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Immune Response in Patients with Mandible Fracture Complicated with Suppuration of a Bone Wound

M.P. Komskyi[1,a], Ye.G. Romanenko[2,b]

[1]Dniprovskyi Medical Institute of Traditional and Non-Traditional Medicine Dental Faculty,

[2]Dnepropetrovsk Medical Academy of the Ministry of Public Health of Ukraine, Dental Faculty,

Dnepr, Ukraine

[a]rolex-50@yandex.ru, [b]helenromanenko2017@gmail.com

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Abstract. Modern clinical observations focused on the increasing complications at the open mandible fracture, which indicated about severe immune suppression in patients with this pathology. For estimation cellular immunity in a peripheral blood there were examined 25 patients in basic group (15 men, 10 women) with mandible fracture, complicated with abscess of a bone wound; control group included 20 practically healthy persons (10 men and 10 women). To assess cellular immunity in the peripheral blood were determined: absolute number of leukocytes, relative amount of lymphocytes; phagocytic activity. Determination relative number of T–lymphocytes (phenotype CD_{2+}, CD_{3+}) and B – lymphocytes (phenotype CD_{22+}) was performed by method of immunofluorescence with monoclonal antibodies. In a case of mandible fracture, complicated with abscess of bone wound and leukocytosis, in the patients were reduced parameters of cellular immunity: on 40.3% – phagocytic index, on 47.7% – phagocytic number, on 43.9% – content of CD_{2+}– and CD_{3+}– lymphocytes. Content of null cells was increased on 76.8 %.

On the 7th day of postoperative treatment in patients from basic group was decreased level of leukocytes on 16.5%, increased level of monocytes on 28.6%, and lymphocytes – on 13.4%, which indicated about decreasing of inflammatory process and active current of reparative process. After 14 day of clinical observations, leukocytes and lymphocytes were in the normal values. It was noted increasing indicators of phagocytic activity, but number of T–lymphocytes was reduced. Content of null cells did not have significant difference from it initial level, which was before treatment. Indicators of cellular immunity demonstrated incompleteness of inflammatory process and immunosuppression activity, which was carried out two weeks after traditional treatment of the bone wound suppuration in a case of mandible fracture. This requires development of a new approach of pathogenetic therapy.

Introduction

The numerous studies were focused on the treatment of mandible fracture. On the one hand, there are numerous innovative methods of diagnostics and treatment of the given pathology. On the other hand, at the patients was shown a lot of complications, especially at the open mandible fractures [1, 2, 3]. Frequency of the purulent inflammatory complications of mandible fractures ranges from 4.4 % to 40 % cases [4, 5, 6].

According to the literature review, attention of researchers was focused on the fact, that purulent-inflammatory diseases of maxillofacial region were associated with changes of the immune parameters, having a significant diagnostic value [7, 8, 9, 10, 11].

The key problem should be focused on the adequate clinical assessment of patient in the dynamics of treatment, such as changes of the cellular immunity parameters in a peripheral blood.

Material and Methods

In the basic group – 25 patients with mandibular fracture (15 men, 10 women) undergone medical examination. Control group included 20 absolutely healthy persons (10 men and 10 women). In both groups amount of leukocytes and indicators of cellular immunity in dynamics were research. Indicators of peripheral blood in patients with mandible fracture had been taken three times: at the hospitalization, after 7 and 14 days of treatment.

In a peripheral blood stream indicators of cellular immunity were determined: absolute number of leukocytes, amount of lymphocytes in percent; phagocytic activity. Coefficients of ratio monocytes to lymphocytes – M/Lf and neutrophils, monocytes to lymphocytes (N+M)/Lf were calculated. To determine number of neutrophils, the blood, which was obtained from a cubital vein, stabilized with heparin (final concentration 10 U/ml) and diluted with Hanks solution in a ratio 1:1. It was used a double gradient for the selection of immunocompetent cells: ficoll ("Pharmacia", Sweden) – verografin ("Spopha", Czech Republic) with a density 1.077 g/cm^3 and 1.115 g/cm^3 respectively. In the centrifuge tubes were sequentially layered on 2.5 ml of a gradient component and 4 ml of diluted blood. There were received three layers of cells after centrifugation: an upper layer – macrophages and lymphocytes, middle – granulocytes and lower – erythrocytes. Neutrophils were taken with Pasteur pipette, washing twice in buffered physiological saline solution (pH-7.4). After washing, neutrophils were weighed in Hanks solution 5x10^5 cells/ml. All manipulations were performed in the silicone dishes. Relative number of T–lymphocytes (phenotype $CD_{2+,}$ CD_{3+}) and B–lymphocytes (phenotype CD_{22+}) was conducted by immunofluorescence method with monoclonal antibodies. Coefficient of ratio T–and B–lymphocytes (T/B) was determined. We concerned lymphocytes without markers of B– and T–cells to the null cells.

Results of Research and their Discussion

Patients with mandible fracture and abscess of a bone wound, have leukocytosis at the hospitalization. Coefficient (N+M)/Lf was shown activity of inflammatory reactions in the given group of patients. It was increased on 22.7%, compare with indicators in control group (table 1).

Table 1. Content and coefficient of leukocytes' ratio in the blood of patients (M±m)

Indicators/Ratios	Groups			
	Control (n=20)	Basic (n=25)		
		before treatment	on 7 day of treatment	on 14 day of treatment
Leukocytes (x10^9/l)	6.52±0.61	9.22± 0.13*	7.62±0.21	6.63±0.41**
Neutrophils (%)	58.51±2.63	62.35±2.51*	60.83±1.62**	58.52±2.01
Monocytes (%)	6.40±0.52	5.61±0.64	7.23±0.42**	6.42±0.53
Lymphocytes (%)	28.91±0.20	25.83±2.10	30.20±1.61*	29.40±1.71
Lf/M (s.u.)	4.53±0.12	4.63±0.12	4.33±0.11	4.83±0.12
(N+M)/Lf (s.u.)	2.20±0.11	2.75±0.11	2.32±0.12	2.20±0.14

Note: * – $p < 0.05$ in comparison with healthy persons; ** – $p < 0.05$ compare with indicators in the basic group before treatment.

According to the results of our research was shown severe inflammatory process in the patients from basic group before treatment.

On the other hand, on this stage of observation was shown the reduction of phagocytic index on 40.3%, and phagocytic number on 47.7% (table 2), compared with indicators in the control group.

Table 2. Subpopulation of lymphocytes and phagocytic activity in dynamics in both groups of patients (M±m)

Indicators		Groups		
		Control (n=20)	Basic (n=25)	
			before treatment	on 14 day of treatment
T–lymphocytes (CD_{2+} , CD_{3+}) (%)		54.51±2.12	30.62±2.41*	29.43±1.72*
B–lymphocytes (CD_{22+}) (%)		14.52±2.61	13.42±1.85	11.56±1.12
Null cells (%)		32.73±0.62	57.83±1.64*	60.23±1.72*
T/B (s.u.)		3.93±0.64	2.34±0.32	3.20±0.11
Phagocytic activity	Phagocytic index (%)	54.83±1.61	32.71±1.80*	44.24±1.65**
	Phagocytic number (s.u.)	6.52±0.14	3.41±0.10*	5.64±0.93**

Note: * – $p < 0.05$ in comparison with healthy persons; ** – $p < 0.05$ compare with indicators in the basic group before treatment.

Indicators of cellular immunity in the basic group of patients were decreased. Firstly, content of CD_{2+} and CD_{3+} cells decreased on 43.9%, compared with indicators in the control group. As the result, ratio T– and B–lymphocytes reduced on 41.0%. Content of null cells increased on 76.8 %. On the 7th day of postoperative treatment in the basic group of patients level of leukocytes decreased on 16.5% ($p < 0.05$), but monocytes increased on 28.6% ($p<0.05$). Number of lymphocytes increased on 13.4% ($p < 0.05$), in comparison with initial indicators. It was indicated about reduction of an inflammatory and active current of the reparative processes.

After 14 day of clinical observations, amount of leukocytes and ratio of (N+M)/Lf were decreased to the normal value. The given process described degree of inflammation. On this stage of treatment, in the basic group of patients were noted signs of the cellular immunity recovery. The phagocytic index was significantly increased on 35.2% and phagocytic number – on 64.7%, in comparison with initial level. However, amount of T–lymphocytes and null cells was significantly different from indicators in the control group.

Discussion

Suppuration of a bone wound at the mandible fracture was observed after 3-7 days of injury. Hematoma infection was situated between the bones' ends. A poor body resistance in patient carried out to the infection of the fracture gap. However, this process did not involve inflammation separate parts of a bone tissue and necrosis of the bone beams. They were located in a big distance from the fracture gap and could lead to the formation of sequester.

Standard treatment of the inflammatory disease (opening of an abscess, antimicrobial therapy, infusion therapy, enhancement of nonspecific resistance an organism, etc.) carried out to the decreasing symptoms of inflammation process. Consolidation of the bone fragments carried out in

the standard terms. This clinical situation is commonly regarded as the festering wounds of a bone, which finished with a rejection of the primarily necrotic tissue and its regeneration [12].

Indicators of cellular immunity are objectively characterized a body condition in children and adults with pathology of different systems. It is well-known, that immune system's response to the inflammation should be focused on the changes of lymphocytes number and phagocytic activity.

It is known that all types of the immune system cells were growth from precursors in a bone marrow. Important place takes lymphocyte memory. Firstly, it covered T–lymphocytes, which transfer from a bone marrow and should arrive to another central organ of the immune system – thymus. At the same time, various additional factors occur during immune responses. All of them are affecting on a bone metabolism [13]. Secondary, active forms of T–cells characterized by the formation of interleukin-6, inflammatory cytokine and osteoclastogenesis stimulator. T–cells also directly stimulate formation of osteoclasts on such way, which is not dependent from osteoclastogenic cytokine– receptor activator of the nuclear factor kappa-beta (RANK), and its ligand (RANKL) [14]. Signal RANKL/RANK regulated formation of the multinucleated osteoclasts from their precursors. Primary, it promotes their activation and survival under condition of standard bone remodeling and at the various pathological conditions. Osteoprotegerin ligand (OPG) protects a skeleton from excessive bone resorption by binding with RANKL, preventing binding with its receptor RANK. Thus, ratio RANKL/OPG is an important determinant of bone mass and integrity of the skeleton [15].

OPG regulates the lymph node organogenesis, lymphocyte development and interactions between T –cells and dendrites cells in the immune system. Both systemic and local activation of T–cells can lead to the production of OPG and subsequent bone loss. Generally, T–cells are regulators of a bone physiology [16]. Bone resorption is initiated by leukocytes, activated in the peripheral blood stream [17].

Osteoclast-activating factor (OAF) is a strong stimulator of the osteoclastic bone resorption [18]. OAF should be secreted by mononuclear cells in a peripheral blood stream, when exposed by a specific antigen. Both the lymphocytes and monocytes initiated release of OAF. Results of our research was shown that: (a) activated lymphocyte included the cells or OAF source, (b) prostaglandins produced by monocytes, were necessary for OAF production by the activated lymphocytes, and (c) prostaglandins of monocytes have an indirect effect on the bone resorption, regulating OAF production, and activation of osteoclasts. Interaction of OAF and prostaglandins in the places of bone resorption has an important role at the inflammatory diseases, associated with bone destruction [19].

Decreasing of T–lymphocytes, increasing of null lymphocytes, on a background of increased leukocytes in a peripheral blood stream in patients with mandible fracture could indicate about suppression of numerous factors, leading to the formation of osteoclasts and affects the bone resorption.

Phagocytic activity of neutrophils usually increased at the early development of inflammatory process. Its reduction leads to the chronic inflammation and autoimmune process beginning. As the result of this, destruction function and circulating immune complexes elimination from the body takes place.

Any inflammatory process carried out throughout complete process of decreasing T–lymphocytes. High sensitivity of T–lymphocytes level in the blood should caused by the following activity. The most active T–lymphocytes with granulocytes quickly transfer to the centre of inflammation. T–cells with a poor metabolic activity (young, old or defective cells and cells with blocked receptors, i.e. temporarily inactive) should stay in a bloodstream. They are poorly identified by the routine laboratory methods. That's why they should concern to the category of null cells.

However, some authors considered that increased formation of null cells supports a variety of T–lymphocytes spectrum after regression. Primary, increasing formation of the immature lymphocytes is the reaction of compensation of T– cells deficiency. Production of T– null cells is decreases. Cellular and humoral immunity depends on the T–lymphocytes number, was suddenly decreasing [20].

Patients with mandible fractures, which complicated with suppuration of a bone wound, have the immune deficiency (reduction number of lymphocytes and monocytes; phagocytosis activity, T–lymphocytes) of the functional origin. This process is observed as the result of influence numerous exogenous and endogenous adverse factors. At the same time, decreased of T–lymphocytes and monocytes was shown as protective reaction of the body, carried out to the further damage of a bone, which caused by activation of osteoclastogenesis. Generally, a bone resorption is initiated by activation of leukocytes in peripheral blood stream. Intensive formation of immature lymphocytes is protective reaction of a body, which focused on the suppression of inflammatory processes. After surgical intervention, on a background of leukocytes reduction, was shown high amount of lymphocytes and monocytes and increasing of phagocytic activity. Content of null cells was increased on a background of decreasing proportion of T–lymphocytes.

Conclusions

Patients with mandible fracture, complicated with abscess of a bone wound, have decreased indices of the cellular immunity: phagocytic index – on 40.3%, phagocytic number – on 47.7%, content of CD_{2+} and CD_{3+}lymphocytes – on 43.9%, on a background of leukocytosis. Content of null cells was increased on 76.8 %.

On the 7^{th} day of postoperative treatment in the basic group of patients' level of leukocytes decreased on 16.5%, monocytes increased on 28.6%, lymphocytes increased – on 13.4%. It is indicated about reduction of the inflammatory process and about active current of reparative process. After 14 day of clinical observation, leukocytes and lymphocytes returned to the standard values. It was noted increasing of the phagocytic activity, but quantity of T– lymphocytes was reduced. Content of null cells had not significant differences from initial level before treatment. Indicators of cellular immunity demonstrated incompleteness of an inflammatory process and immunosuppression in two weeks after traditional treatment of suppuration in a bone wound, in a case of mandible fracture. All these findings required primary implementation of a new approach to the pathogenetic therapy of the given diseases.

References

[1] V. A. Malyshev, B. D. Kabakov, Fractures of jaws, SpetsLit, St. Petersburg, 2005.

[2] N. I. Ivashchenko, Facial injuries in the boys: medical and social aspects of a problem, Dentist 2 (2008) 50 – 53.

[3] B.R. Chrcanovic, Factors which influence on the incidence of maxillofacial fractures, Oral and maxillofacial surgery 3(2012) 17.

[4] J. B. Inkarbekov, Improving surgical treatment of the traumatic osteomyelitis of a lower jaw, Dentistry 3(2008) 46 – 50.

[5] N. F. Yashurkova, Structural characteristics of inflammatory diseases in the maxillofacial area by the hospitalized morbidity at the adult population of large city for ten year period and forecasting trends, Dentistry 4(2007) 28 – 34.

[6] Y. I. Vernadsky, Traumatology and reconstructive surgery of the maxillofacial region, Medical literature, Moscow, 2006.

[7] S.C. Yeoh, S. MacMahon, M.Schifter, E.R. Dodwell, Osteomyelitis and septic arthritis in children: current concepts, Current Opinion in Pediatrics 1 (2013) 58–63.

[8] S.R. Myneni, R.P. Settem, A. Sharma, Bacteria take control of tolls and T cells to destruct jaw bone, Immunological investigations 7 (2013) 519–31.

[9] N.U. Zitzmann, T. Berglundh, Definition and prevalence of peri–implant diseases, Journal of clinical periodontology 35 (2008) 286–91.

[10] M. Saaby, E. Karring, S. Schou, F. Isidor, Factors influencing severity of peri-implantitis, Clinical Oral Implants Research 27(2016) 7-12.

[11] J. Derks, C. Tomasi, Peri-implant health and disease. A systematic review of current epidemiology, Journal of clinical periodontology 42 (2015) 158 – 71.

[12] V.V. Afanasiev, Traumatology of the maxillofacial region, GEOTAR–Media, Moscow, 2010.

[13] M.C. Walsh, N. Kim, Y. Kadono, J. Rho, S.Y. Lee, J. Lorenzo, Y.Choi, Osteoimmunology: interplay between the immune system and bone metabolism, Annual review of immunology 24(2006) 33–63.

[14] L. Rifas, M.N. Weitzmann, A novel T cell cytokine, secreted osteoclastogenic factor of activated T cells, induces osteoclast formation in a RANKL–independent manner, Arthritis and Rheumatism 11(2009) 3324–3335.

[15] L.C. Hofbauer, A.E. Heufelder, Role of receptor activator of nuclear factor-kappa B ligand and osteoprotegerin in bone cell biology, Journal of Molecular Medicine 79(2001) 243–53.

[16] Y.Y. Kong, U. Feige, I. Sarosi, B. Bolon, A. Tafuri, S. Morony, C. Capparelli, J. Li, R. Elliott, S. McCabe, T. Wong, G. Campagnuolo, E. Moran, E.R. Bogoch, G. Van, L.T. Nguyen, P.S. Ohashi, D.L. Lacey, E. Fish, W.J. Boyle, J.M.Penninger, Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand, Nature 18 (1999) 304–309.

[17] T.R. Arnett, Acidosis, hypoxia and bone, Archives of biochemistry and biophysics 503 (2010) 103 – 109.

[18] C.L. Trummel, G.R. Mundy, L.G. Raisz, Release of osteoclast activating factor by normal human peripheral blood leukocytes, Journal of Laboratory and Clinical Medicine 6 (1975) 1001-1007.

[19] T. Yoneda, G.R. Mundy, Monocytes regulate osteoclast-activating factor production by releasing prostaglandins, The Journal of Experimental Medicine 2(1979) 338-350.

[20] A.A. Kishkun, Biological age and aging: opportunities and determine ways of correction: GEOTAR-Media, Moscow, 2008, p. 349.