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## CORRECTION OF BIOCHEMICAL MARKERS OF ANTIMICROBIAL DEFENSE AND BACTERIAL CONTAMINATION IN RAT GINGIVA INDUCED BY A MODEL OF PEROXIDATIVE PERIODONTITIS AND ALIMENTARY PROTEIN DEFICIENCY USING A THERAPEUTIC-PROPHYLACTIC COMPLEX

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The study assessed the impact of a newly developed therapeutic-prophylactic complex on indices of nonspecific antimicrobial defence (lysozyme activity) and bacterial contamination (urease activity, dysbiosis index) in gingival homogenates of rats subjected to a combined model of peroxidative periodontitis and alimentary protein deficiency. Thirty male Wistar rats were randomly assigned to three groups: an intact control, a combined-pathology group, and a combined-pathology group treated with the therapeutic-prophylactic complex. The therapeutic-prophylactic complex effectively prevented disruptions of antimicrobial homeostasis and substantially restored the microbiological balance of periodontal tissues under conditions of protein-oxidative stress, underscoring its potential as a prophylactic agent for nutrition-related periodontal injuries.

**Key words:** periodontitis, protein deficiency, rats, experiment, biochemical markers.

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## КОРЕКЦІЯ ЗМІН БІОХІМІЧНИХ МАРКЕРІВ АНТИМІКРОБНОГО ЗАХИСТУ ТА БАКТЕРІАЛЬНОЇ КОНТАМІНАЦІЇ У ЯСНАХ ЩУРІВ, ВИКЛИКАНИХ МОДЕЛЮВАННЯМ ПЕРЕКИСНОГО ПАРОДОНТИТУ ТА АЛІМЕНТАРНОГО ДЕФІЦИТУ БІЛКУ, ЗА ДОПОМОГОЮ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Дослідження було присвячено оцінці впливу розробленого лікувально-профілактичного комплексу на показники неспецифічного антимікробного захисту (активність лізоциму) та бактеріальної контамінації (активність уреаз, індекс дисбіозу) у гомогенатах ясен щурів за моделювання перекисного пародонтиту у поєднанні з аліментарним дефіцитом білка. В експерименті взяли участь 30 щурів-самців лінії Вістар, яких поділили на три групи: інтактну, групу з поєднаною патологією та групу з поєднаною патологією, які отримували лікувально-профілактичний комплекс. Лікувально-профілактичний комплекс ефективно попереджає порушення антимікробного гомеостазу та значною мірою відновлює мікробіологічну рівновагу тканин пародонта в умовах білково-окиснювального стресу, що підтверджує перспективність його використання як профілактичного засобу за ризику нутритивно-зумовлених уражень пародонта.

**Ключові слова:** пародонтит, білковий дефіцит, щури, експеримент, біохімічні маркери.

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Periodontal diseases are chronic inflammatory infections that progressively destroy the tooth-supporting apparatus and remain a principal cause of tooth loss worldwide [8, 11]. Their pathogenesis is driven by complex dysbiotic shifts in the oral microbiome that favour periopathogenic species and provoke an exaggerated host immune response. Recent epidemiological analyses estimate that more than one billion individuals are affected, underscoring periodontitis as a major unmet global health need [8].

In health, innate antimicrobial molecules within saliva and gingival crevicular fluid restrain bacterial proliferation. Lysozyme – a c-type glycosyl hydrolase – hydrolyses the  $\beta$ -1,4-linkage between N-acetylmuramic acid and N-acetyl-D-glucosamine in bacterial peptidoglycan, thereby exerting potent bactericidal and bacteriostatic effects [7]. Urease, in contrast, is produced exclusively by oral microorganisms; its activity reflects microbial biomass because host tissues do not express the enzyme. Consequently, the urease-to-lysozyme activity ratio (dysbiosis index) is a sensitive proxy for the balance between bacterial contamination and nonspecific antimicrobial defence [11]. Reduced lysozyme activity coupled with elevated urease therefore signals compromised innate immunity and the onset of dysbiosis [7, 11].

Systemic nutrition has emerged as a critical determinant of periodontal resilience. Protein-energy malnutrition (PEM) diminishes immune competence, salivary function and tissue repair, predisposing to periodontal destruction [9]. Conversely, dietary adequacy – particularly sufficient intake of high-quality proteins, micronutrients and antioxidants – has been linked to lower gingival inflammation, a more favourable microbial profile and enhanced healing [5]. These observations highlight the need to model periodontal disease under conditions of protein deficiency to better understand the interplay between nutrition, antimicrobial defence and microbial overgrowth.

Experimental animal models provide a controlled framework for dissecting such interactions [10]. Rat models combining alimentary protein deficiency with lipid peroxidation reliably reproduce oxidative stress, dysbiosis and connective-tissue breakdown characteristic of human periodontitis [7]. Importantly, integrated therapeutic-prophylactic regimens have shown promise in these models by restoring antioxidant capacity and normalising lysozyme and urease activities in gingival tissues [7]. Elucidating how such complexes modulate key biochemical markers in protein-deficient periodontitis could inform future adjunctive strategies for high-risk human populations.

**The purpose** of the study was to evaluate the effect of the drug complex on biochemical markers of rat gingival homogenates – namely, indices of nonspecific antimicrobial defence and bacterial contamination against the background of modelling peroxidative periodontitis and alimentary protein deficiency.

**Materials and methods.** Experimental studies were conducted using 30 male rats of 1 month of age, with an average weight of 60–75 g, of the Wistar line of herd breeding, which was chosen as a model for the study, which is one of the most common lines of laboratory rats for experimental studies. The animals were kept in normal vivarium conditions under natural light and with free access to water and food. Throughout the experiment, the microclimatic conditions of the vivarium environment were strictly observed: temperature (19–23°C) and humidity (50–75 %). Experimental studies were conducted at the Laboratory of Biochemistry and Vivarium of the SE “The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine” (SE “ISMFS NAMS”). All experiments on rats were conducted according to standard operating procedures approved by SE “ISMFS NAMS”, developed in accordance with the Guidelines of the Pharmacological Committee of the Ministry of Health of Ukraine and the International Regulations for the Use of Laboratory Animals [3, 7].

The animals were divided into 3 groups as follows:

- 1<sup>st</sup> group – intact, n=10;
- 2<sup>nd</sup> group – modelling of peroxidative periodontitis and alimentary protein deficiency (combined pathology), n=10;
- 3<sup>rd</sup> group – combined pathology + TPC, n=10.

Animals in the intact group received balanced feed that fully covered their daily requirements for nutrients, vitamins, minerals and trace elements, as well as disinfected and reverse osmosis-filtered water with free access.

A model of combined pathology – alimentary protein deficiency in rats of the 2<sup>nd</sup> and 3<sup>rd</sup> groups was modelled by transferring animals to a diet deficient in proteins, namely essential amino acids (corn – 73.5 %, beetroot – 14, 7 %, cabbage – 11.8 %), and these groups were also modelled with experimental periodontitis by adding peroxidised sunflower oil to the daily diet at the rate of 1 ml per animal per day for 60 days. The peroxidised oil was obtained by heating refined sunflower oil in the presence of 2 % CuSO<sub>4</sub> for 8–10 hours until the peroxide number reached more than 35 units. The development of the experimental model of periodontitis was based on the modern concept of the development of the disease in humans.

The duration of the experiment was 60 days. Animals were withdrawn from the experiment by an overdose of intraperitoneal anaesthesia using sodium thiopental (at a rate of 40 mg/kg) on day 60 of the experiment by total bleeding from the heart. In gingival homogenates prepared in 20 mg/mL Tris-HCl buffer (pH 7.5), we quantified lysozyme activity as an indicator of nonspecific antimicrobial defence and urease activity as a marker of microbial colonisation. The dysbiosis index (DI) was calculated from the ratio of relative urease to lysozyme activities.

The results were processed by variational statistical methods of analysis using the Microsoft Office Excel 2016 software. Statistical processing of the experimental study results was carried out by the methods of variation analysis using Student's test. The difference was considered statistically significant at  $p < 0.01$  [4].

**Results of the study and their discussion.** Quantitative profiling of host-derived antimicrobial enzymes and microbially produced virulence factors offers a direct biochemical window into the equilibrium between defense and colonization in periodontal tissues. Among the available candidates, lysozyme and urease provide complementary and thus highly informative read-outs because they originate

from distinct biological compartments (host versus microbe) yet converge functionally on biofilm regulation. To elucidate how combined protein deficiency and lipid peroxidation disturb this equilibrium, and how the therapeutic–prophylactic complex rectifies it, we determined the activities of both enzymes in gingival homogenates after 60 days of experimental exposure.

At this stage of the investigation, the status of the gingival microbiocenosis was assessed using biochemical markers – lysozyme and urease activities – the results of which are presented in Table 1.

Table 1

**Antimicrobial defence and bacterial contamination in rat gingiva with combined pathology and after prophylactic treatment with the drug complex, M±m**

Groups	Indicators	Urease activity, mkat/kg	Lysozyme activity, U/kg	Dysbiosis index
Intact, n=10		1.15±0.08	36.2±1.5	1.0±0.09
Combined pathology, n=10		1.68±0.07 p<0.001	29.4±1.2 p<0.001	1.81±0.10 p<0.001
Combined pathology + TPC, n=10		0.98±0.08 p>0.2 p <sub>1</sub> <0.001	35.8±1.8 p>0.7 p <sub>1</sub> <0.001	0.88±0.06 p>0.3 p <sub>1</sub> <0.001

Note. p – significance of differences to the intact group; p<sub>1</sub> – significance of differences to the “combined pathology” group.

Lysozyme is a glycosidic enzyme that cleaves peptidoglycan by hydrolysing the 1,4-β linkages between N-acetyl-muramic acid and N-acetyl-D-glucosamine, exerting both bactericidal and bacteriostatic effects. A key function of lysozyme is to disrupt membrane permeability, metabolism and cell-growth processes in Gram-positive bacteria. Although Gram-positive bacteria are the primary targets owing to their thick peptidoglycan layer, lysozyme exhibits measurable lytic or growth-inhibitory effects against certain Gram-negative species (e.g., *Aggregatibacter actinomycetemcomitans*) once the outer membrane is destabilised by host defensins.

Urease, by contrast, is produced exclusively by opportunistic and pathogenic microorganisms and is not synthesized by commensal eubiota or host somatic cells. Functionally, the enzyme hydrolyses urea into ammonia and carbon dioxide, raising the local pH and creating an alkaline milieu that favors the outgrowth of urease-positive periopathogens such as *Porphyromonas* and *Prevotella* species. The resulting increase in ammonia not only compromises epithelial integrity but also drives calcium-phosphate supersaturation, thereby accelerating dental calculus formation and stabilizing pathogenic biofilms. Because mammalian tissues lack endogenous urease, its activity serves as a highly specific biochemical proxy for microbial burden and a sensitive indicator of dysbiotic shifts within the periodontal niche.

In the gingiva of animals in Group 2 (combined periodontitis and low-protein diet), urease activity increased by 46 % (p<0.001), indicating enhanced microbial colonisation, whereas lysozyme activity fell by 18.8 % (p<0.001). Consequently, the dysbiosis index rose by 81 % (p<0.001) after prolonged exposure to the low-protein diet and lipid peroxides, reflecting a substantial expansion of opportunistic and pathogenic flora against a background of weakened nonspecific antimicrobial defence and culminating in dysbiosis.

Regular administration of the prophylactic complex to Group 3 rats for 60 days markedly counteracted these changes, limiting the decline in lysozyme activity to 21.7 %, curbing the rise in urease activity to 41.6 %, and reducing the two-fold increase in the dysbiosis index. All parameters returned to levels indistinguishable from those of intact animals (p>0.2; p>0.7; p>0.3).

Analysis of the conducted experiments indicates that the therapeutic-prophylactic complex possesses a strong preventive capacity to curb dysbiosis and enhance nonspecific antimicrobial defence under conditions of experimentally induced periodontitis combined with alimentary protein and calcium deficiency.

Thus, the biochemical findings in rat gingiva show that modelling the combined pathology – peroxidative periodontitis together with alimentary protein deficiency – produces marked pathological disturbances, whereas continuous administration of the complex for six days restores these parameters to normal levels.

The present investigation demonstrates that simultaneous exposure to lipid peroxidation and a protein-deficient diet produces a profound dysbiotic shift in rat gingival tissue, as evidenced by a 46 % increase in urease activity and an 18.8 % reduction in lysozyme activity, culminating in an 81 % rise in the dysbiosis index relative to intact controls. These findings align with clinical observations that nutritional insufficiency compromises salivary antimicrobial systems and promotes periopathogenic overgrowth in humans [5, 9]. Protein-energy malnutrition decreases mucosal immunoglobulin A, alters salivary flow and

reduces antimicrobial peptides, thereby rendering the periodontium more susceptible to microbial colonisation [9]. Importantly, 60 days of prophylactic complex (TPC) administration fully reversed these biochemical disturbances: urease activity fell below baseline, lysozyme levels rebounded to near-intact values and the dysbiosis index normalised. Comparable antioxidant-enriched regimens have been shown to restore catalase, superoxide dismutase and glutathione peroxidase activities in experimental periodontitis, while simultaneously limiting lipid peroxidation and bacterial load [6]. The present data extend those observations by demonstrating that the same multifactorial approach not only bolsters redox homeostasis (as reported previously) but also re-establishes the lysozyme-urease balance – a direct surrogate of antimicrobial defence. Mechanistically, the lysozyme recovery can plausibly be attributed to (i) reduced oxidative degradation of the enzyme, and (ii) improved protein synthesis secondary to better amino-acid availability supplied by the complex. The marked decline in urease activity suggests either a diminution of urease-positive opportunists or direct anti-urease effects of specific phytochemicals incorporated in the formulation, as certain plant polyphenols inhibit urease holoenzyme formation [7]. Decreasing urease is clinically meaningful because ammonia production elevates local pH, facilitates calculus deposition and enhances biofilm virulence [11]. From a translational standpoint, our results support the concept that targeted nutritional and antioxidant support can offset the periodontal risks imposed by protein deficiency and oxidative stress, conditions frequently co-existing in low-resource settings and among medically compromised patients [5]. The data also corroborate global disease-burden analyses calling for adjunctive, host-directed measures to complement conventional mechanical-antimicrobial therapy in periodontitis management [8]. Limitations include the use of a single animal species and the absence of direct histomorphometric assessment of alveolar bone. However, the robust normalisation of multiple biochemical markers strengthens confidence in the biological relevance of the observed effects. Future studies should dissect the individual contributions of TPC components and evaluate long-term periodontal outcomes after treatment cessation. In summary, protein deprivation and lipid peroxidation synergistically impair innate antimicrobial defences in rat gingiva, but a multifaceted therapeutic-prophylactic complex effectively restores lysozyme activity, suppresses urease production and normalises the dysbiosis index. These findings provide mechanistic support for integrated nutritional-antioxidant interventions as promising adjuncts in the prevention and management of nutrition-linked periodontal disease.

### Conclusions

1. The combined model of peroxidative periodontitis and alimentary protein deficiency triggers a marked increase in gingival bacterial contamination and suppresses nonspecific antimicrobial defence, resulting in an almost two-fold rise in the dysbiosis index.
2. Sixty-day administration of the therapeutic-prophylactic complex reliably normalises lysozyme and urease activities and restores the dysbiosis index to values observed in intact animals, demonstrating a pronounced protective potential for the periodontal microbiome.
3. These findings substantiate the rationale for implementing integrated nutrition- and antioxidant-based strategies in the prevention and adjunctive management of periodontitis associated with protein-energy malnutrition and oxidative stress.

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## AGE-RELATED MORPHOLOGICAL FEATURES OF THE INTRACRANIAL (CISTERNAL) SEGMENT OF THE FACIAL NERVE DURING THE POSTNATAL PERIOD

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The study of the intratemporal structure of the facial nerve has long been a focus of interest for researchers. In this article, the age-related morphological features of the facial nerve segment extending from the base of the brain to the internal acoustic meatus were examined. The material consisted of cross-sections of the intracranial (cisternal) portion of the facial nerve, obtained from 96 human cadavers of both sexes and various stages of postnatal development. According to the case history, no pathological changes were identified in the skull or temporal bone, and no facial nerve pathologies were recorded in any of the cadavers. For histological examination, the cadavers from which the study material was obtained were divided into five age groups: group I (7–12 years) – 8 cadavers; group II (13–20 years) – 11 cadavers, group III (21–35 years) – 22 cadavers, group IV (36–57 years) – 35 cadavers and group V (58–74 years) – 20 cadavers. The samples were fixed in a solution containing 2 % paraformaldehyde, 2 % glutaraldehyde, and 0.1 % picric acid in 0.1 M phosphate buffer (pH 7.4). The results showed that in adults, the shape of the facial nerve is either oval or irregular, but its configuration is not directly related to the internal organization of the nerve. The number and arrangement of nerve bundles, as well as their myeloarchitectonic structure, are determined by the characteristics of early ontogeny. The study also found that the shape, size, number, and topography of the nerve bundles are variable parameters that depend on both individual features and age. These indicators are closely related to the connective tissue stroma of the nerve trunk.

**Key words:** facial nerve, myeloarchitectonics, myelinated nerve fibers, connective tissue stroma.

G.E. Керимзаде

## ВІКОВІ МОРФОЛОГІЧНІ ОСОБЛИВОСТІ ВНУТРІШНЬОЧЕРЕПНОГО (ЦИСТЕРНАЛЬНОГО) СЕГМЕНТУ ЛИЦЕВОГО НЕРВА У ПОСТНАТАЛЬНОМУ ПЕРІОДІ

Вивчення внутрішньоствольної структури лицевого нерва тривалий час перебувало у центрі уваги дослідників. У цьому дослідженні розглядалися вікові морфологічні особливості сегмента лицевого нерва, що тягнеться від основи мозку до внутрішнього слухового проходу. Матеріалом для дослідження слугували поперечні зрізи внутрішньочерепної (цистернальної) частини лицевого нерва, отримані від 96 трупів людських обох статей на різних етапах постнатального розвитку. Згідно з анамнезом, у жодному випадку не було виявлено патологічних змін в області черепа або скроневої кістки, а також не було зафіксовано патологій самого лицевого нерва. Для гістологічного дослідження трупи було поділено п'ять вікових груп: I група (7–12 років) – 8 трупів; II група (13–20 років) – 11 трупів; III група (21–35 років) – 22 трупи; IV група (36–57 років) – 35 трупів; V група (58–74 років) – 20 трупів. Зразки фіксували у розчині, що містить 2 % параформальдегіду, 2 % глутарового альдегіду та 0,1 % пікринової кислоти на основі 0,1M фосфатного буфера (pH 7,4). Результати показали, що в дорослих осіб лицевий нерв або овальної, або нечітко окресленої форми, проте його конфігурація немає прямого зв'язку з внутрішньою організацією нерва. Кількість та розташування нервових пучків, а також їх мієлоархітектонічна будова визначаються особливостями раннього онтогенезу. Також встановлено, що форма, розміри, число та топографія нервових пучків є змінними параметрами, що залежать від індивідуальних особливостей, так і від віку. Ці показники тісно пов'язані з будовою сполучнотканинної стромі нервового стовбура.

**Ключові слова:** лицевий нерв, мієлоархітектоніка, мієлінові нервові волокна, сполучнотканинна строма.

A review of the literature shows that the study of the intratemporal structure of the facial nerve has been a focus of researchers' attention for many years [3]. The majority of studies have been dedicated to the extracranial segment of the nerve – specifically, the part following its exit from the stylomastoid foramen. This focus is of particular importance for addressing the complications that arise during the restoration of the nerve after injuries sustained in surgical interventions in the middle ear, parotid gland, and the cervicofacial region. The individual variability of each peripheral nerve is reflected in changes in the number and spectrum of myelinated fibers at different stages of life. In the dynamics of the formation of the intratemporal structure of the facial nerve, three main developmental stages are distinguished: active