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GINGIVAL FLUID AS A POTENTIAL OBJECT FOR DIAGNOSTICS PROCESS

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ABSTRACT — Gingival sulcus is a place where inflammation occurs due to microbial penetration into the periodontal tissue. Gingival fluid can be employed as the fastest and most accurate reflection of pathological changes taking place in the cellular composition, the protein spectrum, and the pH status so this is of interest as a research object. The difference in the outcomes obtained through studying the cytokines level has been attributed to the use of different methods for sampling and storage of fluid samples.

KEYWORDS — chronic generalized periodontitis, gingival fluid, cytokines.

INTRODUCTION

Periodontal diseases belong to multifactorial infections, resulting from disturbed interaction between various bacteria and the periodontal immune system cells. These processes lead to the release of an uncontrolled amount of pro-inflammatory cytokines and chemokines, which ultimately results in destroyed periodontal structures [1–7]. Gingival crevicular fluid (GCF) in persons with periodontal issues contains inflammatory cells, bacteria, tissue disintegration products, antibodies, the complement system proteins and enzymes, as well as many inflammatory mediators [8]. Its preparation and quantitative determination of its immunoregulatory biomarkers can be considered one of the most non-traumatic research methods to obtain details on the status and degree of periodontal tissues destruction, to identify the features of its damage in various periodontal diseases, and to predict their course [9–11].

The microbial biofilm — a generally recognized etiological factor behind the inflammatory periodontal diseases development – involves immune defense mechanisms in the pathogenetic circle. The imbalance between bacterial invasion and the oral cavity immune defense systems is considered the major cause leading to the development of periodontal tissue destruction [12, 13]. The cell walls components in anaerobic periodontal pathogens, while interacting with Toll receptors (TLRs) lead to a change in their structure and function, which, in turn, leads to activation of signaling pathways, an increase in the cytokines/chemokines production by the periodontal tissue local immune system cells, which attract polymorphonuclear cells to the focus of inflammation for effective clearance of bacteria. Activated neutrophils, in turn, cause destruction of the gum tissue, periodontal and alveolar bones, the result of that being an increase in the periodontal pockets depth. GCF is considered to be one of the reliable sources in studying inflammatory processes in case of periodontal diseases [14, 15]. GCF is one of the main objects in which the cytokines content is associated with the development of the immune response of both cellular and humoral type. However, the feasibility of its use for the quantitative determination of immune regulatory mediators as biomarkers for the periodontal disease progression has not been identified finally. Despite the large number of research works published so far, there is no generally approved method for GCF sampling and subsequent processing to do quantitative analysis of cytokines, first of all – based on the enzyme-linked immunosorbent assay, which is the most available currently.

Aim of study

to develop a method for identifying cytokines in GCF in order to evaluate the inflammation degree in patients with chronic generalized periodontitis (CGP).

MATERIALS AND METHODS

40 patients aged 40–55 years were examined, including 20 of them suffering from mild chronic generalized periodontitis (CGP), and 20 basically healthy patients. The inclusion criteria: persons over 40, who signed the awareness consent and protocol regarding the purpose and nature of the study. The exclusion criteria were: age under 40; coagulation and hemostasis disorders; immune system issues; chronic infectious, mental, oncological diseases; HIV-positive; women through pregnancy and lactation, as well as the patient's refusal to undergo examination.

To assess the effect of the preanalytic stage, involving GCF sampling, on the cytokines concentra-

tion in the 20 healthy individuals and 20 patients with CP, the GCF was collected twice, simultaneously.

GCF sampling method. After cleaning the teeth and adjacent gums from plaque, they were isolated from saliva with cotton rolls and dried. The material was taken from the gingival sulcus and/or periodontal pocket using special targets as sterile endodontic absorbent paper points (Absorbent Paper Points, No. 25, Taper 02), which are used in therapeutic dentistry to dry the root canal before filling. The points are sterile, made of paper with high absorbent capacity and free from impurities of binders, of perfect density to be inserted into the gingival sulcus or periodontal pocket. Analytical weighing of 30 paper points inserted into the periodontal pockets and kept until completely saturated revealed that the average amount of the absorbed GCF was 5.0 ± 0.05 mg. Using dental tweezer and a carver, 2 points were placed in the gingival sulcus, each of them getting completely saturated with GCF for 100–120 sec and transferred into two Eppendorff tubes. One of the points contained 1000 μl of 0.155M sodium chloride solution, while the other contained 0.2% of the biocide ProClin 300 series [16]. As a result, GCF samples were obtained with a 1:200 dilution, which were further frozen at –40° C and stored until analysis.

The concentration of IL-1β, IL-8, MCP-1, VEGF, IL-1RA cytokines in the GCF samples was indentified with enzyme-linked immunosorbent assay using the respective reagent kits (Vector-Best CJSC, Novosibirsk, Russia).

The statistical processing of the research results was done with the Statistica v6.0 software. When comparing the results, the nonparametric Wilcoxon-Mann-Whitney test was used in the Multi Experiment Viewer software (P <0.05).

RESULTS AND DISCUSSION

Table 1 offers a view on the obtained results.

The study results showed significant differences in the composition of GCF in individuals without periodontal disease and with CP, the basis of that being the high content of such pro-inflammatory cytokines as IL-1β (5.0 times as high, P <0.05), IL-6 (23.8 times, P <0.05), IL-8 (4.2 times, P <0.05), MCP-1 (1.9 times, P <0.05), TNFα (1.5 times as high). Their concentration increase is due to the simultaneous involvement into the inflammation of all the immune defense cells of the gingival sulcus (neutrophils, lymphocytes and monocytes, epithelial cells).

The presented differences in the increase degree of the content in the main group of pro-inflammatory cytokines in the GCF from patients with CP were obtained through GCF extraction medium with ProClin 300. The cytokines content was significantly higher in the GCF extracted with 0.155 M sodium chloride solution along with 0.2% ProClin 300. To a smaller extent, this medium had its effect on the level of the main pro-inflammatory GCF cytokines of individuals who were basically healthy. Significant concentration preservation in it was observed for IL-8 (1.49 times as high; P <0.05); MCP-1 (1.67 times; P <0.05); TNF-α (2.3 times; P <0.05). As for the patients with CP, in a medium containing ProClin 300, an increase of the entire major pool of pro-inflammatory mediators was registered. The increase in the mediators level in GCF was as follows: IL-1β (1.95 times as high; P <0.05); IL-6 (2.13 times; P <0.05); IL-8 (2.23 times; P <0.05); MCP-1 (2.35 times; P <0.05); TNF-α (2.98 times; P <0.05). It means that the use of a medium with antimicrobial activity for GCF extraction straight after its collection with a paper point allows ensuring stability in identifying the true concentration of the major cytokines group. An advantage of a biocide such as ProClin is the blockage of various types of microorganisms contained in the discharge coming from the gingival sulcus/periodontal pocket. Their presence in the GCF obtained for the study, both in case of intact periodontium and, especially, with CP, leads to rapid destruction of nearly the entire cytokine group, which is currently identified through enzyme immunoassay.

CONCLUSION

Given all of the above, when identifying the cytokines concentration in GCF, the preanalytical stage is of crucial importance in terms of obtaining reliable and reproducible results of the analyses. The introduction of ProClin 300 gingival fluid into the solution for extraction and subsequent storage of gingival fluid can be used to increase the sensitivity and specificity of the entire group of cytokines in the diagnostics of inflammatory periodontal diseases. The introduction of GCF biomarkers examination for assessing inflammatory periodontal diseases is a promising area for the introduction of advanced decision-making systems in practical dentistry.

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Table 1. *Effect of diluent solution at GCF sampling on major pro- and anti-inflammatory cytokines*

Indicators (pg / ml dilution medium)	GCF in individuals without abnormal periodontal changes		GCF in patients with mild chronic generalized periodontitis	
	0.155M sodium chloride solution and 0.2% ProClin 300 solution	0.155M sodium chloride solution	0.155M sodium chloride solution and 0.2% ProClin 300 solution	0.155M sodium chloride solution
IL-1β	4,2 (1,9;7,2)	3,1 (1,2;5,4)	21,1** (12,5;27,8)	10,8* ** (6,3;15,2)
IL- 6	4,9 (2,3;5,7)	3,7 (1,8;4,3)	116,6** (64,4;166,2)	54,5* ** (32,4;83,4)
IL-8	73,3 (65,6;79,7)	48,9* (43,5;52,5)	295,25** (167,9;322,9)*	132,3* ** (65,7;160,8)
MCP-1	28,9 (26,6;32,1)	17,3* (17,9;21,4)	110,4** (78,6; 120,5)	46,9* **(39,3;59,8)
TNFα	7,6 (6,4;9,6)	3,3* (3,1;5,2)	11,3 (5,2;14,3)	3,8* (1,1;4,7)
IL-1RA	4279,5 (3794,25;4885,5)	2139,5* (1897,2;2442,8)	3885 (1894; 4808)*	1928* (946;2403)

** — P <0.05, when comparing the results of the GCF study placed in test tubes with 0.155 M sodium chloride solution and 0.2% ProClin 300 solution, and without it;*
*** — P <0.05, when comparing the results of the GCF study in individuals without pathological changes in periodontal disease and in patients with mild CGP*

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