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LABORATORY MONITORING OF PERIODONT INFLAMMATORY DISEASES

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Abstract: 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-4, IL-6, IL-8, IL-10 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.
Keywords: Chronic disseminated periodontitis, gingivitis, oral fluid, periodontium, biomarkers,cytokines.
Аннотация: Известно, что пародонтит - это многофакторное хроническое необратимое воспалительное заболевание, поражающее опорные структуры зубов, инициируемое и распространяющееся в результате сложного взаимодействия между периопатогенами и иммунной системой хозяина. Все начинается с микробной инфекции, за которой следует повреждение тканей пародонта, вызванное гиперактивностью лейкоцитов, цитокинов, эйкозаноидов и матриксных металлопротеиназ. На основании оценки их диагностических свойств были определены наиболее информативные лабораторные биомаркеры микрофлоры ротовой жидкости, протеолитические ферменты катепсин, эластаза, гаптоглобин, IL-1, IL-4, IL-6, IL-8, IL-10 и TNF-α при гингивите и хроническом пародонтите. Это позволяет оценить диагностическую эффективность этих маркеров у пациентов с заболеваниями пародонта и использовать их для ранней диагностики при неинвазивной диагностике.
Ключевые слова: Хронический диссеминированный пародонтит, гингивит, ротовая жидкость, пародонт, биомаркеры, цитокины.
Annotatsiya: Ma'lumki, periodontit-bu periopatogenlar va xo'jayin immun tizimining murakkab o'zaro ta'siri natijasida boshlangan multifaktorial surunkali qaytarilmas yallig'lanish kasalligi bo'lib, dastlab mikrob infektsiyasidan boshlanadi, so'ngra leykotsitlar, sitokinlar, eikosanoidlar va matritsali metalloproteinazalarning giperaktivligi natijasida periodontal to'qimalarning shikastlanishi kuzatiladi. Ularning diagnostik xususiyatlarini baholash asosida gingivit va surunkali tarqalgan parodontit bilan kasallangan bemorlarda og'iz suyuqligi mikroflorasining eng informatsion laboratoriya biomarkerlari, proteolitik fermentlar katepsin, elastaza, haptoglobin, Il-1, IL-4, IL-6, IL-8, Il-10 va TNF-α o'rganildi.Bu periodontal kasalliklarga chalingan bemorlarda ushbu markerlarning diagnostik samaradorligini baholash va ularni invaziv bo'lmagan diagnostikada erta tashxis qo'yish uchun ishlatish imkonini beradi.

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Kalit so‘zlar: Surunkali tarqalgan parodontit, gingivit, og‘iz suyuqligi, periodont, biomarkerlar, sitokinlar.

INTRODUCTION

Identification of biomarkers of periodontal diseases and early prognosis of the disease will improve the effectiveness of treatment for many patients suffering from periodontal diseases worldwide. The term periodontal disease is a term used to describe inflammatory and painful conditions of the periodontal structures. Prevalence increases with age, with 70.1 percent of adults aged 45 and older having periodontal disease. Beginning with gingivitis, an inflammation of the gum tissue, periodontitis can progress to an irreversible state, leading to the destruction of periodontal tissues, including periodontal pockets and alveolar bone. Continuous treatment is needed to regenerate the tissue around the lost tooth. The first problem of the periodontist in the treatment of periodontal diseases is timely and correct diagnosis, because the loss of connective tissue is a gradual and mostly irreversible process [1]. Therefore, it is very important to detect periodontal disease at an early stage.

Systematic reviews and meta-analyses have identified five promising oral fluid biomarkers as the basis for effective diagnostics for the early diagnosis of periodontal diseases. 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 [8].

ANALYSIS OF LITERATURE ON THE SUBJECT:

Currently, in most clinical settings , periodontitis is diagnosed almost entirely on the basis of various clinical indicators or measurements, among which are the depth of the probe, the degree of clinical attachment, bleeding in the probe, plaque index and radiographic changes or results. These traditional methods have several advantages and disadvantages. Advantages associated with these methods include ease of Use, economy, and relative invasiveness. However, the main disadvantage of these methods is that they are static diagnostic parameters that indicate the history of the disease, and not the current state of the disease. In recent years, in different regions of our country (Vavilova, t.P., Medvedev A.E., Vavilova, 2014., Pajariskaya, M.M. 2001., Gerasimova L.P., Kabirova M.F., Usmanova I.N., Gadiullin A.M. 2016., Elendo M.B., Lomiashvili L.M., Vasileva N.A. 2013., Emelyanov S.S. 2010., Jaludev S.E. 2018., Kubrushko T.V., Korobkin V.A., Milova E.V., Lunѐv M.A., Hein S.S. 2013., . Kostina I.N., Dregalkina A.A., Zaitseva L.N. 2018) studies since the late 1980s have focused on determining and describing salivary gland functions,oral fluid, functional and biochemical composition of salivary, gingvinal fluids, salivary-containing specific proteins, physical peculiarities of salivation, buffering, pH environment, viscosity. When studying the scientific work of scientists in our country, it turned out that even the change in the chemical content of saliva in different diseases, depending on different ages, is of great physiological importance.This study evaluated the diagnostic efficacy of these markers according to their degree of change to create a prognostic panel in patients with healthy, gingivitis, and chronically periodontitis [2].

RESEARCH METHODOLOGY

Researches were conducted in the educational laboratory of the medical and biological chemistry department of the Tashkent state dental institute of the Ministry of Health of Uzbekistan and the therapeutic dentistry department.

68 subjects consisting of somatically 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

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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%).

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 enzymes alpha-amylase, lactate dehydrogenase, alkaline phosphatase, ceruloplasmin, cathepsin, elastase using a colorimetric enzymatic method by "Hosptex" (Switzerland) biochemical analyzer. Set up three control tubes and three reaction tubes as described in section 3 and label these “4°C”, “Room Temperature” and “37°C”. After making the enzyme extract, incubate one tube on ice, at room temperature, and at 37 °C (in a preheated water bath). Allow reactions for 1 min and then terminate reactions by adding 500 µl sodium carbonate 1 M to each tube. Measure the absorbance at 420 nm for each tube as described in Section 3. Record the values by subtracting the control value from the response value in each case. Results are expressed in U/l.

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 an immunosorbent assay using from the company "Human" on the analyzer "Mindrey". They were determined by "sandwich" method using enzyme-linked immunosorbent assay using Cytokine test system.

Samples and standards were poured into microplate wells coated with antibodies to human IL-1B. Antibodies adsorbed to microplate wells capture IL-1, IL-4, IL-6, IL-8, IL-10 during incubation. Unbound antibodies present in the sample are removed during washing. Biotin-conjugated antibodies to human IL were then added. If IL-1B is present in the sample, the biotinylated antibodies will bind to the IL-1B captured by the antibodies. Excess biotinylated antibodies are removed by washing [6]. Horseradish peroxidase-conyugated streptavidin (streptavidin peroxidase) is added, which reacts specifically with biotinylated antibodies bound to IL-1B captured during the first incubation. Excess streptavidin was removed by peroxidase washing, and the substrate tetramethylbenzidine (TMB) was added to the wells. The color develops in proportion to the amount of IL in the sample. The enzymatic reaction is stopped by adding sulfuric acid. Then, the adsorption change was measured in a spectrophotometer at a wavelength of 450 nm.

All reagents were stored at 12 - 8°C before use. Biotinylated LL37 antibodies and streptavidin-peroxidase conjugate are stable for 1 month when stored at 2-8°C. A solution of polyclonal antibodies to IL-1B was prepared immediately before addition to the plate.

Tetramethylbenzidine (TMB) solution was prepared by mixing substrate and reagent in proportion. A 5:1 vial of lyophilized human IL-1B standard was diluted in 800 µl of buffer C and left for 15–20 min, then mixed well. A solution with a concentration of 4000 pg/ml was formed. A series of standards (400, 200, 100, 50, 25, 12.5, 6.3, and 0 pg/ml) was prepared by diluting the standard dissolved in polypropylene tubes [5].

ANALYSIS AND RESULTS

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The level of activity of proteolytic 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 catarrhal 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 [3].

At the next stage, as a result of objective examination of patients with chronic inflammatory processes of periodontal tissues, it was found that high values of KPU and UIG indices have a specific condition in the biochemical indicators of oral fluid.

Table 2

Biochemical indicators of oral fluid in patients with gingivitis and moderate chronic disseminated periodontitis

	Healthy group n =14	Gingivitis group n =26	Periodontitis group n =28
Lactate (mmol/l)	0,36±0,04	2,13±0,27*	3,35±0,29*
Lactate dehydrogenase (ed/l)	214,21±11,3	289,63±12,7*	421,1±23,47*
Alkaline phosphatase (ed/l)	13,78±0,94	16,47±1,43*	30,43±1,72*
Amylase (ed/l)	57,63±4,38	25,57±0,76*	20,29±1,78*
Ceruloplasmin mg/dl	9,83± 0,83	15,43±1,19*	18,74±1,69*

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

According to the results of the study presented in Table 2, the amount of lactate in the oral fluid of patients with gingivitis increased by 5.9 times compared to healthy people. A similar situation was observed in patients with chronic disseminated periodontitis. Thus, the amount of lactate in the oral fluid of patients with periodontitis was 9.3 times higher than the initial level of patients without periodontitis. It is known that lactate is formed in the process

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of anaerobic glycolysis, the main enzyme of this cycle is lactate dehydrogenase. The analysis of the results of the study showed that the activity of this enzyme increased in the examined patients, that is, with gingivitis, it was 289.63±12.72 ed / l, which is 35% higher than in healthy people. In chronic disseminated periodontitis of moderate severity, it is 2 times higher than the initial indicators of the healthy group. Thus, the changes associated with the main enzyme of anaerobic glycolysis - lactate dehydrogenase and the final product of this pathway - lactic acid, indicate a violation of microcirculation and increased hypoxia in periodontal tissues [4].

With inflammation of periodontal tissues, there is a change in the amount of inflammatory cytokines (IL-1, IL-4, IL-7, IL-18, TNF-α) in the oral fluid. IL-17 produced by Th17 cells can cause periodontal soft and hard tissue erosion during chronic P. gingivalis infections. Under conditions of chronic inflammation, neutrophils themselves may act protectively, leading to loss of IL-17-dependent bone resorption. Among the biomarkers used for the development and diagnosis of periodontal disease, cytokines in the inflammatory process associated with gingival complications have received the most attention. Cytokines detectable in oral fluid associated with inflammation include IL-1 beta (β), IL-6, IL-8, and TNF-α. These cytokines in oral fluid can be used as biomarkers to determine the severity of periodontal disease. 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).

Table 33

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*
FNO-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. This inflammatory cytokine, after attaching to special receptors on the surface of local tissues, promotes the production of fibronectin, which is synthesized by the endothelial cells that attract polymorphonuclear granulocytes and monocytes to the area of inflammation. IL-1, bound to fibroblast receptors, induces the synthesis of collagenase, which

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delays the formation of collagen and bone tissue and inhibits osteosynthesis. Also, IL-1 stimulates bone resorption.

Thus, the increase of inflammatory agents IL-1β, 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. The increase of IL-4 and IL-6 ensures the activation of humoral immunity, limiting the importance of cell-mediated immune responses. An increase in IL-4 is a characteristic sign of the intensity of humoral immune reactions. Thus, it can be concluded that the toxic effect of anti-inflammatory cytokines on periodontal tissue is related to their negative effect on tissue repair, especially the reduction of the normal process of resynthesis of connective tissue by fibroblasts, inhibition of osteosynthesis, and reduction of osteoformation potential [6].

According to the results of the study, the level of IL-8 in patients with periodontitis exceeds the initial level by an average of 8.1 times. IL-8 is known to be an inflammatory cytokine. It can also prolong the expression of adhesion molecules, neutrophil activation, and induce bone resorption. When the endothelial cells of the blood vessel wall are damaged, monocytes enter the blood vessels through the endothelial cells. Monocytes located in blood vessels are macrophages. Monocytes increase the synthesis of various inflammatory cytokines, tissue factors, growth factors and metalloproteases. Cytokines and growth factors released by macrophages can activate various immune responses, including T lymphocytes. Therefore, it is not surprising that there is interest in cytokines as laboratory indicators, in particular, to detect disease recurrence and to evaluate the effectiveness of therapy [7].

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. These included the following: oral microflora, proteolytic enzymes (cathepsin, elastase, ceruloplasmin, haptoglobin, IL-1, IL-6, IL-8, TNF-α, MMP-8). It was also shown that the tests with the highest diagnostic value have a positive result value for the development of an algorithm for laboratory monitoring of gingivitis and periodontitis.

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1-stage	2-stage	3-stage
• Microbiology • P. gingivalis 72-86% ↑ • Biochemistry Cathepsin 17.3-26.5 ncat/l Elastase 37.2-46.6 nkat/l	• Immunoenzyme • SCFA - 3.3-3.8 times ↓ • IL-1 - 1.6-2.5 times ↑ • IL-6 - 14.5-26 times ↑ • IL-10 - 13% - 35% ↓ • IL-8 - 3.2 -8.8 times↑	• Immunoenzyme MMP-8- 5.1-10 times ↑ ITP - increased - 40% and 3.6 times ↑

Laboratory monitoring algorithm for gingivitis and chronic periodontitis

CONCLUSIONS

The results obtained during the research have theoretical and practical significance. Informative content of oral fluid biomarkers for assessment of disease severity in patients with gingivitis and periodontitis was demonstrated.

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.

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