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GUM FLUID BIOMARKERS IN PERSONALIZED DIAGNOSTICS OF INFLAMMATORY PERIODONTAL DISEASES

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ABSTRACT — Aim of the study: To evaluate the diagnostic value of crevicular fluid immunoregulatory biomarkers in cases with inflammatory periodontal diseases. The study included a total of 97 patients aged 21 to 55 years with inflammatory periodontal disease (gingivitis — 22 patients; mild periodontitis — 31 patients; moderate periodontitis — 19) as well as 25 persons with healthy periodontium (the control group). A conventionally accepted clinical and instrumental examination revealed that all the patients with inflammatory periodontal diseases featured the most typical signs of periodontal issues: in the crevicular fluid — an increase in the levels of pro-inflammatory cytokines and chemokines (IL-1β, IL-6, IL-17, TNF-α), a decreased level of anti-inflammatory cytokine IL-1RA, and an increased concentration of vascular endothelial growth factor (VEGF). A ROC analysis showed that the increased content of VEGF, IL-8, IL-1β, IL-6 in the crevicular fluid is of a high diagnostic value in terms of detecting initial inflammation in the gum tissues, whereas the biomarkers pointing at periodontal destructive changes included high levels of VEGF, TNF-α and chemokines.

KEYWORDS — gingivitis, periodontitis, cytokines, chemokines, vascular endothelial growth factor, crevicular fluid.

RELEVANCE

The diagnostics of periodontal diseases relies currently on the results of clinical and radiological studies. Moreover, molecular genetic research and advanced computer technologies are gaining more and more of a foothold in diagnosing periodontal pathology. Unlike traditional laboratory technologies the newly developed diagnostic methods in question offer no sufficient evidence confirming their respective effectiveness. However, a number of studies have already been held, which serve evidence to the need of introducing a number of periodontal pathology biomarkers into the dental clinical practice [1, 10–12]. Biomarkers characterizing the periodontal status are considered to be the key to personalized medicine. As far as diagnostics and treatment of oral diseases are concerned, dental practice is currently facing a need to switch from traditional technologies to approaches based on precision medicine [2].

Given immunoglobulins, lysozyme, T-and B-lymphocytes (ratio 1/2,7), electrolytes, etc. contained in gingival crevicular fluid (GCF), this is seen nowadays as an essential factor of oral cavity local protection. Certain authors (N. Brill, V. Krasse, 1958; G. Cimasoni, 1983) claim that the formation of GCF is associated with an increase in the gum tissues permeability during inflammation. The intensity of the crevicular fluid release depends on the connecting furrow epithelium filtration factor, and especially by the pressure gap between the interstitial and the crevicular fluid. From this stance, the use of qualitative and quantitative GCF indicators for diagnosing periodontal issues of inflammatory origin appears well-grounded and promising.

The most common inflammatory periodontal diseases (IPD) are considered currently not only as an effect of the gum tissue response to the biofilm developing due to plaque bacteria, yet also as something taking place through altered epithelial-immune interaction, cytokines being the major agents of this process [3, 4, 5]. An increase in their content in the GCF is due to an influx of neutrophil cells, macrophage monocytes, T-and B-lymphocytes from the peripheral blood into the gingival sulcus in case of inflammatory periodontal diseases. Cytokine production of each of the gingival sulcus cells groups in case of IPD is aimed at both at immune protection and at the destruction of tissues in the inflammation focus. These cells, which make up the focus of local inflammation, lead to the accumulation of numerous cytokines in the contents of the gingival furrow, primarily such as IL-1β, IL-6, TNF-α, IL-8, MCP-1, etc. At the initial stage, there is an accelerated destruction of epithelial cells and collagen structures developing in the inflammation focus. Further on, the inflammation leads to the bone tissue resorption in the interalveolar septae. The GCF cytokine profile is considered one of the

features indicative of the IPD activity and severity [6]. Changing levels of cytokines in the GCF was found to allow adjusting the complex treatment of IPD and identifying the directions of personalized therapy, evaluating the treatment effectiveness and forecasting the disease course [7, 8, 9]. The results of studying the GCF cytokine profile, however, remain diverse and contradictory.

Aim of study:
evaluation of the diagnostic value of crevicular fluid immunoregulatory biomarkers in case of inflammatory periodontal diseases.

Methodology:
the study relied on data from the respective literature focusing on the IPD etiopathogenesis: Elsevier Science, PubMed Central, ClinicalTrials.gov, MedlinePlus.

MATERIALS AND METHODS

The study involved 97 patients aged 21 to 55 suffering from inflammatory periodontal diseases (gingivitis — 22; mild periodontitis — 31; moderate periodontitis — 19) as well as 25 people with healthy periodontitis (the comparison group). Groups of patients with inflammatory periodontal diseases included the patients who featured typical signs of periodontal damage diagnosed through a conventional clinical and instrumental examination. The comparison group included persons with no signs of periodontal pathology. The exclusion criteria were: concomitant diseases of the digestive system in the acute phase; diabetes mellitus; severe concomitant diseases; the patient's refusal.

The study was carried out at the dental clinic at the S.R.Mirovtsev University Clinical Hospital (part of the Clinical Center of the V. I. Razumovsky Saratov State Medical University) with a prior approval of its Committee for Bioethics.

The focus of research was GCF. After cleaning the teeth from plaque, they were isolated from saliva with cotton rolls and dried. The material from the gingival cavity and / or periodontal pocket was taken with special targets made as paper, absorbent, sterile endodontic pins (Absorbent Paper Points, No. 25). Using tweezers and a packer, two pins were immersed sequentially in the gingival cavity (periodontal pocket), whereas after impregnation they were both put in an Eppendorff-type test tube containing 1000 μl of 0.155 M sodium chloride solution and 0.2% ProClin 300 series biocide [8]. GCF samples with a dilution of 1:200 were frozen at -40° C and stored until the analysis. The concentration of IL-1β, IL-6, IL-17, TNF-α pro-inflammatory cytokines, IL-8, MCP-1 chemokines, the IL-1RA anti-inflammatory cytokine and the VEGF in the GCF samples was identified by enzyme immunoassay using commercial reagent kits (Vector-Best, JSC; Novosibirsk, Russia). The statistical processing of the obtained data was performed using the Statistica v. 6.0 set of software. The results of cytokines quantitative analysis are presented as a median with a quarter-scale (25–75 percentile). The statistical analysis of the results obtained from studying cytokines in the compared groups was carried out relying on the Mann-Whitney U-test, graphical analysis of nonparametric statistical indicators and ROC analysis.

RESULTS AND DISCUSSION

Each of the selected groups of patients with IPD featured specific indicators of the periodontal complex index evaluation (Table 1).

The groups of patients suffering from gingivitis and periodontitis had high medians of the OHI-s, PMA and SBI indices associated with inflammation. An increase in the periodontal pockets depth and the Russel periodontal index, which are typical of a high volume of destructive changes in the periodontal complex, were observed in groups where patients had periodontitis of mild and moderate severity.

A comparison of the cytokine study results in the patients divided into groups, revealed an increase in all proinflammatory cytokines and chemokines in cases with the IPD, and a decrease in the level of anti-inflammatory cytokine, IL-1RA, and an increase in the VEGF concentration (Table 2.3 and Figure 1.2).

There was a direct relationship identified between increased content of pro-inflammatory cytokines/chemokines and the severity of the inflammation clinical manifestations. In case of gingivitis, for instance, as well as in case of mild and moderate periodontitis, the respective GCF featured an increased content of proinflammatory cytokines/chemokines IL-1β, IL-6, IL-8, IL-17, TNF-a/IL-8, MCP-1, and a decrease in the IL-1RA levels. The most meaningful factor in the development of a primary immune response to the introduction of periodontal pathogenic microbiota is IL-1β, whose content in the GCF goes up as soon as at the early stages of inflammation. Increasing levels of pro-inflammatory cytokines/chemokines, as well as VEGF in the GCF of patients belonging to the groups with mild and moderate periodontitis pointed at developing degradation of epithelial cells and collagen fibers of connective tissue further passing onto periodontal support tissues, which means it was associated directly with destructive issues affecting the periodontal complex. The group of patients with moderate periodontitis were found to have the highest PI index and the content of IL-6, IL-8, IL-17, TNF-α, IL-8, MCP-1, and VEGF in the crevicular fluid.

Table 1. *Index evaluation of the periodontal tissues status in patients with IPD*

Examined groups	Index score indicators				
	OHI-S (Green J.C., Vermillion J.R., 1964)	SBI (Mühlemann H.R., Son S., 1971)	PMA (C. Parma, 1960)	PI (Russel, 1956)	Periodontal pocket depth, mm
Gingivitis (n=22)	2 (1,8;2,2)	1,5 (1,4; 1,6)	22,1 (21,4; 26,3)	1 (0,9; 1,55)	-
Mild periodontitis (n = 31)	2,2 (2,1; 2,4)	2,3 (2,1; 2,5)	50,3 (46,7; 52,8)	3,6 (3,4; 3,9)	3,4 (2,7; 3,6)
p-level	0,2	0,015	0,002	0,002	–
Moderate periodontitis (n = 19)	2,6 (2,5; 2,8)	2,5 (2,3; 2,7)	70 (64,3; 77,1)	5,3 (4,9; 5,4)	4,3 (4; 4,8)
p-level	0,2	0,002	0,008	0,001	0,001
Comparison group (n = 25)	1,3 (1,2;1,4)	–	–	–	–

Table 2. *Results of studying the cytokine profile of crevicular fluid in patients with IPD*

Indicator (pg / ml)	Examined groups			
	Healthy (comparison group)	Gingivitis	Mild periodontitis	Moderate periodontitis
	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]
TNF-α	1,58 [1,3;1,9]	1,5 [1,4;1,9]	6,7 [4,5;8,9]*	13,9 [12,8;14,9]*
IL-17	7,45 [6,1;10,8]	18,1 [12,6:21,0]*	15,5 [12,8;22,1]*	28,4 [26,6;33,7]*
IL-6	0,17 [0,1;0,9]	2,9 [1,7;3,4]*	4,2 [2,1;5,6]*	9,4 [8,1;11,3]*
IL-1β	4,9 [2,7;5,3]	10,3 [9,6;11]*	19,6 [11,6;23,6]*	16,2 [13,6;20,1]*

** difference between the comparison group and patients with IPD (Mann-Whitney U test) with a confidence level exceeding 0.95.*

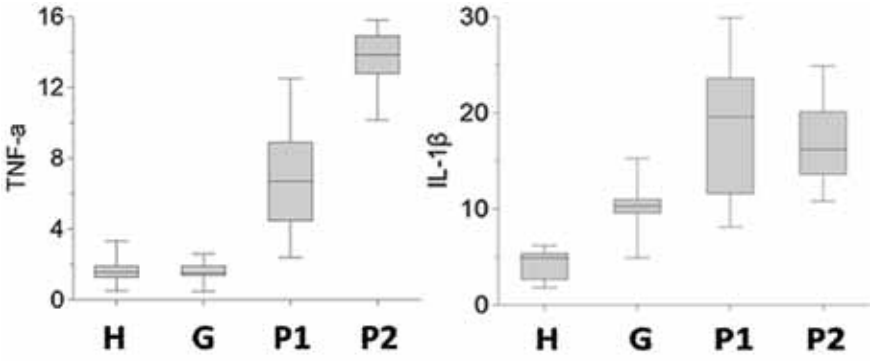

Fig. 1. *The range of TNF-a and IL-1β levels in the crevicular fluid: H — comparison group, G — gingivitis, P1 — mild periodontitis, P2 — moderate periodontitis*

Table 3. *Results of studying the level of chemokines, IL-1RA and VEGF in the crevicular fluid in patients with IPD*

Indicator (pg / ml)	Examined groups			
	Healthy (comparison group)	Gingivitis	Mild periodontitis	Moderate periodontitis
	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]	Me [Q1; Q3]
IL-8	58,7 [23,6;69,5]	75,7 [55,9;189,7]*	122,4 [95,3;173]*	248,2 [213,0;312,7]*
MCP-1	28,2 [21,2;34,4]	26,7 [21,5:31,7]	110,0 [84,1;135,7]*	242,5 [199,1;265,7]*
IL-1RA	3724,7 [2900;4303]	3065 [2772;3478]*	1940 [1559,7;3750]*	2306,2 [1506,0;2759,0]*
VEGF	9,4 [4,9;12,0]	25.1 [18,9;27,8]*	34.5 [30,9;42,7]*	33,5 [27,8;38,0]*

** difference between the comparison groups and patients with IPD (Mann-Whitney U test) with a confidence level exceeding 0.95.*

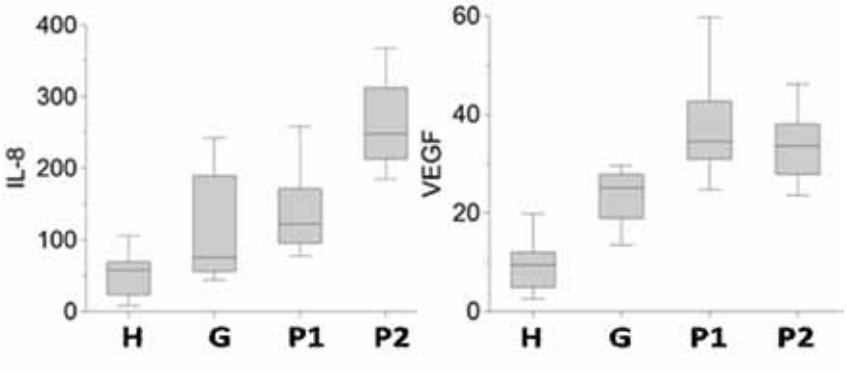

Fig. 2. *The range of IL-8 and VEGF levels in the crevicular liquid: H — comparison group, G – gingivitis, P1 — mild periodontitis, P2 — moderate periodontitis*

An analysis of ROC-curves showed that the increase in the GCF-content of the group of mediators related to immunoregulatory processes is associated with clinical manifestations of inflammatory and destructive changes. Assuming a nonparametric distribution, a confidence level of 95%, including the cut-off value in the positive classification, the increase of VEGF, IL-8, MCP-1, IL-1β, TNF-α, IL-6 in the GCF appears of a reliably high diagnostic value.

Significant binary classifiers were considered those values where the AUC (the area under the ROC curve) was above 0.7 (below 0.3 at a negative dependence) at a significance level of lower than 0.05 when testing the hypothesis stating that the area under the curve was equal to 0.5 (Aundersurve, AUC). Fig. 3–5 offer a view of the respective results.

odontal tissues. During that, biomarkers like VEGF, IL-8, IL-1β, and IL-6 proved of diagnostic value when it came to evaluating the inflammatory process at in the early stages of the disease in case of gingivitis. This proves once again the role of IL-1β as a diagnostic marker of initial inflammation in the gum tissues in response to the plaque bacteria biofilm. High levels of VEGF, chemokines, and TNF-α are to be seen as further biomarkers of inflammation-induced changes that affect the supporting periodontal tissues and lead to the alveolar bone resorption. At thresholds of VEGF — 30.6 pg/ml, MSR-1 — 178.9 pg/ml, IL-8 — 185 pg/ml, and TNF-α — at 11.7 pg/ml, biomarkers reveal sensitivity and specificity from 1.8 to 1.96, which serves proof to their potential use as a group of indicators that allow differential diagnostics of

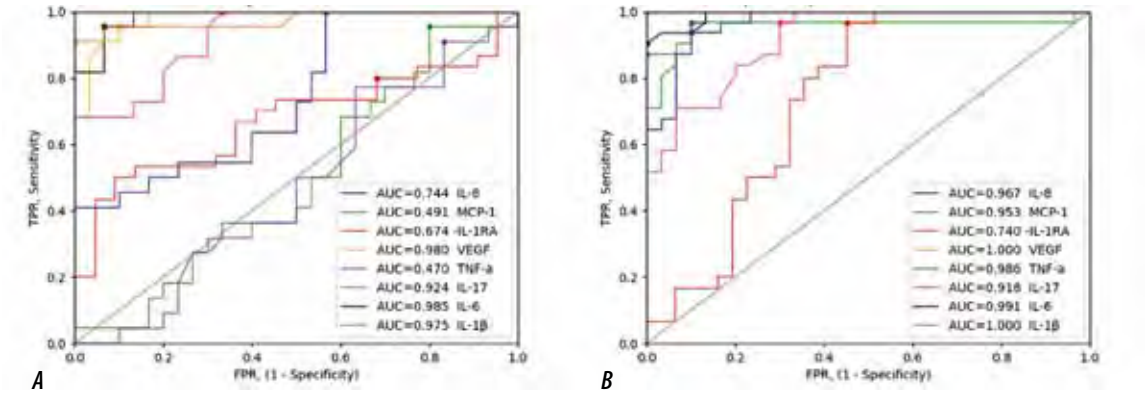

Fig. 3. *ROC curves of the following groups: A — the comparison group and that of patients with gingivitis; B — the comparison group and patients with mild periodontitis*

In patients with mild and moderate periodontitis, the diagnostic value resided in almost the entire group of identified indicators, except IL-1RA and IL-17. That said, an increase in the contents of VEGF, IL-8, MCP-1, IL-1β, TNF-α, IL-6 in the GCF points at a local inflammatory process at the level of periodontitis and gingivitis, as well as monitoring the inflammation passing further to destroy the periodontal ligament and bone tissue.

As of today, there has been a protocol designed for examining patients with IPD. However, diagnosing this pathology at the initial stages still remains

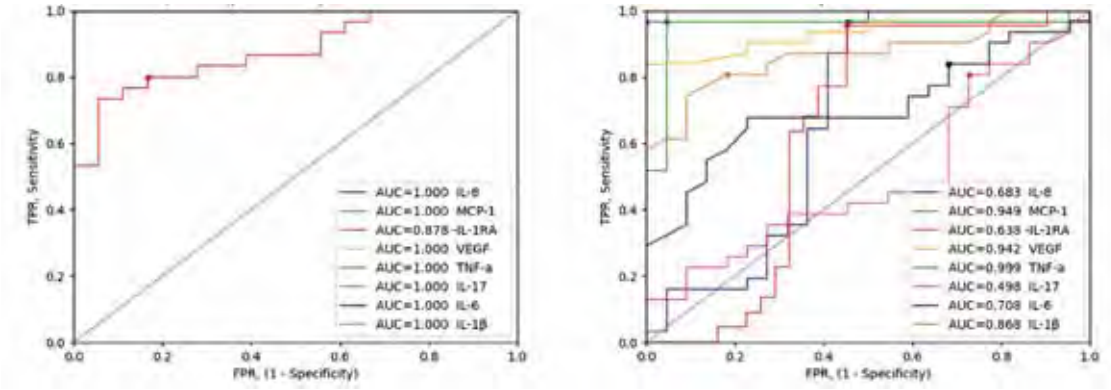

Fig. 4. *ROC curves of the following groups: A — the comparison group and that of patients with moderate periodontitis; B — patients with gingivitis and mild periodontitis*

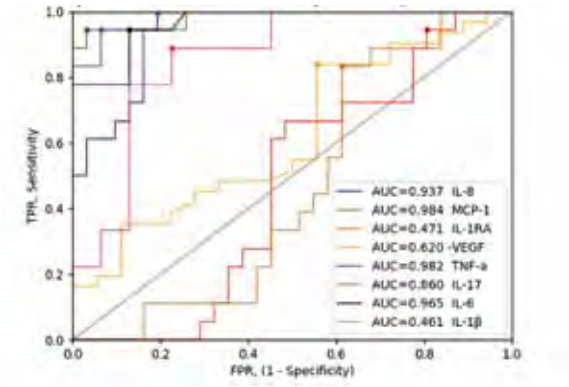

Fig. 5. *ROC curves of groups of patients with mild and moderate periodontitis*

an issue. Patients rarely consult a doctor at the onset of the disease due to poor manifestation of clinical symptoms. Such a latent course of IPD does not allow diagnosing the disease in due time, nor does it facilitate any preventive and treatment measures, as well as supportive therapy. The present study allows proving the diagnostic value of methods employed to obtain and quantify a group of immunoregulatory cytokines in the GCF as biomarkers of early inflammation manifestations affecting periodontal tissues, as well as predictors and indicators to be used for predicting the progression of pain and the development of osteodestructive periodontal changes. This makes it possible to recommend using the quantitative determination of a group of immunoregulatory mediators (VEGF, IL-8, MCP-1, IL-1β, TNF-α, IL-6) in the GCF as personalized biomarkers when developing forecast regarding the disease course as well as the effectiveness of the treatment for IPD. Further developments in the field of saliva proteomic analysis and GCF would pave the way towards new diagnostic tools and personalized medicine. However, their application in dentistry will depend on them being used in daily clinical practice.

CONCLUSION

1. Identification of the immunoregulatory biomarkers levels in the GCF, combined with the results of clinical, laboratory, microbiological, functional and X-ray studies, allows diagnosing the intensity of the damage affecting periodontal tissues, as well as the effectiveness of rehabilitation measures when dealing with patients suffering from inflammatory periodontal diseases.

2. The dynamics of the changes to be observed in the level of immunoregulatory biomarkers contained in the crevicular fluid in case of IPD serves a reliable reflection of the severity of the damage affecting the structural and functional properties of periodontal tissues. As could be seen from the ROC analysis, early stages of inflammation in the gum tissues feature an increase in the contents of VEGF, IL-8, IL-1β, IL-6 in the crevicular fluid, while a high level of VEGF, TNF-α and chemokines in the GCF are indicative of inflammation progress and destructive issues affecting periodontal tissues.

3. The development and shaping of reference intervals related to qualitative and quantitative immunological, physical, chemical, microbiological indicators of the crevicular fluid in case of inflammatory periodontal diseases of various severity, if projected onto the patient's dental status might allow objective evaluation of the degree of disturbances in the oral homeostasis, as well as the imbalance of specific, non-specific factors of local immunity and cytokine profile.

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