



**Analysis of causative factors and cytokine status in traumatic injuries
of the lower jaw and complications thereof**

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lower jaw and complications thereof**

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ABSTRACT

This paper presents the degree of incidence, etiology, traumatic injuries structure, and assessment of local level of TNF α , IFN γ and IL-10 in patients with chronic posttraumatic osteomyelitis and simple jaw fractures. Among the causes for fractures, predominance of civilian trauma is revealed (82.5%) provided that the majority of injured (64.7%) suffered the injury under the influence of alcohol. Main factors aiding occurrence of pyoinflammatory complications in patients with fractures of the lower jaw are as follows: late request for quality medical assistance by the injured person; poor-quality of immobilization; presence of a tooth in the fracture cleft, and comorbidity. The research demonstrates that immunological indicators of mixed saliva reflect the dynamics of a local inflammation. It is found that the level of TNF α , IL-10 may be additional immunological markers for development of posttraumatic osteomyelitis of the lower jaw.

Key words: traumatism, cytokines, osteomyelitis, mixed saliva.



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1. INTRODUCTION

According to the data of various investigations in a general structure of maxillofacial area disease incidence, traumas take one of the leading places amounting up to 90% [1; 10]. One of the most serious complications is the posttraumatic osteomyelitis.

According to maxillofacial surgery clinics, incidence thereof reaches 30% [1; 2; 11].

The following aids to traumatic osteomyelitis development: degree and severity of anatomic injuries; infection contamination of bone tissue due to breakage of mucous coat of alveolar bone; presence of teeth (roots) in a fracture cleft; regional circulation failure and trophism of tissues in the area of injury; late visit of injured person to a medical treatment institution; untimely and ineffective immobilization of jaw fragments, and inadequate medication [1; 9].

In recent years, the researchers pay attention in pathogenesis of chronic osteomyelitis to investigation of cytokine system state, various failures of which may be the diagnostic and predictive markers in a number of diseases [3; 12]. TNF α regulates immunoinflammatory reaction in traumas or infections being the main stimulant for neutrophils and endothelial cells, for interaction thereof and further transfer of leucocytes, increase of quantity of fibroblasts and endothelium in a wound repair [3; 8]. In addition, investigation of the role of interferon gamma (IFN γ), one of understudied proinflammatory cytokines in maxillofacial area diseases, is of particular interest. It is produced by T-lymphocytes and natural killers (NK). It stimulates the activity of T- and B-lymphocytes, macrophages, neutrophils, and NK. In addition, IFN γ regulates apoptosis of a number of normal and some infected and transformed cells [3; 13]. However, increased IFN γ concentration in pyoinflammatory diseases not always correlates with fast and full infection focus elimination [3; 8; 13]. Anti-inflammatory cytokines such as interleukine-10 (IL-10) are no less important in chronic inflammation development. IL-10 stimulates proliferation of B-lymphocytes, thymocytes and mast cells. It inhibits the synthesis of IFN γ antigen-presenting function of macrophages and dendritic cells, reflects the activity of regulatory T-cells [5], by virtue whereof IL-10 acts as essential inhibitor of the cell immunity [2; 3; 8; 12].



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The research was aimed at investigation of matters of incidence, etiology, structure of traumatic injuries, and assessment of local level of TNF α , IFN γ and IL-10 in patients with chronic posttraumatic osteomyelitis of the lower jaw and simple jaw fractures.

2. Materials and methods

91 patients became the investigation subject being treated stationary at the maxillofacial surgery unit of the State Budgetary Healthcare Institution “Regional Clinical Hospital No. 2”, Vladivostok, from October, 2015 to August, 2016. The patients were divided into 3 groups based on a post-event analysis. The I group included 30 patients (25 men, 5 women; average age - 43 years [33;52]) with chronic posttraumatic osteomyelitis of the lower jaw. The II group included 35 patients (28 men, 7 women; average age - 39 years [28;51]) with chronic posttraumatic osteomyelitis of the lower jaw in the exacerbation phase. The III group included 26 injured persons with simple jaw fractures (20 men, 6 women; average age - 33 years [26;43]). Control group consisted of 20 healthy volunteers comparable to patients in respect to gender and age. Clinical and anamnestic, radiographic, and laboratory data were analyzed (Zareinejad et al., 2014). Cytokines level determination was carried out using specific reagents provided by R&D Diagnostics Inc. (USA) by the sandwich-type method of enzyme-linked immunosorbent assay. Sampling 5 ml of mixed saliva was made on the first day of hospital admission and on the 10th day of treatment. Recording of the results was performed with the aid of immunoassay analyzer Multiscan (Finland). Calculation of cytokines quantity was performed by means of calibration curve generation using the computer program. The quantity was expressed in pg/ml in the form of median (Me), 25 % and 75 % quartiles (Q25; Q75), minimum (min) and maximum (max) value (María Paredes Chacin et al., 2017). Statistical significance level at which null hypotheses deviated was less than 0.05. In order to reveal the sensitivity and specificity of alteration of parameters chosen, linear regression method was used with generation of ROC-curves in MedCalc program.

3. Results and discussion



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The quantity of injured persons with traumatic injuries of the lower jaw during the investigation amounted to 39% of total quantity of indoor patients. The patients were distributed by gender; among them men prevailed (76 people; 86.36%); women amounted to 12 people (16.64%). Average age of men was 37.26 [27;52] years; women – 35.5 [26;41] years. The quantity of patients residing in the city was 59%; residents of other cities - 41%. Among the causes for fractures, predominance of civilian trauma is revealed (82.5%) provided that the majority of injured (64.7%) suffered the injury under the influence of alcohol. It should be noted that persons abusing alcohol are at high risk of development of pyoinflammatory complications of maxillofacial area. This state of patients is characterized by decreased phagocytic activity of macrophages, inhibition of complement, bactericidal and lysozyme activity [2]. Terms of visiting the unit by the patients were as follows: up to three days after getting injured – 53.21%, after three days – 32.53%, complications in occurrence revealed – 14.26%. Fracture of the lower jaw was observed more often in the area of angle (48.5%) and body (32.0%) thereof.

Time duration of chronic posttraumatic osteomyelitis of the lower jaw was from 2 months to 1 year. 21 patients treated stationary with regard to the disease for the first time; relapse took place in the rest 16 patients, 6 patients of which suffered from several relapses. Main factors for posttraumatic osteomyelitis development were unsatisfactory fixation of fragments (41.6%), inadequate therapy (30.8%), and late request for medical aid in respect of lower jaw fracture (27.6%).

Immobilization of fragments on outpatient basis was carried for 4 patients only. At that, dental splints were fixed wrong requiring repeated immobilization. Analysis of errors in carrying out of immobilization of the lower jaw with curved wire splints allowed detecting faulty common factors – the use of “shortened” splints (within the premolars). Such splints are not capable to provide for adequate fixation of fragments and immobilization of the lower jaw especially in location of the fracture cleft in the area of molar teeth. The majority of patients (84%) were directed to a hospital even without temporary immobilization.



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In survey patients with chronic posttraumatic osteomyelitis of the lower jaw, the fractures were located within the dental arcade; however, the teeth were removed from the fracture line in 20% of patients only. Meanwhile, the majority (85%) of patients needed the oral cavity sanitation. Abnormal focus location was various (Foroughi and Esfahani, 2012). According to our monitoring, posttraumatic osteomyelitis appeared more often in the area of the lower jaw angle – 48.5%. The second place by incidence of injuries was occupied by the body thereof – 32%, and the third – by genial area (19.5%). The main part of patients with complicated fractures of jaw was admitted to hospital in the exacerbation phase of the chronic process.

Table 1. Level of pro- and anti-inflammatory cytokines of mixed saliva of patients with posttraumatic osteomyelitis of lower jaw

Indicators	Chronic posttraumatic osteomyelitis of the lower jaw (I) n=30 Me (Q25; Q75)		Chronic posttraumatic osteomyelitis of the lower jaw in the exacerbation phase (II) n=35 Me (Q25; Q75)		Simple fractures of the lower jaw (III) n=26 Me (Q25; Q75)		Control group n=50 Me (Q25; Q75)
	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	
TNFα (pg/ml)	0,69 (0,56;6,96) *** p ₁₋₂ <0,01	2,38 (1,96;7,22) ## p ₁₋₂ <0,01	6,93 (5,18;9,5) *** p ₂₋₃ >0,05	0,87 (0,57;4,90) *** ### p ₂₋₃ <0,01	5,01 (4,75;10,05) *** p ₁₋₃ <0,01	3,11 (2,65;4,95) ## p ₁₋₃ >0,05	2,25 (1,41;3,42)
IFNγ (pg/ml)	12,88 (10,19;15,26) p ₁₋₂ >0,05	11,67 (11,22;13,01) p ₁₋₂ <0,01	11,77 (10,02;13,88) p ₂₋₃ >0,05	8,14 (6,76;9,59) * ## p ₂₋₃ <0,01	11,90 (10,41;12,45) p ₁₋₃ >0,05	10,31 (9,84;11,11) p ₁₋₃ >0,05	10,42 (10,02;11,32)
IL-10 (pg/ml)	18,55 (11,77;23,32) *** p ₁₋₂ >0,05	2,63 (0,70;6,81) ### p ₁₋₂ <0,01	12,24 (0,67;23,67) * p ₂₋₃ <0,05	0,63 (0,61;2,15) ### p ₂₋₃ <0,01	4,62 (2,51;25,14) ** p ₁₋₃ <0,01	7,55 (6,84;23,20) * # p ₁₋₃ <0,001	3,88 (1,82;5,28)



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Notes. * - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$ – statistical confidence of differences with control group; # - $p < 0,05$; ## - $p < 0,01$; ### - $p < 0,001$ – statistical accuracy 1 – 10 day; $p_{1,2,3}$ – groups of examined persons.

While evaluating the TNF α local content (Table 1) in patients with chronic posttraumatic osteomyelitis of the lower jaw (I group), its deficit was revealed within the first day ($p < 0,001$). However, in II group of examined patients in the exacerbation phase of chronic osteomyelitis and in III group with simple fractures, a reliable TNF α increase in comparison with a control group was recorded. No reliable differences between indicators of II group and III group were found. At the second stage of monitoring in II group of examined patients there was decrease of TNF α level in comparison with reference values and with testing results at the first stage. While in I group and III group the indicators reached normal values by the 10th day.

On admission to hospital the local indicators of IFN γ in all examined patients did not statistically significantly differ in comparison with a control group. By the tenth day the indicators in I group and III group preserved within the norm, and did not statistically significantly differ from each other. In patients with chronic posttraumatic osteomyelitis of the lower jaw in the exacerbation phase (II group) there was reliable decrease of IFN γ in comparison with the first day and indicators of the control group (Table 1).

The highest IL-10 content was recorded in I group before sequestrectomy in comparison with II group and III group (Table 1). At that, the IL-10 content in patients with aggravated chronic posttraumatic osteomyelitis of the lower jaw and simple fractures of the lower jaws was reliably higher than target values. At the second stage the IL-10 level in I group (with chronic posttraumatic osteomyelitis of the lower jaw) decreased in comparison with the first day and reached the limits of target values. The lowest level of this indicator was recorded in II group as compared to other examined patients and control group. Patients of III group after metallic osteosynthesis with titanium plates maintained the IL-10 level at the 10th day higher than the target values, and the level did not statistically significantly differ from the first stage of study.



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A decreasing tendency of the average age of patients with traumatic injuries is not only medical but a social problem. The greater number of patients are urban dwellers, moreover, hospitalized in a drunken state, which can result from the presence of a great number of urban stress factors contributory to getting injured. Formation of alcoholic attraction and dependency can be preceded by depressions, infections which as a whole are factors of a risk of the development of secondary immune-deficient conditions. Specific regularities were found while analyzing a mode of life, about 42% patients were unemployed. It should be noted that the persons abusing alcohol for a long time demonstrated the suppression of anti-infectious protection mechanisms and considerable decrease of natural resistance to *Staphylococcus aureus* and its toxin [7; 8]. 36% were the patients being indifferent to their health (seeking medical assistance only in case of abscesses and phlegmons and, respectively, in case of worsening of general well-being, and increased pain syndrome), and were not ready to be hospitalized routinely for radical interferences. Probably, this is associated with a low level of basic medical knowledge, in particular, due to a lack of social and health education. It is recorded that residents from another cities were admitted some time later after an injury, this is connected with initial hospitalization of patients to other units due to underlying abnormality. Long communication of the osteal wound with the oral cavity noted concurrently, ongoing injury of ends of bone fragments and surrounding tissues apparently contributed to the development of inflammatory complications as well. Probably, with a timely professional treatment of injuries, development of inflammatory complications can be avoided.

In persons with chronic posttraumatic osteomyelitis of the lower jaw a TNF α deficit was recorded all over the whole period of treatment, probably this is associated with hyper-production of anti-inflammatory cytokine IL-10. It is known that IL-10 inhibits a synthesis of anti-inflammatory cytokines, increases proliferation of mast cells, and participates in differentiation of B-lymphocytes [7]. Local IFN γ content at two stages of monitoring corresponded to reference values.

In the group with chronic posttraumatic osteomyelitis in the exacerbation phase (II group), reliably high values of TNF α and IL-10 were found at the first day of



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hospitalization in comparison with the control group. By the 10th day the TNF α level reached normal values, and IL-10 content in mixed saliva was reliably low in comparison with reference values. It is proved that IL-10 is an important regulator of resorption of the alveolar bone [2; 4; 5; 7]. Respectively, the revealed changes can disturb the operation of phagocytosed cells, thereby contributing not only to the advance of destructive processes within a fracture zone but chronization of an inflammatory process. A reliable decrease of IFN γ was noted at the second stage of monitoring in comparison with the control group. Interferones and IL-10 are important immunomodulatory agents which are able to produce various biological responses depending on a “program” of different cells of the body on which they make an influence [7]. Theoretically, IL-10 and IFN have the same possibility to activate a similar cascade of signals resulting in seminal end effects, however, these cytokines have opposite activities. Their differences lie in the fact that IL-10 inhibits some activities induced by IFN- γ [7; 8].

At the first day of hospitalization, a high TNF α content was recorded in patients with simple fractures of the jaw, however, at the second stage of study the indicators reached reference values. A IFN γ level over the period of 10 day maintained within the norm. IL-10 content within the whole study was higher than target values.

For analysis of reveal of sensitivity and peculiarity of selected parameters, ROC-analysis of differences of TNF α level was made (Fig.к 1) in the group with chronic posttraumatic osteomyelitis in the exacerbation phase (II group) and simple fractures of the lower jaw (III group), and a high AUC was revealed – an area limited by ROC curve and axis of false positive fractions, - equal to 0,891, which is illustrative of a proper quality of the model and possibility to use this diagnostic sign.



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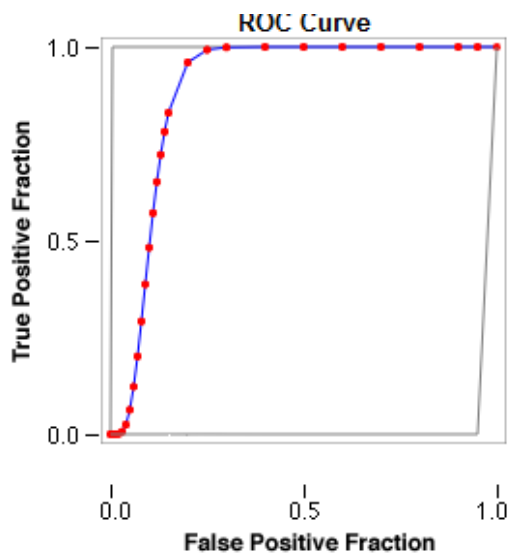


Fig. -1. TNF α level in mixed saliva in patients with chronic posttraumatic osteomyelitis and simple fractures of the lower jaw.

A ROC-analysis of differences of IL-10 level (Fig. 2) was made in the group with chronic posttraumatic osteomyelitis in the exacerbation phase (II group) and simple fractures of the lower jaw (III group), and a high AUC was revealed – an area limited by ROC curve and axis of false positive fractions, - equal to 0,963, which is illustrative of a proper quality of the model and possibility to use this diagnostic sign.

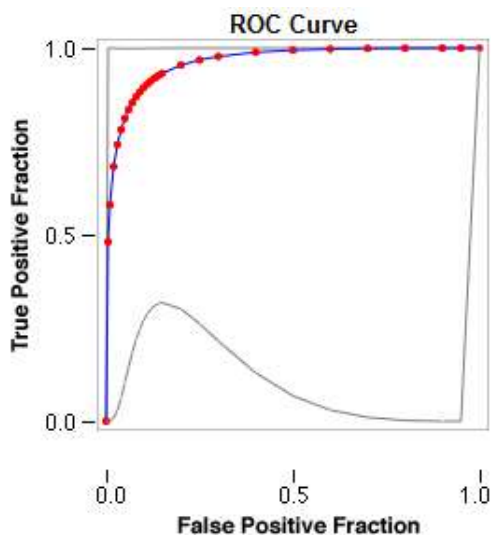


Fig. -2. IL-10 level in mixed saliva in patients with chronic posttraumatic osteomyelitis in the exacerbation phase and simple fractures of the lower jaw.



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The above details attest to the fact that the basic factors contributing to the onset of purulent-inflammatory complications in patients fractures of the lower jaw are: late seeking professional medical assistance by injured persons, improper immobilization, availability of a tooth in the fissure of fracture as well as underlying abnormality. The study proves that in pathogenesis of osteomyelitis, the imbalance in the system of cytokines plays an important role: level of pro- and anti-inflammatory cytokines directly depend on a degree and stage of development of osteomyelitis. Immunological indicators of mixed saliva represent the behavior pattern of local inflammation. Thus, increased value of TNF α , IL-10 can be early diagnostic signs of the onset of posttraumatic osteomyelitis of the lower jaw.

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