DEVELOPMENT OF DIAGNOSTIC ALGORITHM OF ORAL FLUID BIOMARKERS FOR EARLY DIAGNOSE OF PERIODONTAL DISEASES

Rustamova S.M., Xadjhimetov A.A.
Tashkent State Dental Institute
Doctor of biology, professor, Tashkent State Dental Institute
[1]rustamova.sabogul@mail.ru, hadjimetov.aa@mail.ru .

Introduction. It is known that periodontitis is a multifactorial chronic irreversible inflammatory disease that affects the supporting structures of the teeth, initiated and spread by a complex interaction between periopathogens and the host's immune system. It begins with a microbial infection, followed by periodontal tissue damage caused by leukocyte hyperactivity, cytokines, eicosanoids, and matrix metalloproteinases. Based on the assessment of their diagnostic properties, the most informative laboratory biomarkers of oral fluid microflora, proteolytic enzymes cathepsin, elastase, haptoglobin, IL-1, IL-6, IL-8, and TNF-α were determined in gingivitis and chronic periodontitis. It allows to evaluate the diagnostic efficiency of these markers in patients with periodontal diseases and to use them for early diagnosis in non-invasive diagnostics.

According to the results of our research, the markers change sequentially in different stages of periodontitis, so the combination of biomarkers gives a more effective result for the diagnosis of the disease state [3].

The purpose of the study: to determine the diagnostic value of biochemical and metabolic biomarkers in patients with periodontitis.

Research materials and methods: 68 subjects consisting of healthy individuals and patients with chronic disseminated periodontitis (STP) of moderate severity were observed. The control group consisted of 16 healthy, 30.3±2.1-year-old, non-physiological forms of chronic periodontal disease involving oral mucosa, without bad habits and taking any medications. The gender distribution in this group was as follows: 45 men (66.1%) and 23 women (33.9%).

General clinical, biochemical and immunological research methods were used in the examination of patients.

Participants were asked to refrain from eating, drinking, smoking, or engaging in oral hygiene procedures for at least two hours prior to oral fluid collection. The mouth was rinsed for 30 seconds approximately 10 minutes before oral fluid collection and then excreted into sterile tubes while sitting upright. 5 ml of unstimulated oral fluid samples were collected, and then the oral fluid samples were centrifuged at 5000 rpm for 5 min. Supernatants were removed. Aliquots of 0.5 ml of the resulting supernatant were stored at -60° until analysis.

Determination of the proteolitic enzymes cathepsin and elastase a colorimetric enzymatic method by "Hosptex" (Switzerland) biochemical analyzer. Detection of inflammatory and anti-inflammatory cytokines (IL-1, IL-4, IL-6, IL-8, IL-10 and TNF-α) in oral fluid was performed using from the company "Human" on the analyzer "Mindrey". They were determined by "sandwich" method using enzyme-linked immunosorbent assay using Cytokine test system [4].

Obtained results and their discussion: The level of activity of enzymes plays an important role in the development of periodontal diseases. It can be used in the treatment of inflammatory-periodontal diseases of soft tissues with the pathogenetic method of identifying this condition.

Table 1

Activity level of proteolytic enzymes in oral fluid in patients with gingivitis and moderate periodontitis

	Healthy group n =14	Gingivitis group n =26	Periodontitis group n =28
Cathepsin activity (ncat/l)	6,72±0,61	17,28±1,53*	26,48±2,13*
Elastase activity (nkat/l)	25,89±2,83	37,24±2,97*	46,58±3,87*

Note: *- significantly different from the healthy group (P<0.05)

The results of the presented study show that cathepsin activity in patients with gingivitis increased 2.6 times from the initial values, and in patients with periodontitis, the studied indicator was 26.48±2.13 nkat/l, which is 3 times higher than the initial values of patients without periodontitis. Similar dynamics were noted in connection with the activity of the elastase enzyme. Thus, elastase activity in patients with chronic gingivitis was 37.24 ± 2.97 nkat/l, which is 44% higher than in individuals with intact periodontal tissue. The studied indicator in patients with moderate chronic periodontitis increased by 80% from the initial level of healthy people. Therefore, it was confirmed that the process of chronic inflammation in the periodontal tissues is accompanied by the activation of proteolytic enzymes in the oral fluid.

With inflammation of periodontal tissues, there is a change in the amount of inflammatory cytokines (IL-1, IL-4, IL-6, IL-8, IL-10, TNF-α) in the oral fluid. This study can be included in the series of basic biomarkers by comparing the level of cytokines in the oral fluid of patients with periodontal disease of soft and hard tissue inflammation (primary gingival cause) [1].

Table 2

Level of variation in oral fluid cytokine levels in patients with gingivitis and periodontitis

Indicators	Healthy individuals (controls) n =14	Gingivits n =26	Periodontits n =28
Interleukin-1 (IL-1) (pg/ml)	81,80 ± 7,53	132,48 ± 11,51*	205,3± 12,23*
Interleukin-4 (IL-4) (pg/ml	13,87±1,54	36,43±2,58*	51,83±4,52*
Interleukin-6 (IL-6) (pg/ml)	0,87±0,06	12,74±1,38*	22,67±2,13*
TNF-α, (pg/ml)	31,28±2,69	52,67±4,81*	118,76±9,81 *
Interleukin-10 (IL-10) (pg/ml)	10,45±0.86	9,06±1,14*	6,82±0,51*

Interleukin-8 (IL-8) (pg/ml)	80,24 ± 7,68	254,13±11,43*	656,31±15,2 *

Note: *- significantly different from the healthy group (P<0.05)

The analysis of the results of the study presented in the table shows that the amount of IL-1β in the oral fluid of patients with periodontal diseases is significantly increased compared to that of the healthy people.The increase of inflammatory agents IL-6 and TNF-α in the oral fluid of patients with gingivitis and chronic periodontitis activates destructive processes in the periodontal tissue. Increased levels of IL-4 in patients with periodontal disease may be considered as an anti-inflammatory mediator that stimulates V-lymphocytes and inhibits T-helper cells. Consequently, the local humoral anti-inflammatory protective activity is maintained at a high level due to increased IL-4 levels in patients with periodontal pathology [2].

During the study, based on the evaluation of several laboratory indicators of oral fluid, we determined the most informative laboratory biomarkers of oral fluid in gingivitis and chronic periodontitis.

CONCLUSIONS

The results obtained during the research have theoretical and practical significance. Cathepsin activity was found to increase by 2.6 times in the oral fluid of patients with gingivitis, and by 3.9 times in patients with intact periodontal tissue. Elastase activity was 37.24±2.97 nkat/l, which is 44% higher than that of periodontitis subjects, and the studied index in periodontitis patients was 80% higher than the baseline level of healthy subjects. Agents involved in inflammation and anti-inflammation: IL-1 increased by 1.6-2.5 times, IL-6 increased by 14.5-26 times, IL-8 increased by 3.2-8.8 times, IL- 10 - 13% - 35% decreased.

The study of the parameters of the oral cavity fluid expands the possibilities of monitoring the effectiveness of the treatment of patients with periodontal disease and early diagnosis with the help of screening tests using non-invasive methods.

LITERATURE

. Ратнаяке, С. Акерман, Б. Клинг и др., «Слюнные биомаркеры здоровья полости рта – поперечное исследование», *Журнал клинической пародонтологии* , том. 40, нет. 2, стр. 140–147, 2013.

”Parodont kasalliklari bilan kasallangan bemorlar og’iz suyuqligida

3. Rustamova S.M., Xadjimetov A.A., Akhmadaliyev N.N., Atakhodjayeva M.A. “Correlation relations of the composition of saliva and blood plasma in the norm” BRITISH VIEW Volume7,Issue 4,2022.101-108 бет

Рустамова С.М., Хаджиметов А.А.,Ахмадалиев Н.Н “Оғиз суюқлиги ва сўлакнинг физик-кимёвий ва метаболик параметрлари тана ҳолатининг кўрсаткичлари сифатида” “Medicine and innovations”.(8) декабр, 2022