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DYNAMICS OF THE CONTENT OF MARKERS OF BONE TISSUE METABOLISM IN THE ORAL LIQUID OF PATIENTS WITH INJURY OF THE JAW AND FACIAL AREA.

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The introduction of minimally invasive, specific and informative methods of diagnosis and monitoring the effectiveness of treatment in modern dental practice is an urgent task of medical science. One of such methods is the study of specific molecules in biological fluids, which are markers of various pathological conditions. Specificity and their informativeness are firstly determined by their participation in the pathogenesis of diseases; secondly – by their appearance during the pathobiochemical reactions [1, 2].

It is widely known, that inflammation and oxidative stress are among the main links in the pathogenesis of many diseases, including diseases of the oral cavity. This fact determines the great scientific interest of many researchers in this issue. Free radicals formed during oxidative stress have a direct cytotoxic effect and damage functionally active protein molecules and nucleic acids, cell organelles and ultimately lead to damage to all tissue and systemic inflammation. The formation of reactive oxygen species and tumor necrosis factor (TNF) can potentially affect oral healing, the response to bacterial attack, and directly affect the state of bone fibroblasts and osteoblasts by reducing the expression activity of genes encoding collagen, or indirectly through apoptosis or necrosis. Proinflammatory mediators (cytokines and chemokines), ROS, prostagalandins and cysteine proteases stimulate the release of MMP8 from the matrix and in turn activate epithelial cells [3-5]. Activated cells synthesize proinflammatory cytokines IL-1β, IL-6 and TNF-a, and collagen degradation occurs. It was found, that the collagenolytic process is mediated, primarily MMP. In addition, each form of MMP damages a certain type of substrate. Thus, today there are 28 forms of MMP, of which 24 are found in humans and are divided into 6 subtypes: collagenases, gelatinases, stromelysins, matrilysins; MMP of membrane type and others. MMP8 has been shown to play an important role in bone and connective tissue damage and to activate other MMPs - MMP-2, MMP-13 and MMP-14 [6, 7]. In addition, studies by Baron R., Kneissel M., 2013, Wei J., Karsenty G., 2015 showed, that one of the markers of restoring bone integrity of bone metabolism is osteocalcin, which is a low molecular weight short osteoblast-specific non-collagen protein, that produced and secreted by osteoblasts and osteocytes [8, 9]. In addition, an important marker of bone remodeling is fibroblast growth factor, which is a protein with molecular mass 32 kDa and a length of 251 amino acid residue, which is proteolytically cleaved between residues of arginine 179 and serine 180 to form N-terminal and C-terminal fragments. FGF23 is mainly secreted by osteocytes, regulates vitamin D synthesis and calcium homeostasis (phosphatonin). Fibroblast growth factors play a key role in the processes of proliferation and differentiation of a wide range of cells and tissues [10].

Taking into account the above-mentioned, the introduction of biological markers in the diagnosis and screening of the effectiveness of treatment of patients with traumatic injuries of the maxillofacial area is an urgent task of modern dentistry and clinical laboratory diagnosis. The introduction of biological markers in practical dentistry is important for the development of integrated, informative criteria for assessing the condition of the oral cavity, which are based not only on instrumental and radiological, but also on laboratory parameters.

The aim of the study was to investigate the concentration of MMP8, osteocalcin, FGF23 in the oral fluid of patients with traumatic injury of the maxillofacial area and to evaluate the effectiveness of autoplasma treatment by the dynamics of their changes.

Materials and methods. The study was conducted on 60 patients with injuries of the maxillofacial area, who were treated at the MI "City Clinical Hospital of Emergency and Ambulance" in Zaporizhzhia. In order to evaluate the effectiveness of treatment of patients with lesions of the maxillofacial area, we performed an enzyme-linked immunosorbent assay of oral fluid MMP8, osteocalcin and FGF23 for 1 day; 1 and 3 months after surgery. Patients were divided into groups:

Group 1 - patients with damage to the maxillofacial area, 1 day after surgery;

Group 2 - patients with damage to the maxillofacial area, 1 day after surgery on the background of treatment with autoplasma;

Group 3 - patients with damage to the maxillofacial area, 1 month after surgery;

Group 4 - patients with damage to the maxillofacial area, 1 month after surgery on the background of treatment with autoplasma;

Group 5 - patients with damage to the maxillofacial area, 3 months after surgery;

Group 6 - patients with damage to the maxillofacial area, 3 months after surgery on the background of autoplasma treatment.

In order to establish the norm for experimental markers, we selected somatically and dentistically healthy young people (n = 50) - (group 7).

For enzyme-linked immunosorbent assays, oral fluid was taken on an empty stomach in the morning by spitting into a sterile glass tube. Biomass was centrifuged and stored at -800C, MMP8 Matrix Metalloproteinase-8 (Human MMP8, ELISA kit Elabscience), FGF 23 (Human FGF23, ELISA kit Elabscience) and osteocalcin (Osteocalicin) (Osteocaliccin ELISA, Immunodiagnostic Systems) were determined in the test samples. Detection of markers was performed on the enzyme-linked immunosorbent assay ImmunoChem-2100 (USA). The analysis was performed by adding a colorimetric reagent, the resulting signal was measured spectrophotometrically at 450 nm. The concentration of the experimental parameters was registered in ng / ml [11].

Therefore, in order to verify the results of the study, which was implemented by traditional determination of arithmetic mean values and deviations for each of the quantitative indicators M (s), standardized error of the average M m, Student's criterion for quantitative indicators, the universal value of statistical probability p. The hypothesis about the normality of the distribution of the studied indicators was tested using the Shapiro-Wilk test. To compare the statistical characteristics in different groups, multiple comparison was used according to the one-way analysis of variance by Kruskal-Wallis (Kruskal-Wallis ANOVA), with pairwise comparison according to Whitney-Mann criterion(Whitney-Mann U test). Statistical processing of the study results was performed using the programs "SPSS 15.0" and "Excel" from the package "Microsoft Office 2003".

Results. Studies have shown, that against the background of damage to the maxillofacial area for 1 day after surgery there was a significant increase in the concentration of all experimental parameters in patients of both 1 and 2 groups, compared with the group of dentistically healthy patients (group 7, table 1). Such dynamics of changes is typical, and was due to general bone damage, cell destruction processes and generalized release of a large number of markes of bone metabolism. In our opinion, during this period of time, osteocalcin together with FGF23 play only the role of markers of cell damage, i.e. their increase is not associated with protective function against bone tissue.

After 1 month of follow-up, patients in group 3 had a gradual decrease in osteocalcin and FGF23 in comparison with patients in group 1 (82% and 51%, respectively). At the same time, the decrease in these indicators in patients of group

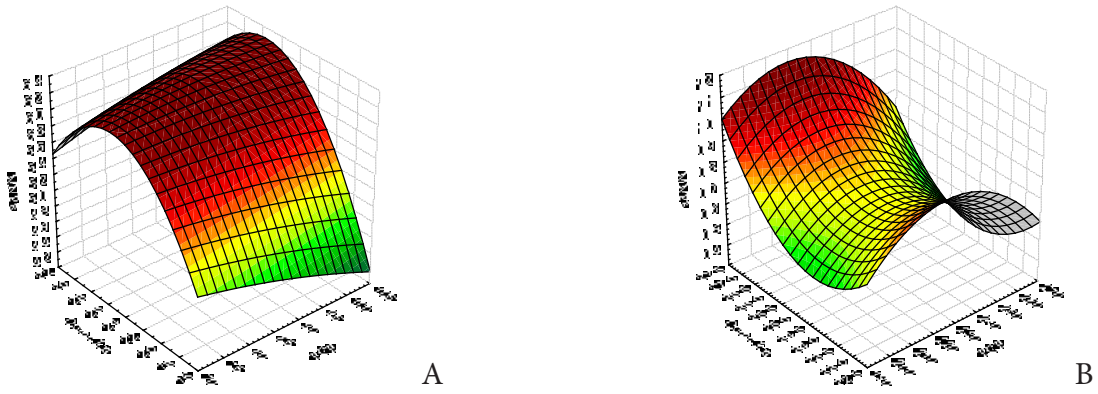
4 was less slow and amounted to 62% and 16%, respectively. A significant decrease of more than 80% in the concentration of MMP8 in this category of patients was noted, while in patients of group 3 the decrease in the content of this marker was only 10%. In addition, the indicators of markers of bone regeneration in patients of group 4 were unidirectional with the pathological process, and reflected a more rapid healing of the wound surface with the improvement of the biochemical picture of the oral fluid. At 3 months, a decrease in the concentration of MMP8 in the oral fluid was registered, and the dynamics of changes in patients of group 6 was more significant than in patients of group 5. The increase in FGF23 concentration was directly correlated with an increase in osteocalcin and inversely with the content of MMP8 (table 1).

Table 1. The content of MMP8, osteocalcin, FGF23 in the oral fluid of patients with damage to the maxillofacial area of different observation groups

Group/ indicator	MMP8, ng/ml	Osteocalcin, ng/ml	FGF23, ng/ml
Group 1	82,0 [8,5 8,7]	82,0 [80,8 86,1]	233,0 [200,0 265,5]
Group 2	7,9 [7,8 8,6]	92,6 [[91,1 95,8]	236,2 [211,7 258,7]
Group 3	7,7 [7,0 7,9]	14,9 [13,9 15,0]	113,4 [100,3 124,7]
Group 4	1,7 [1,5 1,6]	31,3 [30,1 32]	195,6 [185,4 200,1]
Group 5	5,35 [5,1 5,4]	18,6 [18,0 19,3]	92,1 [85,7 97,7]
Group 6	0,77 [0,7 0,8]	34,8 [33,7 36,5]	231,7 [211,4 241,7]
Group 7	0,41 [0,38 0,44]	27,5 [26,4 28,1]	38,2 [34,1 41,5]

Analysis of the graphical image comparing the obtained levels of MMP8, osteocalcin and FGF using 3D XYZ graph revealed the following patterns. The highest levels of MMP8 were found in group 5, i.e. with the lowest levels of osteocalcin and FGF (fig. 1A). While the lowest values of MMP8 were found in the group with the highest levels of osteocalcin and FGF - group 6 (fig. 1B).

It is important to note, that established biochemical parameters of oral fluid of patients were unidirectional with their clinical picture.



A B

Figure 1. 3D graphs of the surface effect of the degree of accumulation in the oral fluid FGF23 on the content of osteocalcin and MMP8 in patients with trauma with damage to the maxillofacial area for 3 months after surgery with autoplasma (B) and without (A).

The analysis of the studied indicators showed, that against the background of damage to the maxillofacial area, the introduction of autoplasma to patients led to a decrease in markers of bone destruction in parallel with increasing concentrations of markers of osteosynthesis and bone regeneration. In our opinion, this is primarily due to the presence in the autoplasma enriched in platelets, autogenous growth factors - growth factors of platelets, fibroblasts, chemokines, arachidonic acid, fibrinogen, fibrin [7, 9, 10]. They are deposited in granules, lysosomes and released at the site of damage by exocytosis and after activation of platelets by thrombin and calcium chloride.

Thus, enzyme-linked immunosorbent assays have established molecular biochemical changes in the oral fluid of patients with traumatic injuries of the maxillofacial area. The dynamics of the content of bone markers was different in patients treated with platelet-rich autoplasma and without, and corresponded to the severity of the pathological process. Thus, it was found that on the background of treatment decreased the increase in MMP8 on the background of increased osteocalcin and FGF23, especially for a 3 month after surgery. This effect, in our opinion, is due to the presence in the autoplasm of various growth factors and regulatory proteins, that can limit the development of oxidative stress, reduce the concentration of cytokines and modulate the processes of proliferation and differentiation of osteoclasts. In addition, by increasing the content of FFGF23 on the principle of feedback, decreased the concentration of the marker of bone destruction - MMP8. Studies of the last decade consider MMP8 and osteocalcin as integral biochemical indicators of functional bone metabolism in the oral fluid of dental patients. Based on our own research, these markers should be considered as markers for diagnosis, assessment of the severity of the course and monitoring the effectiveness of treatment for traumatic injuries of the maxillofacial area.

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