DOI 10.26724/2079-8334-2026-1-95-98-102
UDC 616.314.17-002:615.242-085

Lazarieva K.A., Skrypnykov P.M., Shynkevych V.I., Kolomiiets S.V., Kaidashev I.P.
Poltava State Medical University, Poltava

CYTOKINE CHANGES FOLLOWING ADJUNCTIVE ADMINISTRATION OF EPIGALLOCATECHIN-3-GALLATE OR CURCUMIN IN PERIODONTITIS

e-mail: v.shynkevych@pdmu.edu.ua

This clinical study assessed changes in periodontal parameters and tissue levels of TNF-α, IL-1β, IL-6, and TGF-β in patients with periodontitis following adjunctive epigallocatechin-3-gallate or curcumin therapy alongside professional mechanical plaque removal over six months. Cytokine levels were measured by ELISA. Adjunctive epigallocatechin-3-gallate or curcumin reduced moderate and deep pockets and bleeding on probing, with curcumin also producing CAL reductions of ≥3/≥4 mm compared to periodontal treatment alone. Gingival IL-10 levels increased while TGF-β1 levels decreased significantly under both nutraceuticals, suggesting a link between polyphenol supplementation and immunomodulation. These findings suggest that targeted modulation of pro- and anti-inflammatory cytokines supports sustained clinical improvements and highlight the potential of nutraceuticals as adjuncts in periodontal therapy, with cytokine shifts paralleling clinical recovery and persisting beyond the treatment period.

Key words: curcumin, epigallocatechin-3-gallate, interleukin-1β, interleukin -6, periodontitis therapy, transforming growth factor-β, tumor necrosis factor-α.

Лазарєва К.А., Скрипников П.М., Шинкевич В.І., Коломієць С.В., Кайдашев І.П.

ЗМІНИ ЦИТОКІНІВ ПІСЛЯ ДОДАТКОВОГО ЗАСТОСУВАННЯ ЕПІГАЛОКАТЕХІН-3-ГАЛАТУ АБО КУРКУМІНУ ПРИ ПАРОДОНТИТІ

У цьому клінічному дослідженні, яке тривало шість місяців, оцінювалися зміни періодонтальних показників та рівнів тканинних цитокінів TNF-α, IL-1β, IL-6 і TGF-β у пацієнтів із пародонтитом після додаткового застосування епігалокатехін-3-галату або куркуміну разом із професійним зняттям зубних відкладень. Рівні цитокінів визначали методом ELISA. Додаткове застосування епігалокатехін-3-галату або куркуміну зменшувало кількість середніх і глибоких періодонтальних кишень та індекс кровоточивості при пробі, причому куркумін також забезпечував зменшення клінічної втрати прикріплення у вигляді пропорції порогових значень ≥3/≥4 мм порівняно з лише періодонтальним лікуванням. Рівень IL-10 у біоптатах ясен підвищувався, тоді як рівень TGF-β1 значно знижувався під впливом обох нутрицевтиків, що свідчить про зв'язок між додатковим прийомом поліфенолів та імуномодуляцією. Ці результати свідчать, що цілеспрямована модуляція прозапальних та протизапальних цитокінів сприяє стабільним клінічним покращенням і підкреслюють потенціал епігалокатехін-3-галату та куркуміну як додаткових засобів у терапії періодонтиту, при цьому зміни цитокінів узгоджуються з клінічним відновленням і зберігаються після завершення лікування.

Ключові слова: куркумін, епігалокатехін-3-галат, інтерлейкін-1β, інтерлейкін-6, терапія періодонтиту, трансформуючий фактор росту-β, фактор некрозу пухлини-α.

Funding. *The study is a fragment of the research project: "Development of new methods for diagnosis and personalised treatment of respiratory and comorbid diseases during wartime and post-war periods", state registration No. 0126U000355.*

Since it has become evident that chronic periodontitis (PD) represents a destructive inflammatory response to a dysbiotic subgingival biofilm, limiting excessive inflammation has emerged as an important target of adjunctive therapy, in addition to mandatory professional mechanical plaque removal (PMPR). Recently, the concept of metabolic modulation in the management of chronic diseases, including PD, using nutraceuticals such as bioflavonoids, has gained increasing attention [1–10, 14, 15]. Among these agents, curcumin has been proposed as a promising candidate for PD therapy and has been evaluated in formulations ranging from mouth rinses to systemic administration [4, 5, 14]. Epigallocatechin-3-gallate (EGCG), the most biologically active catechin found in green tea leaves, has demonstrated significant therapeutic potential across multiple diseases, including PD. Owing to its excellent biocompatibility, antioxidant properties, and anti-cariogenic effects, EGCG has been widely investigated for its potential oral health benefits, including applications in oral cancer and dental biomaterials [6–8, 15]. EGCG exerts its effects through molecular targets such as ESR1 and MMP13, supporting its potential role in host-modulation therapy for PD [7].

Among the various mechanisms through which polyphenols act, modulation of cytokines represents an important pathway [3]. Previous studies have demonstrated that pro-inflammatory cytokines, including IL-1β and IL-6 [3, 8], as well as anti-inflammatory cytokines such as IL-10 and TGF-β, serve as reliable biomarkers of PD severity [11, 13].

Despite substantial advances in elucidating cytokines as targets of polyphenol effects, most available evidence is derived from animal models and cell culture studies [4, 8, 10, 14, 15]. Given that numerous polyphenolic compounds are already approved for human use, establishing their clinical efficacy in periodontal therapy represents an

important translational priority. Therefore, clinical trials assessing cytokine modulation during PD therapy may provide deeper insight into the underlying mechanisms of periodontal recovery.

The purpose of the study was to evaluate the effects of adjunctive epigallocatechin-3-gallate or curcumin in periodontitis therapy by assessing clinical outcomes and changes in gingival levels of IL-1β, IL-6, IL-10, and TGF-β1 six months after a two-month supplementation period.

Materials and methods. For this prospective, 6-month, randomized, open-label, controlled clinical trial, patients were consecutively recruited from May 2023 to March 2025 at the Department of Postgraduate Dental Education, Poltava State Medical University (PSMU). The study was approved by the PSMU Biomedical Ethics Committee (Protocol No. 207, August 23, 2022) and was conducted in accordance with the Declaration of Helsinki (1975, revised 2013). Participants in this study did not include any vulnerable populations according to ICH-GCP guidelines. Informed consent was obtained from each patient prior to commencement of the study.

Screening for eligibility was performed in patients, who met the following inclusion criteria: (age ≥18 years, stage II–III/IV, grade B periodontitis, good general health with controlled systemic conditions, and ≥15 remaining teeth. The exclusion criteria were grade C periodontitis, purulent exudation from pockets, recent (within the past three months) antibiotic, anti-inflammatory, or periodontal therapy, pregnancy or lactation, and severe uncontrolled systemic or neuropsychiatric disorders.

A total of 42 patients (31 women, 11 men; mean age 48 years, range 21–74) were enrolled and assigned to the two test groups: the first, who received epigallocatechin gallate (EGCG, Now Foods, USA), 400 mg once daily for 60 days adjunctive to PMPR (the first group, n=14), the second, who received curcumin (Turmeric Curcumin 1000 mg with Bioperine, Puritan's Pride, USA), 1 capsule daily for 60 days adjunctive to PMPR (the second group, n=14), and the control group, who received PMPR along (the control group, n=14). The randomization was stratified by age, sex, smoking status, systemic conditions, and periodontitis severity.

In all groups, evaluation of clinical parameters (PPD, CAL, Plaque index, BoP, Oral Health Impact Profile-14 (OHIP-14) and cytokine analysis assessments were performed at baseline (T1) and at 6 months follow-up (T3). The general study flow diagram is presented in Fig. 1.

The clinical measurements and the protocol for mandatory periodontal treatment were described previously by our group [9]. On the day of the first PMPR session, administration of EGCG or curcumin was initiated according to group allocation, under the supervision of the examiner.

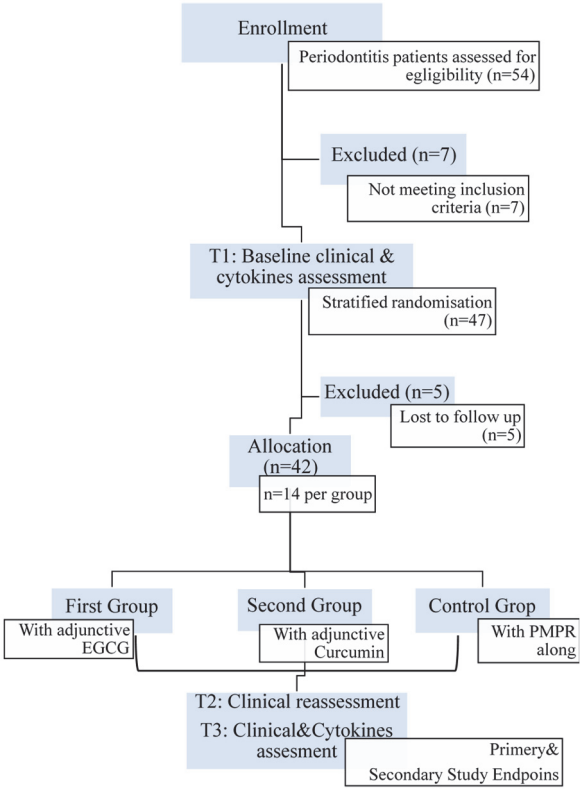

Fig. 1. Flowchart of the study design.

Gingival biopsies (~30 mg) were obtained under local anesthesia at baseline and at 3- and 6-month follow-ups during routine periodontal surgery from sites with PPD ≥6 mm. Although the repeated samples were obtained from the same patients, they were not necessarily collected from the same sites. Samples were transported individually at +4°C in 1.5 mL of modified DMEM (Dulbecco's Modified Eagle Medium, Gibco, USA) supplemented with 10 % fetal bovine serum, 1 % penicillin–streptomycin, and 0.2 % Fungizone (Gibco, USA) for up to 2 hours. Upon arrival, biopsies were immediately incubated for 48 h (37°C, 5 % CO_2) in the same supplemented medium in individual wells of 24-well plates (Costar®, Corning, NY, USA). After incubation, tissues were removed, and media were centrifuged at 800 g at 4°C. Concentrations of IL-1β, IL-6, IL-10, and TGF-β in culture supernatants were measured using ELISA kits (MyBioSource, USA), following manufacturer instructions.

The primary outcome was the change in PPD, assessed as changes in the distribution of probing-depth categories. Secondary outcomes included changes in CAL, BoP, plaque index, and gingival levels of IL-1β, IL-6, IL-10, and TGF-β.

The sample size calculation was described previously [9]. Statistical analyses were conducted using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

The null hypothesis stated that adjunctive EGCG or curcumin would not provide additional clinical or cytokine changes compared to PMPR alone.

Results of the study and their discussion. Cytokine protein levels in gingival tissues were analyzed using a model in which all baseline samples from patients with periodontitis were compared with each post-treatment group (first, second, or control) using pairwise independent tests. IL-1β and TGF-β1 showed significant reductions in both the first and second groups, whereas IL-6 decreased across all groups. In contrast, IL-10 increased significantly in the first and second groups, compare to T1 (Fig. 2).

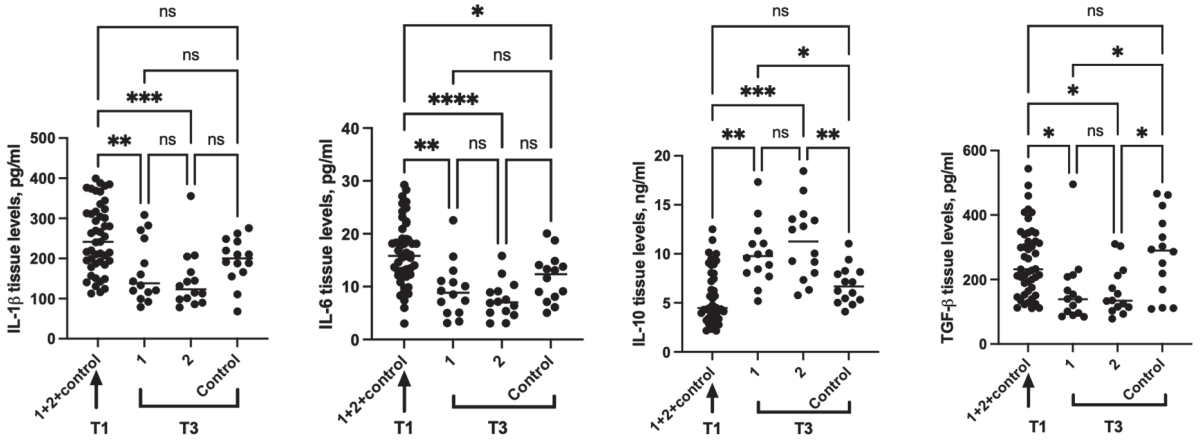

Fig. 2. Comparative analysis of gingival tissue cytokine levels across time points and between treatment groups at the end of the observation period (Brown–Forsythe and Welch ANOVA with Dunnett T3 correction for multiple comparisons: (a) IL-1β; (b) IL-6; (c) IL-10, (d) TGF-β. Note: * (P < 0.05), ** (P < 0.01), *** (P < 0.001), **** (P < 0.0001).

In the between-group comparison at the post-treatment time point T3, no statistically significant differences were detected for IL-1β or IL-6. However, both IL-10 and TGF-β1 levels in the first and second groups differed significantly from those in the control group, suggesting that EGCG and curcumin may enhance IL-10 expression and modulate TGF-β1 levels.

Clinical parameters expressed as proportions of threshold values showed significant positive correlations with IL-10 and TGF-β levels (Table 1).

Table 1

p-values and Spearman's *r* for significant correlations between clinical parameters and cytokine levels at baseline

Clinical parameters Cytokines levels	Ratio of CAL ≥3/≥4 mm	Proportion of sites ≥6 mm	Proportion of BoP+	IL-10	TGF-β
IL-1	0.522	0.144	0.067	0.045* (r=-0.29)	0.002* (r=-0.43)
IL-6	0.933	0.778	1	0.10	0.29
IL-10	1	0.033* (r=0.88)	0.267	1	
TGF-β	0.1	0.556	0.022* (r=0.96)	0.06	1

In addition, inverse correlations between IL-1β and both IL-10 and TGF-β were also statistically significant. After treatment, significant correlations persisted in the first group between the ratio of CAL≥3/≥4 mm and TGF-β levels, as well as between BoP+ sites and IL-6 (Table 2).

In the second group, only the inverse correlation between TGF-β and IL-10 remained significant. In the control group, again the ratio of CAL≥3/≥4 mm showed direct correlated with TGF-β, while the proportion of PPD≥6 mm showed negative correlation with IL-6.

In general, clinically, both the first and second groups demonstrated improvements, including pocket closure and reduced BoP, with the second group showing an additional CAL≥3/≥4 mm reduction compared to the control group. These clinical parameters were consistent but did not correlate with significant changes of IL-10 and TGF-β1 levels.

This study shows that a two-month supplementation with EGCG or curcumin at physiologically relevant doses may have lasting effects on both clinical outcomes and local cytokine profiles in patients with PD, observed six months later.

The clinical parameters reported in [9] were used in this study to correlate with interleukin levels. The interpretation of cytokine changes was based on established patterns observed in PD, in which pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) drive tissue destruction, whereas IL-10 and TGF-β1 exert anti-inflammatory effects, maintaining immune homeostasis and supporting tissue remodeling [12].

In this study, baseline gingival levels of IL-1β were inversely correlated with those of IL-10 and TGF-β1. This is consistent with previously reported data indicating that the IL-1β/TGF-β1 ratio may serve as a potential indicator of periodontitis progression [1]. In addition to TGF-β, IL-10 plays a

well-recognized role in both immune modulation and the maintenance of periodontal tissue homeostasis [13]. A meta-analysis reported higher gene expressions of both IL-1β and the anti-inflammatory IL-10 in PD [2]. Our observed correlations between deep PPD and IL-10 are consistent with these findings. Since current evidence highlights TGF-β as a central mediator of periodontal inflammation, tissue remodeling, and repair [11], the observed correlation between BOP and TGF-β in this study likely reflects its role primarily in the inflammatory process.

Table 2

p-values and Spearman's *r* for significant correlations between clinical parameters [9] and gingival cytokine expression levels at T3

First group				
Clinical parameters Cytokines levels	Ratio of CAL≥3/≥4 mm	Proportion of sites ≥6 mm		Proportion of BoP+
IL-1β	0.197	0.619		0.619
IL-6	0.197	0.488		0.015*(r=0.83)
IL-10	0.360	0.976		1
TGF-β	0.028*(r=0.79)	0.077		0.216
Second group				
Clinical parameters Cytokines levels	Ratio of CAL≥3/≥4 mm	Proportion of sites ≥6 mm	Proportion of BoP+	IL-10
IL-1β	0.892	0.951	1	0.733
IL-6	0.707	0.799	0.492	0.973
IL-10	0.892	0.965	0.537	-
TGF-β	0.387	0.341	0.733	0.035*(r=-0.69)
Control group				
Clinical parameters Cytokines levels	Ratio of CAL≥3/≥4 mm	Proportion of sites ≥6 mm		Proportion of BoP+
IL-1β	0.197	0.228		0.671
IL-6	0.639	0.02* (r=-0.64)		0.511
IL-10	0.078	0.072		0.25
TGF-β	0.03* (r=0.61)	0.195		0.859

Following a course of EGCG or curcumin gingival levels of IL-10 increased compared with periodontal therapy alone and were positively correlated with TGF-β1 in the EGCG group. These findings align with the known biological effects of EGCG and curcumin. In a rat model, PD treatment with EGCG delivered as a mucoadhesive gingival patch for 3 to 21 days consistently increased IL-10 expression in periodontal tissues ($p<0.05$) after 7, 14, and 21 days ($p<0.05$), as demonstrated by immunohistochemistry [8]. Recent studies have shown that EGCG can counteract TGF-β1–induced transformation in various cell types and modulate key signaling pathways, including PI3K/Akt/AMPK, TGF-β/Smad, Nrf2, NF-κB, and ROS/MAPK, highlighting its multifaceted mechanisms of action [3, 6], which may explain a correlation with TGF-β observed after EGCG.

Curcumin has been shown to induce the expression and production of IL-10, thereby enhancing its anti-inflammatory effects across a wide range of tissues [10]. This finding is clinically relevant, as elevated IL-10 protein levels are associated with periodontal health [13], although previous studies have reported that higher IL-10 gene expression can be observed in periodontitis, albeit not consistently [2].

The finding that BOP directly correlated with gingival IL-6 levels after the EGCG course suggests that residual inflammation may be primarily driven by IL-6, given its well-established pro-inflammatory role in periodontitis and its potential modulation by EGCG [8].

In this study, gingival levels of TGF-β decreased following a course of EGCG or curcumin, in contrast to periodontal therapy alone, where a tendency toward increased TGF-β levels was observed despite certain clinical improvement. These findings are consistent with the previously mentioned potential of EGCG to counteract TGF-β1–induced events [3, 6]. Similarly, curcumin has been shown to inhibit phenytoin-induced gingival overgrowth mediated by TGF-β1 activation in human gingival fibroblasts, highlighting its capacity to modulate TGF-β1-dependent pathways in periodontal tissues [4]. This data can explain that the direction of TGF-β changes was favorable. The observed correlation between CAL ≥3/≥4 mm and TGF-β levels may be explained by its role in tissue remodeling in PD [11, 13].

Overall, the modulation of pro- and anti-inflammatory cytokines observed in this study aligns with investigated role of NF-κB in metabolic alterations and cytokine responses [3]. Indeed, TGF-β1 directly modulates NF-κB, along with other signaling pathways [3, 6]. IL-10 prevents the phosphorylation and activation of key upstream signaling components (such as IκB kinase), thereby reducing NF-κB translocation into the nucleus and limiting transcription of pro-inflammatory genes like IL-6 and TNF-α [10, 13].

The clinical benefits of adjunctive EGCG (reduction in the proportion of 4–5 mm PPD) and curcumin (improvement in threshold CAL values), along with their combined effects such as enhanced pocket closure, decreased BoP, reduction of sites with PPD ≥ 6 mm, and improved quality of life according to OHIP-14 [9], suggest that cytokine modulation may represent a key underlying mechanism.

Limitations. A limitation of this study is that clinical measurements reflect the full mouth, whereas cytokine levels were assessed at a single biopsy site and not in all surrounding areas, potentially underestimating local inflammatory heterogeneity. Additionally, cytokine production can vary by cell type, and the biopsy's cellular composition was not evaluated, which should be considered when interpreting the findings.

Conclusion

Adjunctive use of EGCG and curcumin in periodontitis demonstrated significant immunomodulatory effects. Specifically, IL-10 levels increased while TGF-β1 levels decreased, whereas changes in IL-1 and IL-6 were not treatment-specific, suggesting that modulation of anti-inflammatory cytokines may represent a key mechanism underlying their clinical benefits. These alterations in IL-10 and TGF-β1 likely reflect the capacity of EGCG and curcumin to regulate local inflammatory and tissue repair pathways, contributing to a more balanced periodontal microenvironment and supporting enhanced therapeutic outcomes. Compared with mandatory professional mechanical plaque removal alone, these nutraceuticals induced cytokine shifts that paralleled clinical recovery trajectories, including pocket closure, reduced bleeding on probing, and improvement in patient-reported quality of life. The observed effects suggest sustained biological activity beyond the active supplementation period, highlighting the potential of EGCG and curcumin as adjunctive agents in personalized periodontal therapy.

References

1. Balu P, Balakrishna Pillai AK, Mariappan V, Ramalingam S. Cytokine levels in gingival tissues as an indicator to understand periodontal disease severity. Curr Res Immunol. 2024 Jun 23;5:100080. doi: 10.1016/j.crimmu.2024.100080.
2. Caldeira FID, Hidalgo MAR, De Carli Dias ML, Scarel-Caminaga RM, Pigossi SC. Systematic review of ratios between disease /health periodontitis modulators and meta-analysis of their levels in gingival tissue and biological fluids. Arch Oral Biol. 2021 Jul;127:105147. doi: 10.1016/j.archoralbio.2021.105147.
3. Chekalina N, Burmak Y, Petrov Y, Borisova Z, Manusha Y, Kazakov Y, et al. Quercetin reduces the transcriptional activity of NF-kB in stable coronary artery disease. Indian Heart J. 2018 Sep-Oct;70(5):593-597. doi: 10.1016/j.ihj.2018.04.006.
4. Deng YT, Wu KJ, Kuo MY. Phenytoin induces connective tissue growth factor (CTGF/CCN2) production through NADPH oxidase 4-mediated latent TGFβ1 activation in human gingiva fibroblasts: Suppression by curcumin. J Periodontal Res. 2022 Dec;57(6):1219-1226. doi: 10.1111/jre.13058.
5. Divya Bharathi S, Aiswarya SP, Sankar AR. Comparative evaluation of the efficacy of Triphala mouthwash and Curcumin mouthwash in the treatment of gingivitis – A randomized controlled study. J Oral Biol Craniofac Res. 2024 Jul-Aug;14(4):407-414. doi: 10.1016/j.jobcr.2024.05.002.
6. Fan Q, Zhou XH, Wang TF, Zeng FJ, Liu X, Gu Y, et al. Effects of epigallocatechin-3-gallate on oxidative stress, inflammation, and bone loss in a rat periodontitis model. J Dent Sci. 2023 Oct;18(4):1567-1575. doi: 10.1016/j.jds.2023.02.019.
7. Kamaraj B. Exploring the Therapeutic Potential of Epigallocatechin-3-gallate (Green Tea) in Periodontitis Using Network Pharmacology and Molecular Modeling Approach. Int J Mol Sci. 2025 Sep 19;26(18):9144. doi: 10.3390/ijms26189144.
8. Lashari DM, Aljunaid MA, Ridwan RD, Diyatri I, Lashari Y, Qaid H, et al. The ability of mucoadhesive gingival patch loaded with EGCG on IL-6 and IL-10 expression in periodontitis. J Oral Biol Craniofac Res. 2022 Sep-Oct;12(5):679-682. doi: 10.1016/j.jobcr.2022.08.007.
9. Lazarieva K, Skrypnykov P, Shynkevych V, Kaidashev I. Evaluation of a microRNA expression panel and clinical outcomes in periodontitis following adjunctive curcumin or epigallocatechin-3-gallate therapy. Ukrainian Dental Almanac 2025; (4):5-17. DOI 10.31718/2409-0255.4.2025.01.
10. Mollazadeh H, Cicero AFG, Blesso CN, Pirro M, Majeed M, Sahebkar A. Immune modulation by curcumin: The role of interleukin-10. Crit Rev Food Sci Nutr. 2019;59(1):89-101. doi: 10.1080/10408398.2017.1358139.
11. Pawłaszek T, Grabarek BO. Genetic and Molecular Insights into Transforming Growth Factor-Beta Signaling in Periodontitis: A Systematic Review. Genes (Basel). 2025 Oct 1;16(10):1165. doi: 10.3390/genes16101165.
12. Relvas M, Mendes-Frias A, Gonçalves M, Salazar F, López-Jarana P, Silvestre R, et al. Salivary IL-1β, IL-6, and IL-10 Are Key Biomarkers of Periodontitis Severity. Int J Mol Sci. 2024 Aug 1;25(15):8401. doi: 10.3390/ijms25158401.
13. Skrypnyk M, Spahr A, Berkovsky S, Yatsenko T, Xu C, Zuieva O, et al. Chronic stress and the IL-10-mediated immunoregulatory loop in the pathogenesis of periodontitis. Clin Sci (Lond). 2025 Nov 25;139(22):CS20256843. doi: 10.1042/CS20256843.
14. Yetkin Ay Z, Bakır B, Bozkurt ŞB, Kayis SA, Hakki SS. Positive effect of curcumin on experimental peridontitis via suppression of IL-1-beta and IL-6 expression level. Int J Vitam Nutr Res. 2022 Jul;92(3-4):231-239. doi: 10.1024/0300-9831/a000672.
15. Yuliati Y, Mahdani FY, Margaretha SA, Yastuti WTP, Surboyo MDC, Aljunaid MA, et al. The EGCG and α-Mangosteen Stimulate SHED-IL10 and SHED-LL37 Metabolite Concentration. Eur J Dent. 2024 Feb;18(1):138-142. doi: 10.1055/s-0043-1761460.

Conflict of interest. The authors have no conflicts of interest to declare.

ORCID: Lazarieva K.A. https://orcid.org/0000-0003-3992-2793, Skrypnykov P.M. https://orcid.org/0000-0002-4473-2284, Shynkevych V.I. https://orcid.org/0000-0002-2436-9449, Kolomiiets S.V. https://orcid.org/0009-0000-6962-0214, Kaidashev I.P. https://orcid.org/0000-0002-4708-0859.

Article received: 26.02.2025.