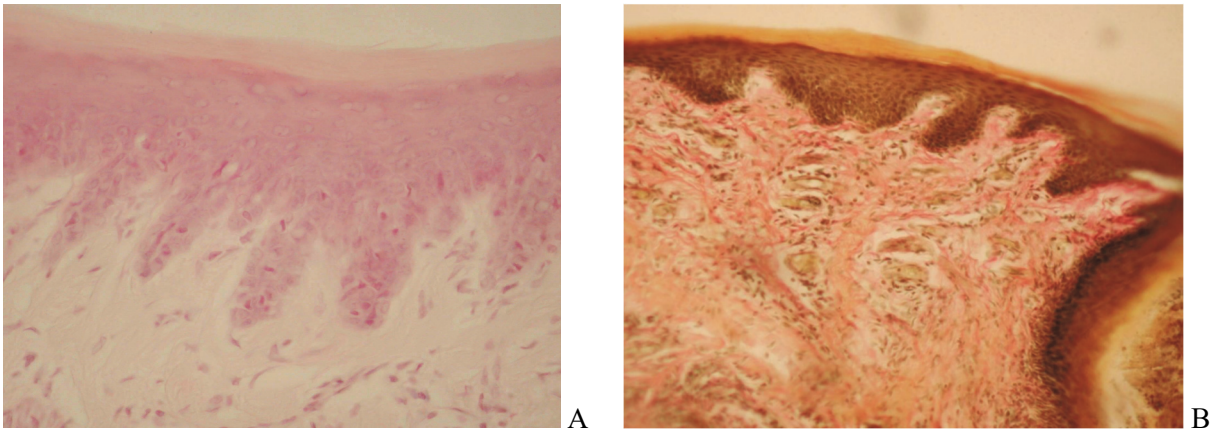
Therefore, while studying the histological sections of the oral mucosa of rats in the Control Group, it can be stated that the morphological structure of the mentioned structural components corresponded to their normal structure.

The multi-layered squamous keratinized epithelium of the oral mucous membrane of animals in the Main Group also counted 16–18 rows of cells, differentiated into stratum basale, stratum spinosum, stratum granulosum and stratum corneum. Cells of the stratum basale wee round, cytoplasm was weakly eosinophilic, the nuclei were moderately basophilic.

Compared to the Control Group, the cells of the stratum spinosum were a larger in size of both the cells themselves and the nuclei, which were basophilic and sometimes pyknotic and surrounded by a narrow rim of cytoplasm. In some cells, nuclei were defined as "shadows", intercellular connections between them were disturbed (Fig. 4A).

The stratum corneum was loose and "voluminous" with foci of parakeratosis, where the intensity of the PAS-reaction was greatest. In the basement membrane of the epithelium, there were both areas of thickening, and thinning up to the point of no visualization.

The papillary layer of the lamina propria is somewhat thickened due to the elongation of the papillae, and the widening of the spaces between the collagen fibers observed.



A B

Fig.4. Fragment of the oral mucous membrane of the Main Group animal: A – karyopyknosis in the foci of the stratum basale and stratum spinosum. Nuclei in the form of "shadows" in the cells of the stratum basale, hematoxylin and eosin staining, x 400; B – swelling of the lamina propria, moderately fuchsinophilic collagen fibers, congested venules, small focal perivascular infiltrates, Van Gieson's stain, x 200.

Collagen fibers were weakly fuchsinophilic with foci of swelling and fibrillation. Endotheliocytes of the capillary protrusion were with swollen nuclei. The vascular bed also showed signs of changes: stasis in some capillaries, or conversely, the remaining capillaries had a slit-like lumen due to their collapse. Perivascular spaces were slightly expanded with small focal clusters of macrophages and lymphocytes. In the reticular layer of the mucous membrane in the Main Group animals, collagen fibers with signs of homogenization, disintegration, and decreased fuchsinophilia were encountered. Between the fibers, a moderate number of fibroblasts were visualized, the number of which compared to the Control Group was reduced. Arterioles were with signs of uneven spasm, loci of desquamation, and proliferation of endotheliocytes. Venules in the reticular layer were unevenly dilated and engorged in places. The endotheliocytes of the venule walls had a flattened shape and elongated basophilic nuclei. The vascular basement membrane of endotheliocytes during the PAS reaction was unevenly thickened, partially fibrillated. In the perivascular spaces of the reticular layer, occasional small foci of lymphohistiocytic infiltrates were encountered (Fig.4B).

The optical density of the nuclei of basal epitheliocytes of Control Group (intact) animals was 0.235±0.017 c.u. of optical density. The similar indicator in the animals of the Main Group was 0.162±0.014 c.u. of optical density, which was by 1.5 times less than the index of the Control Group ($p<0.01$). In addition, the optical density of the cytoplasm of basal epitheliocytes of the mucous membrane of the rats in the Main Group was in histological sections 1.3 times smaller compared to the corresponding measure in the Control Group (0.146±0.016 and 0.183±0.012 c.u. of optical density respectively ($p<0.01$) (Table 1).

Table 1

Morphometric Parameters of Epitheliocytes of the Oral Mucosa Stratum Basale in Test Animals (c.u., M±m), for p<0.01

Groups under Study / Parameters (c.u. of optical density)	Control Group	Main Group
Nuclei of Stratum Basale Epitheliocytes	0.235±0.017	0.162±0.014
Cytoplasm of Stratum Basale Epitheliocytes	0.183±0.012	0.146±0.016

The data obtained from the study histological sections of the oral mucosa of rats indicated signs of morphological transformations in all structural components of the oral mucosa in response to the action of low-frequency EMR. There were observable thickening and swelling of the connective tissue, capillary atrophy, and initial stages of necrobiotic changes. However, a small portion of the nuclei still retained their contours, which may indicate a preserved regenerative potential in the cells of the oral mucosa of experimental animals and the development of adaptive-compensatory processes. Also, there was evidence of decreased morphofunctional activity, confirmed by the reduction in optical density of the nuclei and cytoplasm of epitheliocytes of the stratum basale of the oral mucosa in rats under the influence of EMR due to lower DNA content in the nucleus and RNA in the cytoplasm of these cells.

The morphological changes in the oral mucosa of experimental animals correlate with the results of the clinical examination of individuals who experienced prolonged professional exposure to industrial frequency EMR. It is probable that the latter can facilitate the realization of pathogenetic links in the development of the diagnosed pathology of the oral mucosa in patients of the Main Group. Specifically, in the mentioned group, diseases of the oral mucosa were diagnosed in 81.5 % of individuals, which was by 4.2 times higher than the indices of the Comparison Group ($p<0.05$).

It should be noted that our earlier studies of the impact of EMF on laboratory animals indicated deterioration of oral fluid parameters of rats [2].

Considering the above mentioned, today humanity widely uses electromagnetic energy in various technological processes, leading to the accumulation of EMF-effects, which can no longer be ignored [13, 14]. Undoubtedly, in response to damage, adaptive and compensatory processes develop concurrently because the human body has considerable reserve capabilities. Yet, they are finite, consequently, the disturbance of adaptive processes triggers homeostasis instability and advancement of abnormality at various levels: molecular, subcellular, cellular, tissue, and organ [4], and can even modify the functionality of all body systems [6, 15]. Our research aligns with the opinions of previous researchers [5, 9, 10, 11, 12].

Conclusions

1. The negative effects of non-ionizing low-frequency EMR on the oral mucosa state of test animals relate to the reduction in morphofunctional activity and suppression of the regenerative potential of this structural component. This is a risk factor for the development of oral mucous membrane diseases.

2. The results of the clinical examination correlate with the findings of the experimental study and confirm the negative impact of prolonged professional exposure to industrial frequency EMR on the oral mucosa of patients. This has led to the development of mucosal pathology in 81.5 % of individuals, which was higher by a factor of 4.2 than in the Comparison Group. This situation requires implementation of therapeutic and preventive measures.

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