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CORRECTION OF MICROBIAL CONTAMINATION PARAMETERS AND LOCAL NONSPECIFIC IMMUNE STATUS IN THE ORAL FLUID OF PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS, OSTEOPENIA, AND OSTEOPOROSIS USING A THERAPEUTIC AND PROPHYLACTIC COMPLEX

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Changes in oral fluid urease and lysozyme activities were assessed in patients with chronic generalised periodontitis associated with osteopenia and osteoporosis. Thirty-five adults aged 25–55 years were examined: 10 healthy individuals, 12 comparison-group patients receiving standard therapy, and 13 main-group patients receiving an adjunctive therapeutic and prophylactic complex. Assessments were performed before treatment and at 3 and 8 months, 1.5 years, and 2 years. In the main group, urease activity decreased from 0.181 ± 0.010 to 0.055 ± 0.003 $\mu\text{kat/L}$ at 8 months and was 0.070 ± 0.004 $\mu\text{kat/L}$ at 2 years. Lysozyme activity increased from 68 ± 3 to 165 ± 12 U/L at 1.5 years and was 152 ± 11 U/L at 2 years. The comparison group showed no sustained urease reduction and less pronounced lysozyme changes. The findings describe favourable biochemical changes with the complex.

Key words: chronic generalised periodontitis, osteopenia, osteoporosis, oral fluid, urease, lysozyme, therapeutic and prophylactic complex.

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КОРЕКЦІЯ ПОКАЗНИКІВ МІКРОБНОГО ОБСІМЕНІННЯ ТА СТАНУ МІСЦЕВОГО НЕСПЕЦИФІЧНОГО ІМУНІТЕТУ У РОТОВІЙ РІДИНІ ПАЦІЄНТІВ З ХРОНІЧНИМ ГЕНЕРАЛІЗОВАНИМ ПАРОДОНТИТОМ, ОСТЕОПЕНІЄЮ ТА ОСТЕОПОРОЗОМ ЗА ДОПОМОГОЮ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Оцінено динаміку активності уреаз та лізоциму у ротовій рідині пацієнтів із хронічним генералізованим пародонтитом на тлі остеопенії та остеопорозу. Обстежено 35 осіб віком 25–55 років: 10 здорових осіб, 12 пацієнтів групи порівняння з базовою терапією та 13 пацієнтів основної групи з додатковим лікувально-профілактичним комплексом. Показники визначали до лікування, через 3 і 8 місяців, а також через 1,5 та 2 роки. В основній групі активність уреаз зменшилася з $0,181 \pm 0,010$ до $0,055 \pm 0,003$ мккат/л через 8 місяців і становила $0,070 \pm 0,004$ мккат/л через 2 роки. Активність лізоциму зросла з 68 ± 3 до 165 ± 12 Од/л через 1,5 року та становила 152 ± 11 Од/л через 2 роки. У групі порівняння тривалого зниження уреаз не спостерігали, а зміни лізоциму були менш вираженими. Результати характеризують сприятливу біохімічну динаміку під час застосування комплексу.

Ключові слова: хронічний генералізований пародонтит, остеопенія, остеопороз, ротова рідина, уреаз, лізоцим, лікувально-профілактичний комплекс.

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Chronic generalised periodontitis develops through sustained interactions between dysbiotic biofilms and the host inflammatory response [2]. Disturbance of this relationship contributes to connective tissue attachment loss and alveolar bone resorption, while the inflammatory microenvironment supports unfavourable changes in the microbial community [11]. Current concepts integrate

local mechanisms of tissue damage with systemic factors capable of modifying inflammation and tissue recovery [5, 6]. This approach provides a rationale for supplementing treatment assessment with functional characteristics of oral fluid that reflect selected aspects of the interaction between microorganisms and host defence systems.

Patients with reduced bone mineral density require particular attention. Osteopenia and osteoporosis constitute a systemic background against which local inflammatory and destructive processes may differ in intensity. Contemporary evidence indicates an association between periodontal disease and skeletal status, although its magnitude depends on population characteristics and accompanying factors [14]. Reduced bone mass should therefore not be considered a sufficient explanation for all periodontal changes; rather, it calls for an integrated assessment of local and systemic mechanisms.

Altered regulation of bone remodelling is a potential link between these conditions. Proinflammatory mediators and changes in signals associated with the RANK/RANKL/OPG system may favour osteoclastic resorption. Age, hormonal status, nutrition and other risk factors also influence alveolar bone health [15]. These considerations have encouraged interest in treatment approaches that address tissue metabolism alongside dental biofilm control. Such approaches nevertheless require evaluation in the specific patient populations for which they are proposed.

Experimental research provides further evidence of interactions between microbial and skeletal disturbances. Wang et al. demonstrated that periodontitis-associated salivary microbiota exacerbated systemic osteoclastogenesis in ovariectomised mice through changes in the intestinal environment and tryptophan metabolism [12]. These findings broaden understanding of possible effects of oral microbiota but do not establish the same causal pathway in humans. Their clinical relevance lies primarily in identifying mechanisms for further investigation, rather than supporting direct extrapolation of animal findings.

Oral fluid is an accessible biological material for repeated non-invasive assessment. Its protective properties depend on interactions among electrolytes, proteins, enzymes and other constituents, while its composition reflects contributions from salivary glands, oral tissues and microorganisms [10]. This complexity makes it informative but also requires cautious interpretation of individual parameters. Consistent sample collection, preparation and analytical conditions are important when comparing measurements over time.

Urease activity describes one aspect of oral microbial metabolism. Urea hydrolysis and ammonia formation influence acid–base conditions and the mineralisation of dental deposits. D'souza et al. examined relationships among salivary urea, pH, ureolytic microflora and dental calculus formation in a clinical study [3]. Urea concentration, ureolytic microbial abundance and urease activity are, however, different measurements. Changes in enzyme activity may therefore serve as a functional indicator but cannot replace direct assessment of bacterial abundance or species composition.

Lysozyme is an innate defence factor involved in maintaining mucosal barrier function. Its

enzymatic action involves hydrolysis of bacterial cell-wall peptidoglycan; additional antimicrobial mechanisms have also been described [4]. Measurement of lysozyme in oral fluid characterises one component of local non-specific defence. A single enzymatic parameter does not represent the full range of immune responses, and its changes should consequently be interpreted alongside the disease course and other measurements.

The biological material examined is also important. In a pilot study by Katsiki et al., differences in lysozyme measurements between patients with periodontitis and controls showed different directions in saliva and gingival crevicular fluid [7]. Ukrainian researchers Yarov et al. reported that postoperative changes in local immunological parameters depended on the type of host reactivity [13]. These observations emphasise the contextual nature of biomarkers. A systematic review of salivary protein biomarkers likewise supports assessment of their diagnostic potential in combination, rather than isolated interpretation of a single result [1].

Biofilm control, subgingival instrumentation and supportive periodontal care remain the foundation of treatment within the stepwise EFP approach [9]. Adjunctive agents should be evaluated against this background of standard care. In patients with altered bone metabolism, the effects of vitamin D on mineral metabolism and immune regulation are also of interest [8]. Nevertheless, the biological rationale for an individual component does not establish the efficacy of a multicomponent complex; this requires assessment using defined outcomes and sufficiently long follow-up.

Combined assessment of urease and lysozyme can therefore compare changes in microbial enzymatic activity with those in one component of local defence. Evaluation beyond the initial treatment period is particularly relevant because an early response does not necessarily indicate sustained change. Studying these parameters in patients with coexisting periodontal and skeletal disorders may clarify biochemical features of the response to comprehensive treatment and identify questions for subsequent clinical investigation.

The purpose of the study was to assess changes in oral fluid urease and lysozyme activities in patients with chronic generalised periodontitis associated with osteopenia and osteoporosis receiving an adjunctive therapeutic and prophylactic complex during two-year follow-up.

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).

Urease activity was expressed in µkat/L and lysozyme activity in U/L. Norm supplied with the materials were 0.055±0.003 µkat/L and 186±12 U/L, respectively; these served as common norm across time points. Urease activity was interpreted as an indirect measure of microbial ureolytic activity, whereas lysozyme activity characterised one component of local non-specific defence.

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 $p<0.003$.

Results of the study. At baseline, both patient groups showed elevated oral fluid urease activity (Table 1). Values were 0.191±0.010 µkat/L in the comparison group and 0.181±0.010 µkat/L in the main group, against a norm of 0.055±0.003 µkat/L. The respective means were 3.47 and 3.29 times the norm; $p<0.001$ was reported for both comparisons. The baseline between-group comparison was reported as $p_2>0.2$.

At 3 months, urease activity in the comparison group decreased to 0.152±0.010 µkat/L, a 20.4 % reduction from baseline. This direction of change was not sustained: values were 0.194±0.012 µkat/L at 8 months, 0.208±0.013 µkat/L at 1.5 years and 0.220±0.015 µkat/L at 2 years. The group means therefore indicated a transient early reduction, while urease activity remained elevated relative to the norm at later assessments.

In the main group, urease activity was 0.094±0.003 µkat/L at 3 months, corresponding to a 48.1 % reduction, and 0.055±0.003 µkat/L at 8 months, a 69.6 % reduction from baseline ($p_1<0.001$ reported at both time points). The mean coincided with the norm specifically at 8 months. At 1.5 and 2

years it increased slightly to 0.063 ± 0.005 and 0.070 ± 0.004 $\mu\text{kat/L}$, respectively, but remained 65.2 % and 61.3 % below baseline. At all post-treatment assessments, urease activity was lower in

the main group than in the comparison group, with author-reported $p_2 < 0.001$. At 2 years, the between-group difference was 68.2 % of the comparison-group mean.

Table 1

Oral fluid urease activity during treatment and follow-up, $\mu\text{kat/L}$

Assessment	Comparison group (n=12)	Main group (n=13)
Normal control, n=10: 0.055 ± 0.003 $\mu\text{kat/L}$		
Baseline	0.191 ± 0.010 $p < 0.001$	0.181 ± 0.01 $p < 0.001$ $p_2 > 0.2$
At 3 months	0.152 ± 0.010 $p < 0.001$ $p_1 < 0.01$	0.094 ± 0.003 $p > 0.2$ $p_1 < 0.001$ $p_2 < 0.001$
At 8 months	0.194 ± 0.012 $p < 0.001$ $p_1 > 0.7$	0.055 ± 0.003 $p > 0.7$ $p_1 < 0.001$ $p_2 < 0.001$
At 1.5 years	0.208 ± 0.013 $p < 0.001$ $p_1 > 0.4$	0.063 ± 0.005 $p > 0.7$ $p_1 < 0.001$ $p_2 < 0.001$
At 2 years	0.220 ± 0.015 $p < 0.001$ $p_1 > 0.2$	0.070 ± 0.004 $p < 0.01$ $p_1 < 0.001$ $p_2 < 0.001$

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.

Lysozyme showed a different baseline pattern (Table 2). Activity was 78 ± 5 U/L in the comparison group and 68 ± 3 U/L in the main group, against a norm of 186 ± 12 U/L. The respective reductions were

58.1 % and 63.4 % ($p < 0.001$ in both groups). Elevated urease activity was thus accompanied by reduced activity of one enzymatic component of local defence.

Table 2

Oral fluid lysozyme activity during treatment and follow-up, U/L

Assessment	Comparison group (n=12)	Main group (n=13)
Normal control, n=10: 186 ± 12 U/L		
Baseline	78 ± 5 $p < 0.001$	68 ± 3 $p < 0.001$ $p_2 > 0.1$
At 3 months	94 ± 7 $p < 0.001$ $p_1 > 0.1$	97 ± 7 $p < 0.001$ $p_1 < 0.002$ $p_2 > 0.5$
At 8 months	90 ± 7 $p < 0.001$ $p_1 > 0.2$	120 ± 10 $p < 0.001$ $p_1 < 0.001$ $p_2 < 0.001$
At 1.5 years	86 ± 5 $p < 0.001$ $p_1 > 0.2$	165 ± 12 $p > 0.2$ $p_1 < 0.001$ $p_2 < 0.001$
At 2 years	84 ± 5 $p < 0.001$ $p_1 > 0.5$	152 ± 11 $p > 0.1$ $p_1 < 0.001$ $p_2 < 0.001$

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.

In the comparison group, lysozyme activity increased to 94 ± 7 U/L at 3 months and subsequently measured 90 ± 7 , 86 ± 5 and 84 ± 5 U/L at 8 months, 1.5 years and 2 years, respectively. The supplied materials reported $p > 0.1$ or larger lower bounds at all assessments, while differences from the norm persisted ($p < 0.001$). At 2 years, mean activity was only 7.7 % above baseline.

In the main group, lysozyme activity increased to 97 ± 7 U/L at 3 months, a 42.6 % increase ($p_1 < 0.002$), to 120 ± 10 U/L at 8 months, a 76.5 % increase, to 165 ± 12 U/L at 1.5 years, a 142.6 % increase, and to 152 ± 11 U/L at 2 years, a 123.5 % increase from baseline ($p_1 < 0.001$ reported at the last three assessments). The groups had similar values at 3 months ($p_2 > 0.5$). At 8 months, 1.5 years and 2 years, main-group means were 1.33, 1.92 and 1.81 times the comparison-group values, respectively. These between-group comparisons are marked $p_2 < 0.001$ in the table; the 8-month entry in particular requires verification against the primary data.

The highest mean lysozyme activity was observed at 1.5 years. At 2 years, it was 7.9 % below this maximum but remained appreciably above baseline. Over the corresponding period, urease activity increased slightly from its minimum at 8 months. These comparisons between follow-up assessments are descriptive because separate statistical tests for them were not supplied.

Discussion. Taken together, the findings describe differences in the duration of the biochemical response to the two treatment approaches. Standard therapy was associated with an initial reduction in urease activity, but the mean had returned to its baseline level by 8 months. With adjunctive TPC use, the reduction persisted until the end of the two-year follow-up and was accompanied by increased lysozyme activity. This direction of change is consistent with the interdependence of microbial processes and host defence in the periodontal environment [6]. Nevertheless, the findings show an association with a combined intervention and do not determine the contribution of its individual components.

Baseline urease activity exceeding the norm more than threefold indicates pronounced differences in the ureolytic function of oral microbiota in these patients. It would, however, be excessive to infer a proportional increase in opportunistic bacterial abundance, because ureolytic properties depend on community composition and metabolic conditions. D'souza et al. investigated relationships among ureolytic microflora, urea and pH [3], whereas the present study measured enzyme activity. The reduction in urease should therefore be interpreted as a change in a functional parameter that warrants comparison with quantitative microbiological findings.

Lysozyme changes complement this assessment but do not directly mirror urease changes. At 3

months, the between-group difference in urease was already pronounced, whereas lysozyme activity remained similar in the two groups. Subsequently, lysozyme increased progressively in the main group, reaching its highest mean at 1.5 years. The two enzymes therefore followed different temporal profiles. Different rates of change in microbial metabolism and local defence are a possible explanation, but additional measurements are needed to test it; these results alone do not establish that mechanism.

Reduced baseline lysozyme activity is compatible with the lower salivary lysozyme measurements in patients with periodontitis reported by Katsiki et al. [7]. Their gingival crevicular fluid findings showed a different direction of difference. This limits direct comparisons across biological materials and between enzyme activity and protein concentration. The increase in lysozyme observed here can be considered a favourable change in a specific defence component, but does not demonstrate complete restoration of local immunity.

The Ukrainian study by Yarov et al. showed that immunological responses following periodontal intervention depended on host reactivity [13]. Although it examined surgical treatment with a different follow-up design, it illustrates the importance of systemic context when interpreting local measurements. This context requires specific characterisation in patients with osteopenia or osteoporosis. Reviews of the relationship between periodontal and skeletal disease describe shared inflammatory and metabolic pathways [14, 15], but their direct mediators were not measured in the present analysis.

Including a cholecalciferol-containing preparation has a biological rationale given the role of vitamin D in mineral metabolism and immune regulation [8]. However, this analysis provides no measurements of 25(OH)D, changes in bone mineral density or osteoclastogenesis. Improved oral fluid enzyme profiles cannot therefore be attributed to correction of vitamin D deficiency or restoration of bone tissue. Similarly, the effects of local antiseptics, oral hygiene products and other systemic components cannot be separated because these agents were administered together.

Two-year follow-up permits assessment of whether the favourable direction of change persisted, but does not establish complete normalisation of all functions studied. At 2 years, mean urease activity in the main group was 27.3 % above the norm, while lysozyme activity was 81.7 % of the norm. A non-significant difference in an individual test is not evidence of equivalence to normal. The small increase in urease and decrease in lysozyme relative to their most favourable follow-up values also support continued observation.

The practical contribution of these findings is to identify two accessible parameters for further assessment of treatment response. Their use should supplement clinical evaluation rather than replace measurements of probing depth, attachment loss and bleeding. This approach is consistent with the need for supportive care after active therapy [9] and combined assessment of salivary biomarkers [1]. Further research should compare enzyme trajectories

with clinical outcomes, microbiological profiles, bone densitometry and treatment adherence.

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

Conclusions

1. In patients with chronic generalised periodontitis associated with osteopenia and osteoporosis, baseline urease activity was 3.29–3.47 times the norm, while lysozyme activity was 58.1–63.4 % lower.
2. In the comparison group, the 20.4 % reduction in urease activity at 3 months was not sustained at later assessments; lysozyme activity remained below the norm throughout follow-up.
3. With adjunctive TPC use, urease activity decreased by 48.1 % at 3 months and by 69.6 % at 8 months. At 2 years, its mean remained 61.3 % below baseline.
4. In the main group, lysozyme activity increased from 68 ± 3 to 165 ± 12 U/L at 1.5 years and was 152 ± 11 U/L at 2 years. At the final assessment, its mean was 1.81 times the comparison-group value.
5. The combination of reduced urease and increased lysozyme activities describes favourable biochemical changes during comprehensive treatment. Conclusions about clinical efficacy require verification of the statistical data and comparison with periodontal and skeletal outcomes.

Further research should focus on the long-term evaluation of oral fluid urease and lysozyme activities in patients with chronic generalised periodontitis associated with osteopenia and osteoporosis in order to determine whether changes in microbial enzymatic activity and local non-specific defence correlate with clinical periodontal parameters, microbiological profiles and bone mineral density during comprehensive treatment and subsequent follow-up.

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