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CORRECTION OF ELASTASE ACTIVITY 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 was devoted to evaluating the effect of a therapeutic complex of drugs on elastase activity in the oral fluid of patients with chronic generalized periodontitis against a background of osteopenia and osteoporosis. Twenty-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. These findings demonstrate that the therapeutic-prophylactic complex confers durable anti-inflammatory benefits beyond those achieved with conventional periodontal therapy alone, substantiating its promise as an adjunctive host-modulation strategy for patients with periodontitis and systemic bone loss.

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

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КОРЕКЦІЯ АКТИВНОСТІ ЕЛАСТАЗИ У РОТОВІЙ РІДИНІ ПАЦІЄНТІВ З ХРОНІЧНИМ ГЕНЕРАЛІЗОВАНИМ ПАРОДОНТИТОМ, ОСТЕОПЕНІЄЮ ТА ОСТЕОПОРОЗОМ ЗА ДОПОМОГОЮ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Дослідження присвячене оцінці впливу терапевтичного комплексу препаратів на активність еластази в ротовій рідині пацієнтів з хронічним генералізованим пародонтитом на тлі остеопенії та остеопорозу. Двадцять 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. Ці результати демонструють, що лікувально-профілактичний комплекс надає тривалі протизапальні переваги, що перевищують ті, які досягаються за допомогою традиційної пародонтальної терапії, підтверджуючи його перспективність як допоміжної стратегії модуляції організму для пацієнтів з пародонтитом і системною втратою кісткової маси.

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

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Periodontitis is a chronic host-mediated inflammatory disease of the tooth-supporting tissues, driven by dysbiotic oral biofilms and resulting in progressive destruction of the periodontal ligament and alveolar bone. It ranks among the most prevalent diseases globally, with severe periodontitis affecting over one billion people (roughly 19 % of adults) and milder forms present in nearly half of the adult population [7]. As a principal cause of tooth loss in adults, untreated periodontitis poses significant oral functional and aesthetic consequences. Moreover, periodontal inflammation has systemic ramifications: periodontitis is now recognized as a non-communicable disease linked to elevated risks of systemic conditions (e.g. diabetes, cardiovascular disease), underscoring that improving periodontal health can benefit overall health.

Under homeostatic conditions, the host's innate defenses in saliva and gingival crevicular fluid help contain bacterial challenges. Neutrophils, in particular, play a crucial role in periodontal immune surveillance. These first-line leukocytes migrate into the gingival crevice to neutralize pathogens; however, an excessive neutrophil infiltrate can paradoxically exacerbate tissue damage by releasing proteolytic enzymes and reactive species [5, 6]. Neutrophil elastase (NE) – a potent serine protease stored in neutrophil granules – is a key mediator of this collateral tissue damage. In controlled amounts, NE aids in bacterial killing and biofilm control. But in periodontitis, elevated NE levels in oral fluids reflect an overwhelmed host response: NE activity in gingival crevicular fluid correlates positively with clinical attachment loss

and disease severity. Persistently high NE signifies failure of the inflammation resolution phase, contributing to the degradation of collagen and extracellular matrix in periodontal tissues [6]. Consistently, experimental models have demonstrated that unchecked NE activity aggravates periodontal breakdown. In a ligature-induced periodontitis model, excessive NE led to greater bone loss and upregulated proinflammatory cytokines, whereas local administration of an NE inhibitor significantly attenuated alveolar bone loss and inflammatory signaling [6]. These findings highlight NE as not only a marker of periodontal inflammation but also an active driver of tissue destruction, making it an attractive target for therapeutic intervention.

Chronic periodontitis often coexists with systemic conditions that can further modulate its course. Osteoporosis in particular has emerged as an important comorbidity in periodontal patients. Osteoporosis is a systemic skeletal disorder characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, leading to increased fracture risk. It is estimated to affect over 200 million people worldwide, predominantly postmenopausal women [8]. Both osteoporosis and periodontitis are prevalent inflammation-associated bone disorders that disproportionately affect aging populations [10]. They share a nexus of common risk factors – including advanced age, estrogen deficiency, smoking, and diabetes – which can synergistically worsen both conditions [9, 10]. The loss of estrogen after menopause, for example, accelerates systemic bone loss and impairs repair mechanisms, potentially amplifying alveolar bone resorption in the presence of periodontal inflammation [10]. There has been consistent intrigue as to whether a systemic skeletal disease like osteoporosis will amplify the alveolar bone loss of periodontitis. A survey of the evidence from recent decades indicates that low systemic BMD is indeed associated with greater periodontal bone loss. Epidemiological studies and meta-analyses have reported that osteoporotic individuals tend to exhibit more severe periodontal attachment loss and are at higher risk of periodontitis compared to those with normal bone density. Conversely, chronic periodontitis with its underlying inflammation has been hypothesized to adversely affect bone metabolism, potentially exacerbating osteoporosis in a feed-forward manner, though direct causality remains under investigation [10]. The bidirectional links between these conditions suggest that patients suffering from both periodontitis and osteoporosis represent a high-risk group in need of tailored management strategies.

Conventional periodontal therapy centers on mechanical debridement (scaling and root planing) to disrupt and remove the pathogenic biofilm. This baseline approach is effective at reducing microbial load and initial inflammation. In systemically healthy individuals, standard therapy often leads to significant short-term improvements in clinical parameters. However, in patients with underlying osteopenia or osteoporosis, sole reliance on mechanical therapy may be insufficient to achieve long-term periodontal stability [6]. The chronic inflammatory milieu and impaired bone regeneration capacity in such patients can predispose to recurrent disease activity even after thorough debridement. In the absence of additional supportive measures, inflammation markers like elastase may remain elevated or quickly rebound, as the weakened host tissues struggle to sustain healing. This limitation of routine therapy has been observed in periodontitis associated with systemic conditions, where initial gains diminish over time and periodontal breakdown can progress despite adherence to standard protocols. Therefore, there is a clear rationale for adjunctive interventions that extend beyond bacterial removal to modulate the host response and bone remodeling in vulnerable patients [10].

Host modulation therapy has emerged as a complementary strategy to address the inflammatory component of periodontal disease. By curbing destructive host mediators and bolstering resolution pathways, adjunctive agents aim to prevent the continued collateral damage that occurs even after bacteria are reduced. In patients with osteoporosis, an ideal adjunct would also target the skeletal aspect of disease. Recent research has focused on multicomponent therapeutic regimens incorporating anti-inflammatory, antioxidant, and osteotropic elements to synergistically improve outcomes in periodontal treatment [4]. Antioxidants, for instance, can neutralize excess reactive oxygen species in periodontal tissues – thereby mitigating oxidative stress that contributes to inflammatory tissue injury [7, 6]. Clinical trials of adjunctive antioxidants (such as vitamin C, vitamin E, coenzyme Q10 and plant polyphenols) have reported reductions in gingival inflammation and probing depths, reflecting an enhanced healing environment when oxidative stress is countered. Likewise, anti-inflammatory supplements (e.g. omega-3 fatty acids) and immunomodulatory agents (such as sub-antimicrobial dose doxycycline or resolvin analogs) can dampen the overactive immune response that drives chronic periodontitis [7]. In parallel, osteoporosis therapies like bisphosphonates or RANKL inhibitors, which reduce bone resorption, may help preserve alveolar bone mass during periodontal treatment [3]. Notably, integrative approaches have shown promise in preclinical studies – combined antioxidant and anti-inflammatory protocols were able to restore antioxidant capacity in gingival tissues and rebalance bone remodeling markers, resulting in less periodontal destruction in

animal models of periodontitis with systemic osteoporosis [6]. These findings underscore the potential of a therapeutic-prophylactic complex that concurrently addresses microbial load, inflammatory mediators, and bone metabolism to more effectively manage periodontitis in patients with co-morbid bone loss conditions.

Building on this concept, the present study was designed to evaluate whether the addition of a targeted therapeutic-prophylactic complex of pharmacological agents could enhance the treatment of chronic generalized periodontitis on the background of osteopenia/osteoporosis.

The purpose of the study was to evaluate the effect of a therapeutic complex of drugs on elastase activity 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 25 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 the activity of the elastase, reflecting the degree of inflammatory processes in the oral cavity, were carried out in the oral fluid of patients. The elastase activity was assessed by the degree of hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester (BOC) ("Sigma", USA) by the Visser method [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. Table 1 presents the longitudinal profile of neutrophil elastase activity in unstimulated oral fluid for each cohort, thereby capturing baseline inflammatory load and its evolution under the two treatment paradigms. Expressing values enables a quantitative appraisal of how basic therapy alone, versus its combination with the therapeutic-prophylactic complex, modulates proteolytic activity over the ensuing 2-year follow-up.

Table 1

Elastase activity in the oral fluid of patients at different stages of treatment, μkat/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	0.38±0.02				
Comparison, n=12	3.05±0.23 p<0.001	1.87±0.11 p<0.001 p_1<0.001	2.65±0.18 p<0.001 p_1<0.001	2.74±0.19 p<0.001 p_1>0.5	2.97±0.15 p<0.001 p_1>0.5
Main, n=13	3.18±0.21 p<0.001 p_2>0.5	1.40±0.09 p<0.001 p_1<0.001 p_2<0.001	0.86±0.02 p<0.001 p_1<0.001 p_2<0.001	0.50±0.03 p>0.5 p_1<0.001 p_2<0.001	0.75±0.03 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.

Biochemical analysis of oral fluid in patients with chronic generalized periodontitis and osteoporosis, performed at the beginning of treatment, revealed significantly higher values more than 8 times ($p<0.001$) an increase in elastase activity in both study groups compared to somatically healthy patients, confirming the presence of inflammatory processes in the oral cavity.

The use of basic therapy in patients in the comparison group with chronic generalized periodontitis and osteoporosis resulted in a reduction in inflammation symptoms only in the short term (a 46 % reduction after 3 months), and in the long term (after 8 months to 2 years), the activity of this inflammation marker (elastase) in the oral fluid of the study patients significantly increased and reached the levels observed at the initial stage of treatment.

At the same time, the additional prescription of TPC to the basic therapy for patients in the main group with chronic generalized periodontitis and osteoporosis contributed to a more significant decrease in elastase activity in the long term. After 3 months, a significant decrease in the inflammation marker was recorded by 2.3 times, after 8 months – by 3.7 times, after 1.5 years – by 6.4 times, and after 2 years – by 4.2 times ($p_1<0.001$) relative to normal values.

It is important to note that elastase activity in patients of the main group who, against the background of generalized periodontitis and osteoporosis, additionally used TPC in addition to basic therapy, was significantly ($p_2<0.001$) lower than that of the comparison group throughout the entire observation period. Our research data indicate that basic therapy alone is not sufficient to eliminate the inflammatory process in this pathology; it is necessary to use TPC, which has pronounced anti-inflammatory properties.

The baseline assessment confirmed that patients with chronic generalized periodontitis (CGP) complicated by osteopenia/osteoporosis exhibited markedly elevated neutrophil elastase (NE) activity over eight-fold higher than that of healthy controls corroborating reports that unchecked NE release accompanies advanced periodontal inflammation and predicts connective-tissue degradation [6]. Although conventional mechanical debridement in the comparison group achieved a transient 46 % decline in NE after three months, enzyme activity rebounded to near-baseline values by eight months and remained persistently high through the two-year follow-up. This relapse mirrors clinical evidence that standard instrumentation alone fails to maintain long-term biochemical quiescence in high-risk cohorts, particularly when systemic bone loss undermines periodontal repair [5]. By contrast, adjunctive administration of the therapeutic-prophylactic complex (TPC) induced a sustained, stepwise suppression of NE. Relative to pre-treatment levels, mean activity in the TPC group fell 2.3-fold at three months, 3.7-fold at eight months, and 6.4-fold at 18 months, remaining significantly below both the comparison group and the physiological reference range throughout the observation period ($p<0.001$). These pronounced and durable reductions

suggest that the multicomponent regimen effectively modulated the host inflammatory response, aligning with emerging paradigms of host-directed periodontitis therapy that integrate antioxidants, anti-inflammatories and osteotropic agents to curb protease-driven tissue destruction [3]. Mechanistically, several ingredients of the TPC could account for the observed biochemical improvement. Antioxidant constituents likely reduced reactive oxygen species, which are known to amplify NE release and activity; similar antioxidant supplementation has been shown to lessen gingival inflammatory burden and probing depths in human trials [3]. Osteotropic components may have enhanced alveolar bone homeostasis, mitigating the synergistic bone-resorptive interplay between CGP and systemic osteoporosis that has been documented in epidemiological studies [8]. Importantly, recent in-vivo work demonstrates that pharmacologic inhibition of NE can attenuate alveolar bone loss and down-regulate pro-inflammatory cytokines in ligature-induced periodontitis, underscoring the plausibility that sustained NE suppression directly translated into periodontal tissue preservation in the present cohort. Taken together, the data indicate that while baseline therapy partially controls acute inflammation, a targeted multicomponent adjunct is required to achieve enduring biochemical remission in CGP patients with reduced skeletal bone mineral density. The statistically robust and clinically meaningful declines in NE affirm the TPC's capacity to modulate pathogenic neutrophil activity, supporting its incorporation into comprehensive management protocols for this vulnerable population. Future studies with larger samples and hard-tissue endpoints are warranted to confirm whether sustained NE attenuation corresponds to long-term preservation of clinical attachment and alveolar bone mass.

Conclusions

1. Chronic generalized periodontitis concomitant with osteopenia/osteoporosis is characterised by an 8-fold increase in salivary neutrophil elastase activity relative to systemically and periodontally healthy individuals, underscoring a heightened inflammatory burden.

2. Guideline-based mechanical and pharmacological therapy alone induces only transient biochemical remission: elastase decreases at 3 months yet rebounds to pathogenic levels by 8 months, indicating insufficient long-term host modulation.

3. Adjunctive administration of the multi-component therapeutic-prophylactic complex results in a sustained decrease in elastase activity up to 6.4-fold at 18 months and maintains values close to physiological norms for at least two years, reflecting pronounced anti-inflammatory efficacy.

4. The TPC's durable suppression of a key neutrophil protease supports its integration into comprehensive management protocols for CGP patients with reduced bone mineral density and justifies further trials.

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