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ANALYSIS OF MARKERS OF BONE TISSUE METABOLISM IN PATIENTS WITH GENERALIZED PERIODONTITIS

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The search for effective methods of early diagnosis and rational treatment of inflammatory and dystrophic diseases of periodontal tissues is determined by the significant prevalence of this pathology among the population, untimely diagnosis, rapid development of the disease, and the need for long-term, complex treatment [1].

Generalized periodontitis is a complex multifactorial disease that presumes a variety of inflammatory reactions and affects periodontal tissues, ultimately causing tooth loss [2]. Periodontitis can develop independently or result from somatic diseases. Numerous scientific studies have proved the relationship between chronic generalized periodontitis and systemic diseases. The degree of correlation between the level of biological markers in the oral fluid and indicators of periodontium condition in patients with periodontitis against the background of endocrine and cardiovascular pathology is controversial and requires careful study [3, 4].

It has been proved that one of the leading etiological factors in the development of inflammatory periodontal diseases is the microbial one, namely, qualitative and quantitative changes in the microflora of the oral cavity, as well as the activation of periodontal pathogenic microorganisms [5]. From the point of view of pathogenesis, periodontitis destroys the connective tissue of the periodontal complex against the background of impaired collagen metabolism and proteoglycans and results in bone resorption and the formation of periodontal pockets [6].

There are classical clinical, instrumental and radiological methods for diagnosing the condition of periodontal tissues, including measuring the depth of periodontal pockets, determining gum bleeding, the level of oral hygiene and the degree of bone tissue destruction [7]. The use of standard methods for the diagnosis of generalized periodontitis does not present difficulties in case of pronounced clinical symptoms. It is very difficult to identify the prerequisites for pathological process development, its transition from the inflammatory to the destructive stage, which is characterized by damage to the bone tissue of the alveolar ridge and requires the introduction of additional diagnostic methods. In this regard, non-invasive diagnostic methods, which include a biochemical examination of saliva and gingival fluid, are promising [8, 9].

In modern medicine, laboratory diagnostic methods with the use of biological markers are widely employed. The study of biological markers level allows to assess the risk and identify the disease, determine its progression, as well as to evaluate the results of the treatment and the effectiveness of preventive procedures [10].

At present, no algorithms for the use of biological markers for dental diseases diagnosis have been elaborated. Given the complex and not fully understood pathogenesis of periodontal diseases, when selecting diagnostic markers, it is necessary to take into account their prognostic significance for a specific disease [11].

From the point of view of pathogenesis, periodontitis destroys the connective tissue of the periodontal complex, which is characterized by the formation of an inflammatory focus, impaired collagen metabolism, and bone resorption with the formation of periodontal pockets [12]. In the focus of inflammation immune cells accumulate, which secrete cytokines and proteases, thus promoting the release of matrix metalloproteinases, which are a key link in bone matrix resorption [13]. Gingival sulcus epithelial cells, gingival and periodontal ligament fibroblasts, monocytes,

macrophages, plasma cells are sources of MMP-8. This metalloproteinase plays an important role in the destruction of periodontal tissues. Sources of MMP-9 are alveolar macrophages and neutrophils. Clinical studies have shown that in osteoporosis, MMP-9 has a significant influence on reducing mineral bone density [14].

In recent years, more and more attention has been paid to estimating vitamin D concentration in the oral cavity in order to determine the scope of treatment measures and draft a plan for preventive procedures. Apart from playing an important role in calcium-phosphorus homeostasis, vitamin D is proved to play a role in cell differentiation, cell growth inhibition, and immunomodulation. This property can explain the mechanism of vitamin D action in bone resorption. As a result of recent research, it has been proved that vitamin D not only coordinates bone metabolism but is also able to function as an anti-inflammatory agent and stimulate the production of antimicrobial peptides [15].

According to scientific data, less than 1% of vitamin D exists in the unbound form [16]. Vitamin D-binding protein (VDBP) is a polyfunctional glycoprotein, whose main role is to transport vitamin D and its metabolites into the cell. At this, albumin-bound and free vitamin D is distinguished under the general term "bioavailable" [17]. When bound, vitamin D and VDBP bind to specific receptors and enter cells where vitamin D is activated and released. It is highlighted that VDBP has a greater association with mineral metabolism compared to total vitamin D level [18].

Based on the above, MMP-8, MMP-9, vitamin D and VDBP play an important role in the pathogenesis of inflammatory-dystrophic diseases of periodontal tissues. The study of bone metabolism markers concentration (MMP-8, MMP-9) and the metabolism of vitamin D (vitamin D and VDBP) has practical value, which is based on the creation of therapeutic and diagnostic methods in the aspect of complex treatment of periodontal diseases.

The study aimed to determine the concentration of markers of bone metabolism (MMP-8, MMP-9) and vitamin D metabolism (vitamin D and VDBP) in the oral fluid in patients with generalized periodontitis of initial, I and II degrees in the course of treatment.

Materials and methods. The study was conducted in the Scientific and Educational Medical Centre "ZSMU University Clinic". A comprehensive examination and treatment of 110 patients, equally men and women, aged 18 to 56 years, was performed. The control group comprised 20 healthy patients, the main group consisted of 90 patients with periodontitis: initial degree (16), I (35) and II (39) degrees. The diagnosis was made grounding on clinical and radiological signs according to the classification of periodontal diseases by Danilevsky [19]. Particular attention was paid to such clinical parameters as measuring the depth of periodontal pockets, gums bleeding during probing, the degree of gingival inflammation and the level of oral hygiene. Clinical and laboratory, as well as X-ray examination, was performed to all patients. They were treated according to the Ministry of Health of Ukraine protocol for the provision of medical care in the specialty "Therapeutic dentistry" dated 2007. Patients with generalized periodontitis received complex treatment according to the generally accepted protocol [20]. Before the medical correction, professional oral hygiene was performed, i.e. the ultrasound removal of supra and subgingival dental plaque. Dental therapy included therapeutic, surgical and orthopedic treatment. The study was performed before the start of treatment for generalized periodontitis, after week 2 and 12 consequently.

The study was performed with the use of enzyme-linked immunosorbent assay (ELISA) in unstimulated oral fluid, the level of biomarkers – vitamin D, VDBP, MMP-8 and MMP-9 – was determined using a set of reagents «Vitamin D - Diasource, KAP 1971», «Immundiagnostik, K2314RF» (Germany) and (Elabscience®, Human MMP-8, MMP-9 ELISA Kit), following the manufacturer's instructions "LabLine-90" (Austria).

The results were statistically processed with Statistica 8.0 software (Statsoft Inc., USA). The hypothesis of the normalness of indicators of the studied parameters was checked with Shapiro-Wilk test. Statistical characteristics in different groups were compared with the use of multiple comparison according to Kruskal-Wallis ANOVA and of pairwise comparison according to Whitney-Mann U test.

Results. Based on the study of the oral fluid aimed at determining the concentration of markers of bone metabolism (MMP-8, MMP-9) and vitamin D metabolism (vitamin D and VDBP) in patients with generalized periodontitis, data, reflecting the course of the pathological process in the oral cavity, were obtained.

The indicator of the MMP-8 content in the oral fluid was increasing according to the severity of the pathological process in the periodontium. Its difference with the values of the control group was significant in patients of all observation groups. The obtained results demonstrated that the concentration of MMP-9 in the oral fluid in patients with generalized periodontitis was also increasing, but statistically significant differences in indicators for MMP-9 in degree II generalized periodontitis were less pronounced.

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Thus, the analysis of metalloproteinases content proved the indicativeness of MMP-8 and MMP-9 content in the oral fluid according to the activity of the pathological process in the periodontium bone tissue. Moreover, more significant changes in the concentration of MMP-8were observed. Simultaneously, changes in this indicator, contrary to MMP-9, were observed at the initial, I and II degrees (Table. 1).

Vitamin D content in the oral fluid decreases as the severity of the pathological process in the periodontal tissues increases. Simultaneously, a noticeable decrease in this indicator was recorded already at the initial degree. The indicators of all groups were statistically significantly different from the control group.

Vitamin D-binding protein (VDBP) values were decreasing sharply against the background of the above-described vitamin D deficiency. This trend was observed in all study groups except the initial degree group. Thus, the progression of the pathological process in the periodontal tissues is explained by a decrease in the transport globulin level in the oral fluid; it triggers a cascade of inflammatory reactions and forms local immunosuppression. The decrease in the VDBP level correlated with the severity of periodontitis, thus the decrease in VDBP concentration in the oral fluid is comparable with the aggravation of periodontitis degree (Table 1).

Table 1. Indicators of the level of bone metabolism markers in the oral fluid prior to treatment

Groups research	Indicators			
	MMP-8 (mkg/l)	MMP-9 (mkg/l)	Vitamin D (ng/ml)	VDBP (ng/ml)
Control group	0,63 [0,51 0,7]	13,34 [11,2 15,7]	34,28 [30,4 36,2]	85,71 [82,9 88,3]
First group	0,71 [0,61 0,84]	15,48 [14,1 17,2]	25,64 [22,7 26,9]	83,85 [79,4 88,6]
Second group	0,84 [0,68 0,97]	18,02 [16,7 20,4]	16,85 [14,7 18,8]	62,79 [58,4 65,6]
Third group	1,17 [0,94 0,41]	21,74 [16,2 24,4]	10,84 [7,6 12,4]	39,03 [35,8 42,5]

After the treatment, MMP-9 indices were decreasing rather rapidly, the fastest tempo was observed in the group of patients with II degree generalized periodontitis, but did not reach the indices of the control group after treatment. On the contrary, MMP-8 concentration in the oral fluid returned to normal in accordance with the stages of treatment, and the final results almost corresponded to the values of the control group. The established pattern is pronounced in all observation groups (Figure 2).

After the treatment, vitamin D concentration was increasing gradually, the fastest tempo was observed in the group of patients with initial and degree I generalized periodontitis, but it did not reach the post-treatment values of the control group in comparison with the pre-treatment concentration. A similar trend was observed with the VDBP concentration. The indicators of this marker in the oral fluid normalized in accordance with the stages of treatment and the final results almost corresponded to the values of the control group (Table 2).

Table 2 Indicators of bone metabolism markers level in the oral fluid in the treatment dynamics

Groups research		Indicators			
		MMP-8 (mkg/l)	MMP-9 (mkg/l)	Vitamin D (ng/ml)	VDBP (ng/ml)
Control group		0,63 [0,51 0,7]	13,34 [11,2 15,7]	34,28 [30,4 36,2]	85,71 [82,9 88,3]
First group	Before treatment	0,71 [0,61 0,84]	15,48 [14,1 17,2]	25,64 [22,7 26,9]	83,85 [79,4; 88,6]
	2 weeks	0,69 [0,5 0,81]	15,11 [12,8 18,4]	27,62 [24 30,2]	76,32 [72,6 80,5]
	12 weeks	0,63 [0,47 0,74]	13,98 [11,4 15,2]	33,96 [31,2 34,8]	84,13 [81,1 87,6]
Second group	Before treatment	0,84 [0,68 0,97]	18,02 [16,7 20,4]	16,85 [14,7 18,8]	62,79 [58,4 65,6]
	2 weeks	0,74 [0,51 0,92]	17,27 [15,6 19,3]	22,48 [19,3 24,4]	78,21 [72,8 81,6]
	12 weeks	0,65 [0,41 0,72]	16,65 [13,4 19,8]	32,53 [29,2 34,7]	82,58 [78,5 84,1]
Third group	Before treatment	1,17 [0,94 0,41]	21,74 [16,2 24,4]	10,84 [7,6 12,4]	39,03 [35,8 42,5]
	2 weeks	0,78 [0,61 0,8]	20,33 [17,8 24,1]	14,91 [12,4 16,5]	44,67 [42,2 47,6]
	12 weeks	0,68 [0,57 0,71]	17,12 [15,7 19,8]	31,82 [27,7 33,6]	73,34 [70,2 76,5]

Conclusions:

1. The indicator MMP-9 concentration in the oral fluid in patients with generalized periodontitis characterizes the severity of the inflammatory and destructive process in the bone tissue but is not indicative for evaluating the effectiveness of treatment.

2. The concentration of MMP-8, vitamin D and VDBP in patients with generalized periodontitis increases in the oral fluid in accordance with the degree of the disease, and decreases during treatment.

3. Analysis of the level of bone destruction markers in patients with generalized periodontitis presumes to determine the key pathogenic mechanism of bone resorption at the initial stage of the disease.

4. The results obtained explain the expediency of using the method of enzyme-linked immunosorbent assay of molecular biological markers of the oral fluid as a minimally invasive method that allows to determine the initial state of periodontal tissues and prevent the risk of complications.

5. Determination of indicators of biological markers of the oral cavity is a promising diagnostic method that enables to choose the right treatment tactics, prescribe the correct pharmacotherapy and draft a plan of preventive measures.

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