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Original article

CCL2/MCP-1 Serum Chemokine Level in Patients with Odontogenic Infectious and Inflammatory Diseases of the Soft Tissues of the Maxillofacial Area and Mediastinum

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SUMMARY

Aim. The paper was aimed at determining the CCL2/MCP-1 level in patients with odontogenic infectious and inflammatory diseases of soft tissues of the maxillofacial area and mediastinum.
Methods. The study involved 46 patients with odontogenic infectious and inflammatory diseases of soft tissues of the maxillofacial area and 12 healthy volunteers. The level of patients' plasma CCL2/MCP-1 level was determined using a kit for specific ELISA.
Results. The findings of the studies showed a statistically significant increase in the concentration of plasma CCL2/MCP-1 in patients of all study groups. Our studiy shows a significant increase in plasma CCL2/MCP-1 level in patients with odontogenic phlegmonas, abscesses and mediastinitis, compared to the group of healthy subjects.
Conclusion. CCL2/MCP-1 may play an important role in the pathogenesis of odontogenic infectious and inflammatory diseases of soft tissues of the maxillofacial area and mediastinum, which requires a careful follow-up study.

Keywords: **odontogenic infections, chemokine, phlegmona, abscess, mediastinitis**

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INTRODUCTION

Odontogenic infectious and inflammatory diseases (OIIDs) account for about 20% of cases in the structure of general surgical pathology and are among the widespread diseases of the maxillofacial area (MFA) with a high (10 - 40%) mortality rate (1 - 3). Apparently, the complex anatomical structures of the MFA interconnected cellular spaces cause the development of life-threatening complications: contact mediastinitis, cavernous sinus thrombosis, brain abscess, ENT-organs damage, sepsis, etc. (2, 4). Tissue spread of suppurative material occurs through the interfascial spaces and natural openings by lymphogenic and hematogenous pathways, the rate of which depends on the state of the local and systemic immune factors of the patient and the virulence of the pathogen (3, 5). In the development of OIIDs, the immune response is triggered by the mechanisms of humoral and cellular immunity, which usually are activated at the early and late phases of the innate immune response to microbial agents (6, 7).

Notably, the development of laboratory diagnostics using such methods as ELISA, real-time PCR, immunochemiluminescence assay, maschromatospectrometry, opened new horizons for researchers and clinicians in elucidating the role of poorly studied factors of innate immune response in the pathogenesis of tumor development, atherosclerosis and autoimmune diseases, sepsis and suppurative inflammatory processes caused by microorganisms (8). Generally, a range of non-specific humoral immune factors are the product of the proper immune cells; however, they play the role of regulators of all types of immune responses and are involved in tissue damage in pathology. Among the above factors, chemokines are of special importance, since they are considered the factors of activation and migration of immune cells (9). Recently, few publications highlighting the role of the powerful chemokine C-C motif ligand 2 (CCL2) or Monocyte Chemoattractant Protein 1 (MCP-1) in the regulation of immune processes and pathological conditions in humans and animals have been found. It is this factor that controls the release of immunocompetent cells from hematopoietic organs and blood vessels by chemotaxis to the site of inflammation (10).

Therefore, the study of the CCL2/MCP-1 level in patients with OIIDs will open new perspectives in expanding the patterns of development of their pathogenesis and management of such patients in the clinic of maxillofacial surgery.

AIM

The paper was aimed at determining the CCL2/MCP-1 level in patients with OIIDs of soft tissues of the maxillofacial area and mediastinum.

PATIENTS AND METHODS

The study involved 46 patients of middle age group (52 ± 7.0 years) (according to the WHO standard age group classification) (11), who received treatment for OIIDs of soft tissues of the maxillofacial area at the Maxillofacial Surgery Unit of PU "M.V. Sklifosofsky Poltava Regional Clinical Hospital of Poltava Regional Council" during 2019, and 12 healthy volunteers of the same age group who were included in the control group. Patients with non-odontogenic inflammatory diseases, diabetes mellitus, congenital and acquired immunodeficiencies, CNS diseases were excluded from the study. The patients were assigned into three groups according to the diagnosis: Group I (n = 12) involved patients with acute odontogenic phlegmonas of the maxillofacial area; Group II (n = 12) involved patients with acute odontogenic abscesses of the maxillofacial area, and Group III (n = 12) involved patients with acute suppurative odontogenic mediastinitis. The studies were carried out in accordance with the Helsinki Declaration of the World Medical Association on the ethical principles of medical research with human participation (12). The study protocol was approved by the Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsia, Ukraine (as of 23.05.19 #5). Prior the study, each subject signed a detailed informed consent.

To determine the CCL2/MCP-1 level in patients with OIIDs of soft tissues of the maxillofacial area and mediastinum, venous blood was taken in vacutainers with EDTA after clinical diagnosis was made before the initiation of antimicrobial therapy. Plasma was obtained from the selected blood samples by centrifugation at low speed, followed by freezing and storage at -80°C until use. The level of patients' plasma CCL2/MCP-1 level was determined using a kit for specific enzyme-linked immunosorbent assay (ELISA), manufactured by Elabscience, USA, according to the manufacturer's protocol. The results were evaluated by the degree of absorption of

the test samples using a microplate reader Humareader (Germany) with a wavelength of 450 nm. The minimum detectable concentration was 1 pg/ml.

The resulting data of the three study groups and the comparison group were statistically processed using the standard SPSS 16.0 software (IBM, Armonk, NY, USA) and expressed both the arithmetic mean (M) and arithmetic mean error (± m). The presence of differences between the values of the studied groups of patients was assessed by the Student's t-test. The results were considered reliable at $p < 0.05$.

RESULTS

The analysis of the demographic data of patient, involved in the study indicated a higher incidence of the development of OIIDs of soft tissues of the maxillofacial area and mediastinum in males compared to females (Table 1). However, no gender-related statistically significant differences in the values were found. The values of the average age of patients in the study groups and the comparison group did not differ statistically, although in Group I patients with odontogenic phlegmons, the average

Table 1. *Demographic data of patients in the study groups*

Demographic data		Control group n = 12	Group I n = 12	Group II n = 12	Group III n = 12	p-value
Gender	Male	6 (50,0%)	7 (58,3%)	7 (58,3%)	8 (66,7%)	0,5
	Female	6 (50,0%)	5 (41,7%)	5 (41,7%)	4 (33,3%)	
Ethnicity	White	12 (100%)	12 (100%)	12 (100%)	12 (100%)	N.A.
	Black	0	0	0	0	
Age	Years	45,2	52,5	48,3	51,4	0,95

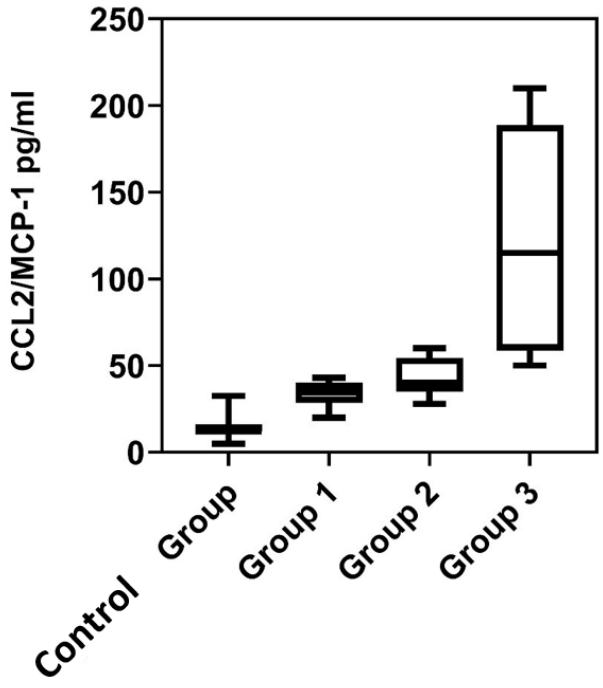

Figure1. *Values of the serum CCL2 / MCP-1 level in patients of the study groups*

age was the highest. This makes it possible to exclude gender and age factors that could affect the findings of the study.

It was established that the plasma CCL2/MCP-1 level in healthy subjects included in the comparison group ranged from 5.0 to 32.5 pg/ml and averaged 14.21 ± 6.9 pg/ml (Figure 1).

The findings of the studies showed a statistically significant increase in the concentration of plasma CCL2/MCP-1 in patients of all study groups with OIIDs of soft tissues of the maxillofacial area and mediastinum compared to the concentration of the chemokine in patients of the control group ($p < 0.05$). Thus, in patients of Group I, II and III, the level of CCL2/MCP-1 was higher by 2.4 times, 3.0 times and 8.7 times ($p < 0,05$), respectively than the value of the group of healthy subject. It should be noted that no statistically significant difference was found between the results of patients in Group I and II.

The highest level of CCL2/MCP-1 was determined in the plasma of Group III patients (123.61 ± 61.07 pg/ml), and in 3 patients (25.0%) this value exceeded 200.0 pg/ml. The concentration of the studied chemokine in patients with mediastinitis significantly exceeded its plasma concentration in patients with odontogenic phlegmons (Group I) by 3.6 times ($p < 0.05$).

DISCUSSION

OIIDs often lead to severe and sometimes fatal, septic complications. Therefore, immediate hospitalization followed by surgery is crucial for patients. Ongoing control of bacterial load (level of microbial colonization of the site of infection) by prompt antibacterial therapy immediately after establishing the diagnosis is essential in management strategy (13 - 15). However, previous studies indicate a close relationship between tissue damage and mortality in patients with the development of an excessive inflammatory response. Apparently, the balance between the protective and harmful effects of the innate immune response is quite complex and ambiguous (15 - 18).

On the one hand, the functional activity of the competent immune cells is the key to effective innate immune response that controls and neutralizes the invading infectious agent. CCL2/MCP-1 is one of the regulators of immune and pathological processes in the body, which is responsible for enhancing T-lymphocyte chemotaxis, activation and chemotaxis of monocytes and type I macrophages and basophils, playing an important role in non-specific acute inflammatory response (16). Therefore, the established significant increase in plasma CCL2/MCP-1 level of patients with soft tissue infection of the maxillofacial area and mediastinum were appropriate. In response to antigen invasion, this chemokine enhances migration and infiltration of monocytes and natural killers and participates in the polarization of Th2, promoting production of IL-4 (19). Consequently, a number of publications highlight the role of CCL2/MCP-1 in reducing bacterial load in septic patients (15, 20).

On the other hand, excessive migration of monocytes to the site of inflammation and hyperproduction of proinflammatory cytokines promotes the expression of tissue factor, which leads to blood clotting disorder and the formation of microthrombi, which results in microcirculation disorder, leading to multiple organ failure and death (21, 22). At the same time, recent studies are correlated with active involvement in the pathogenetic mechanisms of the development and progression of septic conditions and show a direct relationship between CCL2/MCP-1 levels and the severity of sepsis (19). Accordingly, the plasma CCL2/MCP-1 level of patients with OIIDs of soft tissues of the maxillofacial area and mediastinum increased significantly with the severity of the patient's pathology. The highest rates were recorded in severe patients with odontogenic mediastinitis, which coincides with the previously described patterns.

Therefore, studies in plasma CCL2/MCP-1 of patients with OIIDs of soft tissues of the maxillofacial area and mediastinum could be a diagnostic criterion for the severity of the disease and are prognostically unfavorable for critically severe patients.

CONCLUSION

Our studies show a significant increase in plasma CCL2/MCP-1 level in patients with odontogenic phlegmons, abscesses and mediastinitis, compared to the group of healthy subjects. Moreover, the highest rates were characteristic of patients with mediastinitis. Accordingly, CCL2/MCP-1 may play an important role in the pathogenesis of OIIDs of soft tissues of the maxillofacial area and mediastinum, which requires a careful follow-up study.

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Disclosure statement

The authors declare no conflict of interest.

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Nivo serumskog hemokina CCL2/MCP-1 kod bolesnika sa odontogenim infektivnim i inflamatornim bolestima mekog tkiva maksilofacijalne regije i medijastinuma

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SAŽETAK

Cilj. Cilj rada bilo je određivanje nivoa CCL2/MCP-1 kod bolesnica sa odontogenim infektivnim i inflamatornim bolestima mekog tkiva maksilofacijalne regije i medijastinuma.
Metode. Studija je uključila 46 bolesnika sa odontogenim infektivnim i inflamatornim bolestima mekog tkiva maksilofacijalne regije i 12 zdravih volontera. Nivo CCL2/MCP-1 u plazmi bolesnika određen je pomoću specifičnog ELISA testa.
Rezultati. Nalazi studija pokazali su statističko značajno povećanje koncentracije CCL2/MCP-1 u plazmi bolesnika ispitivane grupe. Naša studija je pokazala značajno povećanje nivoa CCL2/MCP-1u plazmi bolesnika sa odontogenom flegmonom, apscesima i medijastinitisom u poređenju sa kontrolnom grupom.
Zaključak. CCL2/MCP-1 može imati značajnu ulogu u patogenezi odontogenih infektivnih i inflamatornih bolesti mekog tkiva maksilofacijalne regije i medijastinuma, što zahteva pažljivo praćenje.

Ključne reči: **odontogene infekcije, hemokin, flegmona, apsces, medijastinitis**