
Metalloproteinases and other enzymes, the production of immunoglobulins specific for microbial antigens and other immunocompetent cells carrying these receptors and induce them to chemotaxis, the secretion of pathogenic microflora through TLR, a continuous flow of signals is provided activating the macrophages interaction with TLR cells differs significantly from that of normal biofilm microflora [2]. With the predominance of monocytes / macrophages, fibroblasts via Toll-like receptors (TLR). The process of parodontopathogens lipopolysaccharides (LPS) capable of interacting with cells of damaged epithelium, neutrophilic granulocytes, macrophages / macrophages, fibroblasts via Toll-like receptors (TLR). The process of parodontopathogens interaction with TLR cells differs significantly from that of normal biofilm microflora [2]. With the predominance of lipopolysaccharides (LPS) capable of interacting with cells of damaged epithelium, neutrophilic granulocytes, monocytes / macrophages, fibroblasts via Toll-like receptors (TLR). The process of parodontopathogens interaction with TLR cells differs significantly from that of normal biofilm microflora [2]. With the predominance of lipopolysaccharides (LPS) capable of interacting with cells of damaged epithelium, neutrophilic granulocytes, monocytes / macrophages, fibroblasts via Toll-like receptors (TLR). The process of parodontopathogens interaction with TLR cells differs significantly from that of normal biofilm microflora [2].

Conclusion: Definite priorities of saliva as diagnostic medium are influenced by contemporary high technology innovations, namely enzyme-associated fluorescence technique, Western blot assays, polymerase chain reaction (PCR).

References:


Key words: saliva, diagnostics, caries, periodontal diseases, biochemical markers;

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USE OF INDICATORS OF OXIDATIVE STRESS AND HSP 70 PROTEIN AS MARKERS IN THE DIAGNOSTICS OF DENTAL DISEASES

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The most important triggering factor in the initiation of the pathological process is the formation of the dental plaque as a multilayered microbial biofilm with parodontopathogenic microorganisms. They include the causative agents of the chronic inflammatory process in the periodontal disease, such as Tannerella forsythensis, Porphyromonas gingivalis, Treponema denticola, and the inducer of acute aggressive inflammation of Actinobacillus actinomycetemcomitans. In these conditions, the resistance of microflora to various protective factors is determined by heat shock proteins HSP70 [1-3]. Paternal-recognition receptors (PRRs) perceive microbes with these new proteins as «strangers.» All these parodontopathogens are a source of lipopolysaccharides (LPS) capable of interacting with cells of damaged epithelium, neutrophilic granulocytes, monocytes / macrophages, fibroblasts via Toll-like receptors (TLR). The process of parodontopathogens interaction with TLR cells differs significantly from that of normal biofilm microflora [2]. With the predominance of pathogenic microflora through TLR, a continuous flow of signals is provided activating the macrophages and other immunocompetent cells carrying these receptors and induce them to chemotaxis, the secretion of pro-inflammatory cytokines (tumor necrosis factor a (TNFα), interleukins (IL) -1,6, -8, the release of matrix metalloproteinases and other enzymes, the production of immunoglobulins specific for microbial antigens...
produced with the involvement of chemokines of T- and B- lymphocytes [1, 3-5]. Release enzyme substances take part in the degradation of collagen and other extracellular matrix proteins, causing the lost contact between the periodontium and the bone. Quite large is the role of pro-inflammatory cytokines, which, in addition to induce inflammatory changes in the tissues, are able to activate osteoclasts and thus promote bone resorption of the alveolar processes. A complicated complex of molecules arises, including electrolytes and other small molecules, proteins, cytokines, antibodies, bacterial antibodies, enzymes, degradation products of connective and bone tissue in the gingival-cervical fluid [6, 7]. Due to the clinical and experimental works, and also, on the basis of the data obtained by us, concerning heat shock proteins, the most interesting is HSP70 as a stress protein, which is an important diagnostic and prognostic indicator of the severity of pathological processes. Of particular interest, for today, is the molecular regulation of HSP70 oxidative and nitrosating stresses, which are unchanged companions of inflammatory diseases of the oral cavity. In order to establish the direction and severity of antioxidant prooxidant processes in the oral cavity, in clinical studies, the determination of the concentration of the nitrosating stress marker - nitrotyrosine, is widely introduced. In connection with the above, the purpose of this work is to evaluate the role of the heat shock protein Hsp 70 and nitrotyrosine in the development of chronic periodontal diseases [8-9].

Materials and methods. 47 patients (25 men and 22 women) with chronic generalized periodontitis aged from 25 to 50 years were examined. The control group consisted of 15 residents of Zaporozhye city of the corresponding sex and age, who did not have somatic or dental pathology. When assessing dental status, the level of oral hygiene was determined using the simplified index OHI-S [Green, Vermillion, 1969], the state of the teeth according to the CFR index. As an integral need measure for the treatment of periodontal diseases, the CPITN index proposed by WHO (1978) was calculated. The severity of gingival inflammation was determined from the reversible PMA index, modified by C. Parma (1960), the severity of the destructive processes in the periodontium was detected according to the periodontal index Pl (A. Russel, 1956), the degree of bleeding gums was identified using the PBI bleeding index according to U.P. Saxer and M.R. Muhlemann (1975). In all the investigated patients, the oral liquid was obtained 10 minutes after rinsing the oral cavity with saline by spitting into plastic tubes. After centrifugation at 3000 rpm./min. 1,5 ml supernatant liquid was collected during 5 minutes, the resulting substrates were placed in eppendorfs and stored at - 20 ° C in the freezer until the time of the study. The HSP70 level in the oral fluid was determined by enzyme immunoassay (IEA) [Enzo Life Science, EKS - 715] nitrotyrosine concentration (ELISA Kit eHyct biotechnology b.v.), the result was expressed in ng / ml and nmol / g. All the patients received a set of basic medical measures.

Results. The study of dental status has revealed a low level of the oral hygiene in 38 (80.9%) patients. In all the patients, concomitant carious lesions of teeth with different severity degree, chronic generalized periodontitis of the 1st and the 2d degrees were diagnosed. In 31 (65.9%) patients the pathology of the occlusion was detected, 42 (89.4%) investigated patients had dentoalveolar anomalies, 23 (48.9%) had orthopedic constructions in the oral cavity. We studied the oral liquid of the patients with periodontitis (ChGP) by nitrotyrosine concentration (Ntz) (ELISA Kit eHyct biotechnology b.v.) and HSP 70 (Enzo Life Science, EKS - 715). Immunoenzymatic determinations of these markers have demonstrated a close relationship between the dynamics of their concentration in the oral fluid and the intensity of the pathological process. As shown in the 1st Figure, at a primary examination of the patients with periodontitis (ChGP) was marked a considerable increase of nitrotyrosine concentration nearly at 8 times in relation to the healthy persons (control group) on the background of a sharp decrease of the HSP 70 protein in oral fluid, responsible for the implementation of molecular mechanisms of protecting macromolecules from oxidative stress. Such a pathobiocchemical shift, in our opinion, indicates the development of oxidative and the disruption of the compensatory mechanisms of the cells. In conditions of oxidative stress significant development, HSP 70 is unable to perform its chaperone function and «correct» oxidatively damaged functionally active protein molecules, which in turn leads to an increase of the pathobiocchemical processes in the periodontitis. On the 10th day of the observation, after the treatment, a decrease in the nitrotyrosine content and a normalization of the HSP 70 proteins concentration was recorded, which occurred on the background of the overall clinical picture periodontitis improvement: good hygienic state of the oral cavity, arrest of the inflammatory process, normalization of the circulation and resumption of the gingival relief.

Conclusions. Thus, the conducted studies have demonstrated that the HSP 70 and Ntz have been able to utilize as biological markers in prognosis and screening efficacy for performed treatment in dentistry. This type of clinical diagnostic study is a minimally invasive and informative method. Further studies in this direction are promising and relevant in modern dentistry and clinical laboratory diagnostics. The technical improvement of the laboratory determination of biomarkers, its transfer to the level of quantitative testing is the most important task of dentistry, because it allows not only to make accurate and timely diagnosis of the disease, but also to predict its progression, complications and outcomes. From this point of view, modern methods of laboratory diagnostics and their capabilities deserve special attention.

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As a result of the conducted biochemical studies, it was proved an increase in the level of MMP-8 in oral fluid in patients with generalized periodontitis (0.4 ± 0.1 ng / ml under the I degree of severity, 0.7 ± 0.2 ng / ml – under the II degree against 0.1 ± 0.03 ng / ml of control, p < 0.05). At the same time, inflammation causes the destruction of the connective tissue of the periodontal complex, characterized by collagen and proteoglycan metabolism disorders, and, consequently, resorption of bone[4]. In this case, the development of inflammatory process in the periodontal tissues leads to increased secretion of proinflammatory cytokines such as interleukin-1α, -1β, -6, tumor necrosis factor-α. Neutrophils produce a large number of enzymes and inflammatory mediators. An increase in their concentration in saliva is a diagnostic sign of inflammatory processes in oral cavity. Therefore, in the search for diagnostic criteria, special attention should be paid to increasing the concentration of collagenases, which include matrix metalloproteinases. They should be considered key in describing the periodontal status, since type I collagen is in the vast majority of the periodontal tissues. Among them matrix metalloproteinase-8 (MMP-8) is the main one in periodontitis, because 90-95% of collagenolytic activity falls on it[5]. All of the above has allowed us to formulate the purpose of the investigation as the study of the level of MMP-8 in oral fluid in patients with generalized periodontitis in the dynamics of the treatment.

Materials and Methods. 30 patients aged 37 - 45 years were included into the study. 15 of patients were diagnosed generalized periodontitis of the I degree of severity, 15 – the II degree of severity. As a control, indicators from a group of 8 persons with intact periodontal tissues, selected similarly for the gender and age characteristics of the observation group, were used. To assess the periodontal condition, a traditional clinical examination, supplemented by the results of an X-ray study, was used. All patients with generalized periodontitis received comprehensive treatment[3]. The content of MMP-8 in the oral fluid was studied using the immune enzyme method (BCM Diagnostics, DMP800, Total MMP8). The research was conducted before and immediately after treatment. The data of the conducted clinical and laboratory studies were to be processed using the eSTATISTICA® for Windows 6.0i [StatSoft Inc., № AXXR712D833214FANS5].

Results. As a result of the conducted biochemical studies, it was proved an increase in the level of MMP-8 in the oral fluid in patients with generalized periodontitis (0.4 ± 0.1 ng / ml under the I degree of severity, 0.7 ± 0.2 ng / ml – under the II degree against 0.1 ± 0.03 ng / ml of control, p < 0.05). At the same time, after a complex treatment, the level of this indicator decreased to 0.2 ± 0.07 ng / ml under the I degree of severity and to 0.5 ± 0.1 ng / ml – under the II degree (p < 0.05). However, it should be noted that the results obtained after the course of treatment outweighed the control (p > 0.05), which, in our opinion, suggests only about inhibition of the pathological process, rather than its complete elimination. Thus, according to the results of the studies, we found that the level of MMP-8 in the oral fluid...