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CORRECTION OF INDICATORS OF MINERAL METABOLISM IN THE ORAL FLUID OF PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS, OSTEOPENIA AND OSTEOPOROSIS USING A THERAPEUTIC AND PROPHYLACTIC COMPLEX

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The study devoted to evaluation the effect of a therapeutic complex of drugs on indicators of mineral metabolism – calcium and inorganic phosphorus concentrations in the oral fluid of patients with chronic generalized periodontitis against a background of osteopenia and osteoporosis. Thirty-five adults (25–55 years) were allocated to three cohorts: an intact healthy control group, a comparison group receiving guideline-based mechanical and pharmacological periodontal therapy, and a main group receiving the same standard therapy supplemented with the therapeutic-prophylactic complex. Findings indicate that the proposed therapeutic complex of drugs exerts a pronounced mineralizing effect and improves the biochemical characteristics of oral fluid in patients with chronic generalized periodontitis associated with osteopenia and osteoporosis.

Key words: chronic generalised periodontitis, osteopenia, osteoporosis, calcium, phosphorus, dental treatment, therapeutic and prophylactic complex.

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КОРЕКЦІЯ ПОКАЗНИКІВ МІНЕРАЛЬНОГО ОБМІНУ У РОТОВІЙ РІДИНІ ПАЦІЄНТІВ З ХРОНІЧНИМ ГЕНЕРАЛІЗОВАНИМ ПАРОДОНТИТОМ, ОСТЕОПЕНІЄЮ ТА ОСТЕОПОРОЗОМ ЗА ДОПОМОГОЮ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Дослідження присвячене оцінці впливу терапевтичного комплексу препаратів на показники мінерального обміну – концентрацію кальцію та неорганічного фосфору в ротовій рідині пацієнтів з хронічним генералізованим пародонтитом на тлі остеопенії та остеопорозу. Тридцять п'ять дорослих (віком 25–55 років) були розподілені на три групи: інтактну здорову контрольну групу, групу порівняння, яка отримувала механічну та фармакологічну пародонтальну терапію відповідно до клінічних настанов, та основну групу, яка отримувала ту саму стандартну терапію, доповнену лікувально-профілактичним комплексом. Результати досліджень свідчать про те, що запропонований терапевтичний комплекс препаратів чинить виражений мінералізуючий ефект та покращує біохімічні показники ротової рідини у пацієнтів із хронічним генералізованим пародонтитом, що супроводжується остеопенією та остеопорозом.

Ключові слова: хронічний генералізований пародонтит, остеопенія, остеопороз, кальцій, фосфор, стоматологічне лікування, лікувально-профілактичний комплекс.

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Periodontitis is a chronic host-mediated inflammatory disease caused by dysbiotic oral biofilms and characterized by progressive destruction of the periodontal ligament and alveolar bone. It remains highly prevalent worldwide, with severe forms affecting about 12.5 % of the population [9], and is a major cause of tooth loss. In addition to its local destructive effects, periodontitis is associated with systemic disorders, including diabetes and cardiovascular diseases [3, 9]. Its pathogenesis reflects an imbalance between microbial challenge and host defense, in which dysregulated inflammatory reactions, excessive proteolytic activity, and oxidative stress contribute to connective tissue and bone destruction.

Osteoporosis is a systemic skeletal disease characterized by decreased bone mineral density and impaired bone microarchitecture. It affects more than 200 million people globally [4, 7], and the risk of osteoporotic fractures rises markedly with age [4]. Periodontitis and osteoporosis share common risk factors and inflammatory mechanisms [4, 9]. Increasing evidence indicates a relationship between them: osteoporosis is associated with greater periodontal attachment and bone loss, whereas advanced periodontitis is often accompanied by reduced systemic bone mineral density [4, 9]. Meta-analyses and reviews have confirmed that low bone mineral density is linked to increased periodontal bone resorption [4, 11]. This relationship is likely

mediated by common pathogenic pathways, including altered RANKL/OPG signaling, pro-inflammatory cytokines, and unfavorable lifestyle factors [4, 9].

Mineral metabolism is essential for both skeletal and periodontal homeostasis. Calcium and phosphate are the main mineral components of bone and are also involved in multiple physiological processes. Their concentrations in oral fluid may reflect local mineralization and demineralization processes. Although correlations between salivary calcium and phosphorus and systemic bone mineral density remain inconsistent [5], these biomarkers may still be useful for assessing periodontal mineral dynamics and treatment effectiveness.

Conventional periodontal therapy is based on mechanical biofilm removal by scaling and root planing and is effective in reducing inflammation in the short term. However, in patients with osteopenia or osteoporosis, this approach alone may be insufficient for stable long-term results because systemic bone loss and chronic inflammation can impair tissue recovery [4]. This has stimulated interest in adjunctive therapeutic strategies aimed at modulating host response and supporting remineralization.

Such approaches include the use of vitamin D, antioxidants, osteotropic agents, and anti-resorptive drugs. Vitamin D_3 is of particular importance because it regulates calcium-phosphate metabolism, stimulates osteogenesis, and exhibits anti-inflammatory activity in periodontal tissues [6]. Antioxidants and micronutrients can reduce oxidative stress and thereby limit inflammatory tissue damage [8], whereas anti-resorptive agents have been proposed to preserve alveolar bone in high-risk patients [10]. Therefore, combined therapeutic regimens integrating antimicrobial, remineralizing, and host-modulating components appear promising [6].

On this basis, the present study evaluated a comprehensive therapeutic-prophylactic complex in patients with chronic generalized periodontitis and osteopenia/osteoporosis, assuming that such an approach may improve mineral metabolism and contribute to more stable periodontal outcomes in this high-risk group.

The purpose of the study was to evaluate the effect of a therapeutic complex of drugs on indicators of mineral metabolism – calcium and inorganic phosphorus concentrations in the oral fluid of patients with chronic generalized periodontitis against a background of osteopenia and osteoporosis.

Materials and methods. Biochemical studies of oral fluid were conducted in 35 patients aged 25–55 years. Biochemical studies were carried out in 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"). The study was carried out from 18 April 2022 to 29 April 2024.

Patients were divided into 3 groups:

– Group 1 – normal control (somatic healthy patients who met systemic and dental health criteria – normal medical exam, oral health and bone mineral density within the normal range), n=10;

– Group 2 – comparison (patients with chronic generalized periodontitis against a background of osteopenia and osteoporosis who underwent basic therapy according to the protocol, n=12);

– Group 3 – main (patients with chronic generalized periodontitis against a background of osteopenia and osteoporosis, who were additionally prescribed a therapeutic and prophylactic complex in addition to the main basic therapy, n=13).

No post-randomisation drop-outs or exclusions occurred, thus group sizes remained unchanged throughout follow-up.

Patients in the main group and the comparison group had chronic generalized periodontitis and a history of concomitant pathology – osteoporosis and osteopenia. Only patients with osteopenia (T-score −1.0 to −2.5) or moderate osteoporosis (T-score −2.5 to −3.0) were eligible, whereas recent osteoporotic fractures were exclusion criteria. The degree of osteopenia/osteoporosis was comparable across groups and is unlikely to confound the observed inter-group differences in periodontal or enzymatic outcomes.

Basic therapy in both periodontitis cohorts followed the national protocol (full-mouth scaling/root planing, professional hygiene instruction and a 0.12 % chlorhexidine rinse for 10 days). The main group additionally received a six-month cyclic therapeutic-prophylactic complex: Orthomol Vitamin D3 Plus (INN: cholecalciferol 20 µg with multivitamins/minerals; Orthomol GmbH, Germany) – two capsules once daily after meals for 60 days; Curaprox Perio Plus Gel (INN: chlorhexidine digluconate 0.9 %; Curaden AG, Switzerland) – pea-sized topical application to gingiva twice daily for seven days; Teraflex (INN: glucosamine hydrochloride 500 mg + chondroitin sulfate 400 mg; Balkanpharma, Bulgaria) – two capsules three times daily for 10 days; Biodent-3 Dental Elixir (herbal antiseptic/remineralising mouth-rinse; SPA "Odeska biotekhnolohiya", Ukraine) – 1–2 tsp diluted in 50 mL water, rinse twice daily for 60 days; Lacalut Sensitive toothpaste (INN: sodium fluoride 0.145 %; Dr Theiss Naturwaren GmbH, Germany) – morning brushing for 60 days; Lacalut Active Herbal 9 toothpaste (same manufacturer; sodium fluoride 0.145 % plus nine herbal extracts) – evening brushing for 60 days. The entire regimen was repeated once at month 6 to sustain therapeutic effects.

Oral fluid was collected in the morning, on an empty stomach, by spitting into sterile centrifuge tubes (without prior cleaning or rinsing of the oral cavity) for 5–10 minutes. Before performing biochemical analysis, the oral fluid was thawed at room temperature, centrifuged at 2,500 rpm for 20

minutes at a temperature of +4°C (bench centrifuge RS-6, MedTech, Ukraine), and the supernatant was collected for biochemical analysis. Biochemical studies of calcium and inorganic phosphorus, reflecting the mineralizing properties of the oral fluid, were carried out in the oral fluid of patients. The calcium concentration was assessed by the reaction with Arsenazo III, resulting in the formation of a colored complex measured spectrophotometrically at 650 nm, using a reagent kit ("Human", Germany).

Biochemical studies of inorganic phosphorus, reflecting the phosphate component of mineral metabolism in the oral fluid, were carried out in the oral fluid of patients. The inorganic phosphorus concentration was assessed by the formation of phosphomolybdic acid in a strongly acidic medium followed by its reduction to molybdenum blue in the presence of iron (II), with subsequent spectrophotometric measurement at 620 nm (570–660 nm), using a reagent kit ("Human", Germany) [1].

All treatment, preventive and diagnostic measures were carried out only after the patients signed a voluntary informed consent in accordance with the principles of bioethics set forth in the Declaration of Helsinki "for Ethical Principles for Medical Research Involving Human Subjects" and "Universal Declaration on Bioethics and Human Rights (UNESCO)". All participants were adults, cognitively competent, and not otherwise classified as a vulnerable population under Good Clinical Practice. Studies recommended by the Commission on Bioethical Expertise (conclusion of the bioethics commission of the SE "ISMFS NAMS", protocol No. 1011 of 04/14/2022).

Data processing was carried out with STATISTICA 6.1. Prior to parametric testing, the Shapiro-Wilk normality test was applied to each continuous variable; none showed significant deviation from a Gaussian distribution ($p>0.05$). Therefore, inter-group comparisons were performed with the two-tailed Student's t-test. When pair-wise contrasts were required (Control × Comparison, Control × Intervention, Comparison × Intervention), the family-wise type-I error rate was controlled with the Bonferroni adjustment. Between-group differences were deemed statistically significant at $p<0.003$ [2].

Results of the study and their discussion. This stage of our work involved determining the levels of mineral metabolism – specifically, the concentrations of calcium and inorganic phosphorus – in the oral fluid of patients in the study groups at various points during the observation period.

Phosphorus is an important structural component of cell membranes and nucleic acids, participating in biological processes such as bone mineralisation, energy production, cell signalling via phosphorylation reactions, and the regulation of acid-base homeostasis (Table 1).

Table 1

Inorganic phosphorus levels in patients' saliva at different stages of treatment, mmol/l (M±m)

Terms / Groups	Terms of the study				
	Initial state	After 3 months	After 8 months	After 1.5 years	After 2 years
Normal control, n=10	5.10±0.12				
Comparison, n=12	3.45±0.15 p<0.001	3.96±0.13 p<0.001 p_1<0.02	3.65±0.12 p<0.001 p_1>0.5	3.74±0.10 p<0.001 p_1>0.5	3.56±0.12 p<0.001 p_1>0.5
Main, n=13	3.76±0.13 p<0.001 p_2>0.2	4.30±0.12 p<0.001 p_1<0.01 p_2>0.1	4.82±0.14 p>0.5 p_1<0.001 p_2<0.001	5.0±0.12 p>0.5 p_1<0.001 p_2<0.001	5.24±0.17 p>0.5 p_1<0.001 p_2<0.001

N o t e . p – significance of differences from the norm; p_1 – significance of differences from the initial state. p_2 – significance of differences from the indices in groups.

Calcium plays a key role in ensuring the proper functioning of the skeletal, endocrine, cardiovascular and nervous systems. In the human body, 99 % of calcium is found in the bones, providing the structure and strength of the skeleton. The remainder is involved in a range of metabolic processes – nerve transmission, muscle contraction, enzymatic activity, transmembrane transport and hormonal function (Table 2).

Table 2

Calcium levels in patients' saliva at different stages of treatment, mmol/l (M±m)

Terms / Groups	Terms of the study				
	Initial state	After 3 months	After 8 months	After 1.5 years	After 2 years
Normal control, n=10	0.96±0.07				
Comparison, n=12	0.69±0.03 p<0.001	0.74±0.05 p<0.05 p_1>0.4	0.78±0.04 p<0.05 p_1>0.1	0.70±0.05 p<0.002 p_1>0.7	0.73±0.04 p<0.02 p_1>0.5
Main, n=13	0.72±0.04 p<0.01 p_2>0.5	0.83±0.05 p>0.2 p_1>0.1 p_2>0.25	0.90±0.04 p>0.5 p_1<0.001 p_2<0.05	1.0±0.05 p>0.5 p_1<0.01 p_2<0.001	0.95±0.04 p>0.7 p_1<0.0001 p_2<0.002

Note. p – significance of differences from the norm; p_1 – significance of differences from the initial state. p_2 – significance of differences from the indices in groups.

The data presented at the initial stage of the study indicate that the levels of key components involved in bone mineralisation processes – inorganic phosphorus and calcium – in both observed groups of patients with chronic generalised periodontitis and osteoporosis was significantly lower than normal values by a factor of 1.4 ($p<0.01$) and 1.3 ($p<0.01$), respectively. The administration of standard baseline therapy according to protocol in patients in the comparison group led to an increase in inorganic phosphorus alone by 14.7 % three months after treatment ($p < 0.02$); at long-term follow-up, these values corresponded to pre-treatment levels. Calcium levels in the saliva of the patients under observation did not undergo significant changes at any stage of treatment under the influence of standard therapy and remained at baseline levels.

Analysis of the oral fluid of patients in the main group, who were being treated for chronic generalised periodontitis and osteoporosis and who received LPC preparations in addition to their standard therapy, revealed a significant improvement, particularly in its mineralising capacity. Thus, a trend towards an increase in calcium concentration was observed at various observation time points: after 3 months by 15.2 % ($p_1>0.1$), after 8 months by 25 % ($p_1<0.001$), after 1.5 years by 38 % ($p_1<0.01$), and by 31.9 % after 2 years ($p<0.001$), compared with pre-treatment data; after 1.5 and 2 years, these values did not differ from normal ranges and were significantly higher than those of the control group at all observation stages. At the same time, a significant increase in inorganic phosphorus was recorded at later observation time points – by 14.3 % after 3 months ($p<0.001$), by 28.1 % after 8 months ($p<0.001$), after 1.5 years by 32.9 % ($p<0.001$), and after 2 years by 39.3 % ($p<0.001$). Calcium and inorganic phosphorus levels in the main group were significantly higher than those in the control group throughout the entire observation period.

The results obtained indicate the high mineralising effect of the developed therapeutic and prophylactic complex of preparations.

The obtained results confirm that chronic generalized periodontitis developing on the background of osteopenia and osteoporosis is accompanied by pronounced disturbances in the mineral composition of oral fluid, particularly by reduced concentrations of calcium and inorganic phosphorus at baseline. These findings are in agreement with the contemporary concept that periodontitis should be regarded not only as a local inflammatory lesion of the supporting tissues of the teeth, but also as a disease closely interconnected with systemic metabolic disorders affecting bone tissue [3, 10]. The high prevalence and multifactorial burden of periodontal diseases, emphasized by Ahmed et al. [3], further support the clinical relevance of identifying biochemical markers that reflect impaired mineral homeostasis in patients with combined periodontal and skeletal pathology.

Our data also correspond to the observations of Moghadam et al. [5], who demonstrated that salivary calcium and phosphate concentrations are altered in patients with chronic periodontitis and may reflect changes associated with bone mineral density. In the present study, both periodontitis groups initially showed significantly lower levels of calcium and inorganic phosphorus in oral fluid compared with normal values, which supports the view that oral fluid may serve as an informative non-invasive substrate for assessing mineral metabolism disturbances in patients with concomitant periodontal and osteopenic/osteoporotic disorders. At the same time, our results extend these findings by demonstrating not only baseline impairment, but also the long-term dynamics of these parameters over a two-year follow-up period.

The limited effectiveness of basic therapy alone observed in the comparison group is also noteworthy. Although a transient increase in inorganic phosphorus was recorded after 3 months, this effect was not maintained at distant observation periods, while calcium levels remained essentially unchanged throughout follow-up. Such findings are consistent with the notion that standard periodontal treatment, while necessary for controlling local inflammation, may be insufficient in patients with impaired systemic bone metabolism [10, 11]. Yu and Wang [10], as well as Zhu et al. [11], emphasized that osteoporosis and periodontal disease share common pathogenic pathways, including enhanced bone resorption, inflammatory activation, and impaired bone remodeling, which may reduce the long-term effectiveness of conventional periodontal therapy when used without adjunctive corrective measures.

In contrast, the use of the proposed therapeutic and prophylactic complex in the main group was associated with a progressive normalization of both calcium and inorganic phosphorus concentrations in oral fluid, with values approaching or reaching normal levels at distant time points. These results may be explained by the multicomponent character of the complex, particularly the inclusion of vitamin D3-containing support, remineralizing agents, and oral hygiene products with fluoride and anti-inflammatory activity. In this regard, our findings are in line with the review by Moszura et al. [6], who highlighted the important role of vitamin D3 in maintaining periodontal health through regulation of calcium-phosphorus metabolism, modulation of inflammatory responses, and support of bone homeostasis. The sustained increase in mineral metabolism indices in our main group suggests that such adjunctive therapy may enhance the mineralizing potential of oral fluid and create more

favorable conditions for stabilization of periodontal tissues in patients with reduced bone mineral density.

Thus, the present results substantiate the pathogenetic validity of combining basic periodontal treatment with a targeted therapeutic and prophylactic complex in patients with chronic generalized periodontitis associated with osteopenia and osteoporosis. In contrast to basic therapy alone, this approach provided a stable corrective effect on oral-fluid mineral metabolism, which may be considered one of the biochemical mechanisms underlying its long-term clinical benefit [5, 6, 10].

Limitations. The study was limited by its single-center design and by the inclusion of only patients with osteopenia or moderate osteoporosis without recent osteoporotic fractures, which may limit the extrapolation of the findings to patients with more severe skeletal pathology.

Conclusions

1. Patients with chronic generalized periodontitis associated with osteopenia and osteoporosis exhibit significant disturbances of mineral metabolism in the oral fluid, manifested by decreased concentrations of calcium and inorganic phosphorus compared with normal values.

2. Basic periodontal therapy according to the protocol provides only limited correction of mineral metabolism indices: it causes a short-term increase in inorganic phosphorus after 3 months, but does not produce significant long-term changes in calcium concentration.

3. Additional administration of the proposed therapeutic and prophylactic complex leads to a marked improvement in the mineralizing properties of oral fluid, as evidenced by a progressive increase in calcium and inorganic phosphorus levels during follow-up.

4. In patients of the main group, calcium and inorganic phosphorus concentrations reached values close to physiological norm at distant time points and remained significantly higher than in the comparison group, indicating the sustained effectiveness of the proposed complex.

5. The obtained results substantiate the high mineralizing potential of the therapeutic and prophylactic complex and support its use as an adjunct to standard treatment in patients with chronic generalized periodontitis combined with osteopenia and osteoporosis.

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