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# Salivary growth factors in patients with chronic periodontitis

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## Abstract

**Aim**. To determine the clinical value of the growth factors concentration in the oral fluid in patients with mild chronic periodontitis.

**Methods**. A prospective study including 30 patients with chronic periodontitis and 20 healthy volunteers was conducted. The diagnosis was made based on standard clinical and radiological criteria. Nerve growth factor β (NGF-β), hepatocyte growth factor (HGF), epidermal growth factor (EGF), vascular endothelial growth factor A (VEGF-A), platelet-derived growth factor BB (PDGF-BB) were determined in oral fluid samples by using multiparametric fluorescence analysis with magnetic microspheres (xMAP technology, Luminex 200, USA). Statistical analysis was performed using nonparametric measures: median (Me) and interquartile range ($Q_1$, $Q_3$). Receiver operating characteristic (ROC) analysis was used to determine the clinical value of the parameters.

**Results**. The chronic periodontitis was accompanied by an increase in the level of nerve growth factor-β by 2.2 times, epidermal growth factor by 3 times, vascular endothelial growth factor A by 1.9 times ($p < 0.05$) compared with the control. The platelet-derived growth factor BB concentration did not change. Using the ROC analysis, diagnostic sensitivity and diagnostic specificity of the studied parameters were determined: 89.1 and 91.1% for nerve growth factor β, 92.3 and 96.1% for epidermal growth factor, 87.1 and 95.3% for vascular endothelial growth factor A, respectively.

**Conclusion**. Salivary growth factors (nerve growth factor β, epidermal growth factor, vascular endothelial growth factor A) can be considered as potential biomarkers of mild chronic periodontitis.

**Keywords**: growth factors, oral fluid, chronic periodontitis.

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**Background**. Chronic periodontitis (CP) is one of the most common diseases worldwide. According to a number of authors, it affects 10–15% of the global population [1]. Dental plaque and poor hygiene are considered as important factors in the development of the disease. However, this may not be enough to cause damage to the periodontal tissue. For this reason, it is generally accepted that the imbalance between the immune system and periodontal microorganisms has the most significant role in its pathogenesis [2–5].

A complex set of intercellular reactions leading to CP in periodontal tissues is regulated by immunocompetent cells (neutrophils, dendritic cells, lymphocytes, macrophages, and plasma cells), and cytokines synthesized by them [6]. Among the latter, growth factors are especially significant [7]. It is a large family of polypeptide signaling molecules that regulate cellular responses during inflammation and regeneration, such as migration, proliferation, and differentiation. They also affect the activity of apoptosis, angiogenesis, and various other physiological and pathological processes in tissues [8–10].

A significant number of publications have focused on the value of cytokines in CP. However, there are significantly fewer works highlighting the growth factors (GF) contained in saliva in this disease [9, 11, 12]. In particular, the level of hepatocyte growth factor (HGF) correlates with the disease activity, and it is also influenced by smoking [13]. Changes in saliva concentration of vascular endothelial growth factor (VEGF), tumor necrosis factor, hypoxia-induced factor, and connective tissue GF in periodontal diseases have been detected [8, 10]. However, a complete pathogenetic role of GF in CP has not yet been clear. The data obtained by different authors are sometimes contradictory.

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In addition, the question of the clinical and diagnostic value of determining the concentration of GF in OF in CP is far from a final decision.

**Aim**. To determine the clinical value of the concentration of GF in OF in mild CP.

**Materials and methods of the study**. In a prospective case-control study, 30 patients with mild CP (main group) and 20 apparently healthy volunteers without periodontal pathology (control group) were examined. The age of patients in the main group was 18–46 years (median 39 years), and 19–38 years (median 37 years) in the comparison group. The ratio of women to men was 1.1 in the main group, and 1.0 in the comparison group. Differences in age and sex composition and structure of concomitant somatic pathology have not been revealed.

In the main group, the diagnosis of mild CP (K05.3 according to the International Classification of Diseases of the 10th revision) was established based on clinical and radiological criteria in accordance with the clinical guidelines (treatment protocols) approved by the Russian Dental Association (2013) as amended and supplemented [http://www.e-stomatology.ru/director/protokols/]. The criteria for inclusion in the main group included age of 18–50 years, the general somatic state of patients in the stage of compensation, and the absence of concomitant somatic diseases in the stage of decompensation.

Patients of the main group complained of bleeding gums when brushing their teeth, dental deposits, and tooth sensitivity. Clinical examination revealed hyperemia and edema of the papillary, marginal gums, and bleeding when probing. The dentogingival junction was impaired (a key clinical and diagnostic sign), and the depth of the periodontal pockets was 4–4.5 mm. In some patients, gingival recession (1–2 mm) was noted, accompanied by the development of increased tooth sensitivity. The mobility of the teeth was physiological. In all these patients, mineralized and non-mineralized dental deposits were identified in the neck area of the teeth.

Patients in the control group did not show complaints related to the condition of the gums and had a conditionally healthy periodontium. On clinical examination, the papillary, marginal gums were pale pink with no visible pathological changes. The dentogingival junction was intact, and the gum was tightly adhered to the necks of the teeth. Gingival recession was not identified. The mobility of the teeth was physiological. All patients in the control group had a good level of individual oral hygiene.

Clinical examination and treatment of patients was performed in the dental clinic of the Ural State Medical University of the Ministry of Health of Russia. The research was conducted in accordance with the ethical principles adopted by the Declaration of Helsinki by the World Medical Association (2000). This study was approved by the Local Ethics Committee of the Ural State Medical University (Protocol No. 3 dated 03/19/2021).

Along with the main methods of clinical examination, dental indices were assessed, namely a Green–Vermillion simplified index of oral hygiene (1964), papillary-marginal-alveolar index modified by C. Parma (1960), and periodontal Russell's index.

In all patients examined, unstimulated OF was analyzed, which was obtained three hours after eating and rinsing the mouth, and collected in Saliva Caps Set tubes, and then frozen. The study was performed in accordance with the protocol developed in our laboratory. The samples were stored at a temperature of–40°C for 3 months maximum. In OF samples, the nerve GFs β (NGF-β, nerve growth factor), HGF, epidermal GF (EGF, epidermal growth factor), VEGF-A, platelet derived GF (PDGF-BB, platelet -derived growth factor) were determined.

The concentration of GF was determined by the method of multiparametric fluorescence analysis with magnetic microspheres (xMAP-technology, Luminex 200, USA) using the ProcartaPlex Human Cytokine/Chemokine/Growth Factor Panel 1 (45 plex) Invitrogen, eBioscience test systems (USA). The study was performed according to the manufacturer's protocol. The biological sample was incubated in a 96-well plate with a mixture of magnetic microspheres stained with infrared fluorescent dye, loaded with monoclonal antibodies specific for the cytokines under study and streptavidin-R- phycoerythrin.

The resulting suspension was passed through a Luminex 200 flow chamber. To detect magnetic particles, the device has two lasers; red laser is used to distinguish the spectral signature and green laser to determine the fluorescence level of streptavidin-R-phycoerythrin, which is proportional to the amount of protein in the sample. The concentration of each GF was calculated based on the average fluorescence intensity of the particles according to the calibration graph using the xPONENT software.

Data processing was performed using the non-parametric criteria. The results in the tables are presented as median (Me) and interquartile range ($Q_1$, $Q_3$). The significance of differences ($p$) was assessed using the Mann–Whitney test.

To determine the clinical value of the parameters, the sensitivity and specificity were calculated, and the ROC-analysis was used in accordance with GOST R 53022.3-2008 "Clinical laboratory tech-

**Table 1**. Concentration of growth factors in chronic periodontitis

| Growth factors, pkg/ml | Control group | Main group (chronic periodontitis) | p |
|---|---|---|---|
| NGF-β. Me ($Q_1$; $Q_3$) | 17.6 (8.8–28.0) | 38.4 (6.3–49.03) | 0.001 |
| HGF. Me ($Q_1$; $Q_3$) | 781.5 (408.7–1405.9) | 341.3 (196.9–790.6) | 0.02 |
| EGF. Me ($Q_1$; $Q_3$) | 116.2 (74.5–303.7) | 357.3 (334.0–510.2) | 0.001 |
| VEGF-A. Me ($Q_1$; $Q_3$) | 144.4 (72.2–858.4) | 269.0 (185.5–814.1) | 0.03 |
| PDGF-BB. Me ($Q_1$; $Q_3$) | 24.4 (12.2–15.2) | 24.0 (6.6–74.7) | 0.07 |

Note: NGF-β —nerve growth factor β; HGF —hepatocyte growth factor; EGF —epidermal growth factor; VEGF-A —vascular endothelial growth factor; PDGF-BB —platelet derived growth factor.

**Table 2**. Diagnostic value of growth factors in sweat fluid in chronic periodontitis

| Indicator | Critical point | Diagnostic sensitivity, % | Diagnostic specificity, % | AUC | Value of a positive result, % |
|---|---|---|---|---|---|
| NGF-β | 32.9 | 89.1 | 91.1 | 0.89 | 85 |
| HGF | 333.1 | 87.2 | 87.6 | 0.78 | 69 |
| EGF | 345.2 | 92.3 | 96.1 | 0.93 | 91 |
| VEGF-A | 284.8 | 87.1 | 95.3 | 0.92 | 90 |
| PDGF-BB | 12.4 | 85.7 | 62.1 | 0.69 | 62 |

Note: NGF-β —nerve growth factor β; HGF —hepatocyte growth factor; EGF —epidermal growth factor; VEGF-A —vascular endothelial growth factor; PDGF-BB —platelet derived growth factor.

nologies." In addition, the predictive value (PV) of a positive result was assessed by the equation:

$$PV = [TP/(TP + FP)] \times 100\%,$$

where TP is the true-positive result; FP is false-positive result.

The Gretal program was used to perform multivariate statistics, while the diagnostic characteristics of the indicators and PV were calculated using the application EXEL 2007 Analyse-it.

**Results and discussion**. Objective indicators of the status of oral cavity are dental indices of periodontal health (papillary-marginal-alveolar index and Russell's periodontal index) and a simplified index of oral hygiene. Their values were noticeably increased in CP patients. For example, the value of the papillary-marginal-alveolar index was 4.5 times higher in patients with CP than in patients of the control group ($p < 0.05$). This confirms the correctness of the diagnosis established and indicates a noticeable periodontal disorder.

The mechanisms of damage to the periodontal tissue are diverse, and, as shown above, GF are involved in them [13–15]. This served as a rationale for determining the concentration of some of them in OF in CP (Table 1). As a result, the NGF-β increased by a factor of 2.2, EGF by a factor of 3, and VEGF by a factor of 1.9 ($p < 0.05$) when compared to volunteers with intact periodontium. The concentration of PDGF-BB was unchanged.

Our results on changes in the concentration of neuropeptides and GF are consistent with that of other authors [8, 13]. However, the question of whether these GFs can be considered as CP biomarkers remains open.

The ROC-analysis is an objective tool for assessing the clinical value of laboratory tests by determining their sensitivity and specificity. This approach showed that NGF-β, EGF, and VEGF-A have the greatest clinical value (Table 2).

In addition to sensitivity and specificity, the PV of a positive result was calculated. The latter depends on the disease prevalence. Considering that the prevalence of CP in the population over 30 years old is quite high, a positive test result depicts that GFs (such as NGF-β, EGF, and VEGF-A) have a key role as biomarkers of inflammation and damage to periodontal tissues in CP. At the same time, the PV of other GFs (HGF and PDGF-BB) is low. Therefore, these parameters cannot be considered as CP biomarkers.

The study of the salivary proteome (especially the determination of the concentration of cytokines in OF) represents a novel field in periodontology. Previous studies illustrate an increase in the levels of cytokines, acute phase proteins, matrix metalloproteinases and other peptides, some of which serve as biomarkers of periodontal damage and other diseases [2, 3, 6].

The authors are currently discussing the issue of the possibility of using GF as biomarkers of CP. For example, Taskan et al. (2019) showed that the concentration of VEGF in CP decreases more in the

CP group [16]. The aforementioned probably contradicts our data, because the study of OF was performed in patients with high CP activity.

One of the cytokines involved in the pathogenesis of CP is NGF-β. It stimulates the proliferation and differentiation of neurons, and the buccal epithelium. It is also one of the mediators of pain accompanying the development of this disease [17–19]. In view of this, we believe that the revealed increase in the salivary level of NGF-β could be predicted.

Similar changes in OF in CP were also revealed in the EGF level and was considered as a compensatory mechanism aimed at increasing the reduced reparative potential of the gums. The level of this indicator probably drops with severe periodontal damage. As noted above, our results were not consistent with that of some authors [14]. This refers to the determination of the PDGF-BB level of OF in CP, which remained unchanged in patients in our study. The result obtained was probably related to the fact that our study was performed in patients with mild CP, and an increase in PDGF-BB concentration is only characteristic of severe CP.

In general, our results correspond to the modern concept of CP pathogenesis, emphasizing the contribution of GF in the mechanisms of inflammation, proliferation, and neoangiogenesis [11].

**Conclusions**. Firstly, we demonstrated a change in the level of HGF in CP, as well as nerve growth factor β, VEGF, and epidermal growth factor in the oral fluid with mild CP. This confirms their active participation in the disease pathogenesis.

Finally, nerve GF β, vascular and epidermal GFs are of great clinical value, and should be considered as biomarkers of mild CP.

**Author contributions**: V.V.B. was the supervisor, developed the study concept, and edited the manuscript; L.G.P. performed immunochemical studies, data analysis, wrote the manuscript, and formatted it; E.A.S. and E.N.S. collected the clinical material, consulted patients; A.Yu.M. performed immunochemical studies and data analysis; Yu.V.M. consulted patients with a clinical point of view.

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**Conflict of interest**. The authors declare no conflict of interest.

## REFERENCES

1. Gross A.J., Paskett K.T., Cheever V.J., Lipsky M.S. Periodontitis: a global disease and the primary care provider's role. *Postgrad Med. J.* 2017; 93 (1103): 560–565. DOI: 10.1136/postgradmedj-2017-134801.

2. Bazarnyy V.V., Polushina L.G., Maksimova A.Yu., Svetlakova E.N., Mandra Yu.V. Pathogenetic substantiation of new approaches to assessing the state of oral cavity tissues in chronic generalized periodontitis. *Problemy stomatologii.* 2018; 14 (4): 14–18. (In Russ.) DOI: 10.18481/2077-7566-2018-14-4-14-18.

3. Polushina L.G., Svetlakova E.N., Sementsova E.A., Mandra Yu.V., Bazarnyi V.V. Clinico-pathogenetic value of some cytokines in periodontitis. *Medical Immunology*. 2017; 19 (6): 803–806. (In Russ.) DOI: 10.15789/1563-0625-2017-6-803-806.

4. Almehmadi A.H., Alghamdi F. Biomarkers of alveolar bone resorption in gingival crevicular fluid: A systematic review. *Arch. Oral Biol.* 2018; 93: 12–21. DOI: 10.1016/j.archoralbio.2018.05.004.

5. Lorenzo-Pouso A.I., Pérez-Sayáns M., Bravo S.B., López-Jornet P., García-Vence M., Alonso-Sampedro M., Carballo J., García-García A. Protein-based salivary profiles as novel biomarkers for oral diseases. *Dis. Markers*. 2018; 2018: 6141845. DOI: 10.1155/2018/6141845.

6. Bazarny V.V., Polushina L.G., Sementsova E.A., Svetlakova E.N., Beresneva N.S., Mandra Yu.V., Tsvirenko S.V. The interleukins in the pathogenesis of periodontitis. *Vestnik Ural''skoy meditsinskoy akademicheskoy nauki*. 2017; 14 (1): 35–39. (In Russ.)

7. Li S., Gu X., Yi S. The regulatory effects of transforming growth factor-β on nerve regeneration. *Cell Transplant*. 2017; 26 (3): 381–394. DOI: 10.3727/096368916X693824.

8. Afacan B., Öztürk V.Ö., Paşalı Ç., Bozkurt E., Köse T., Emingil G. Gingival crevicular fluid and salivary HIF-1α, VEGF, and TNF-α levels in periodontal health and disease. *J. Periodontol.* 2019; 90 (7): 788–797. DOI: 10.1002/JPER.18-0412.

9. Angiero F., Ugolini A., Cattoni F., Bova F., Blasi S., Gallo F., Cossellu G., Gherlone E. Evaluation of bradykinin, VEGF, and EGF biomarkers in gingival crevicular fluid and comparison of PhotoBioModulation with conventional techniques in periodontitis: a split-mouth randomized clinical trial. *Lasers Med. Sci*. 2020; 35 (4): 965–970. DOI: 10.1007/s10103-019-02919-w.

10. Choi Y., Yoo J.H., Lee J.H., Lee Y., Bae M.K., Kim Y.D., Kim H.J. Connective tissue growth factor (CTGF) regulates the fusion of osteoclast precursors by inhibiting Bcl6 in periodontitis. *Int. J. Med. Sci*. 2020; 17 (5): 647–656. DOI: 10.7150/ijms.41075.

11. Smith P.C., Martínez C., Cáceres M., Martínez J. Research on growth factors in periodontology. *Periodontol. 2000*. 2015; 67 (1): 234–250. DOI: 10.1111/prd.12068.

12. Zekeridou A., Mombelli A., Cancela J., Courvoisier D., Giannopoulou C. Systemic inflammatory burden and local inflammation in periodontitis: What is the link between inflammatory biomarkers in serum and gingival crevicular fluid? *Clin. Exp. Dent. Res.* 2019; 5 (2): 128–135. DOI: 10.1002/cre2.162.

13. Guru S., Sam S.E., Rajan S., Padmanabhan S. Comparative evaluation of salivary hepatocyte growth factor in smokers and non-smokers with chronic periodontitis. *J. Investig. Clin. Dent*. 2018; 9 (2): e12306. DOI: 10.1111/jicd.12306.

14. Romano F., Bongiovanni L., Bianco L., Di Scipio F., Yang Z., Sprio A.E., Berta G.N., Aimetti M. Biomarker levels in gingival crevicular fluid of generalized aggressive periodontitis patients after non-surgical periodontal treatment. *Clin. Oral Investig.* 2018; 22 (2): 1083–1092. DOI: 10.1007/s00784-017-2192-1.

15. Li W., Zhang Z., Wang Z. Differential immune cell infiltrations between healthy periodontal and chronic perio-

dontitis tissues. *BMC Oral Health.* 2020; 20 (1): 293. DOI: 10.1186/s12903-020-01287-0.

16. Taskan M.M., Karatas O., Balci Yuce H., Isiker Kara G., Gevrek F., Ucan Yarkac F. Hypoxia and collagen crosslinking in the healthy and affected sites of periodontitis patients. *Acta Odontol. Scand.* 2019; 77 (8): 600–607. DOI: 10.1080/00016357.2019.1624819.

17. Coelho J.M.F., Miranda S.S., da Cruz S.S., Trindade S.C., Passos-Soares J.S., Cerqueira E.M.M., Costa M.D.C.N., Figueiredo A.C.M.G., Hintz A.M., Barreto M.L., Seymour G.J., Scannapieco F., Gomes-Filho I.S. Is there association between stress and periodontitis? *Clin. Oral Investig.* 2020; 24 (7): 2285–2294. DOI: 10.1007/s00784-019-03083-9.

18. Haririan H., Andrukhov O., Böttcher M., Pablik E., Wimmer G., Moritz A., Rausch-Fan X. Salivary neuropeptides, stress, and periodontitis. *J. Periodontol.* 2018; 89 (1): 9–18. DOI: 10.1902/jop.2017.170249.

19. Jasim H., Ghafouri B., Carlsson A., Hedenberg-Magnusson B., Ernberg M. Daytime changes of salivary biomarkers involved in pain. *J. Oral Rehabil.* 2020; 47 (7): 843–850. DOI: 10.1111/joor.12977.