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DOI <https://doi.org/10.32782/2226-2008-2024-5-5>Yu. L. Bandrivsky <https://orcid.org/0000-0002-4103-3664>V. O. Bilan <https://orcid.org/0009-0009-4567-925X>

INVESTIGATION OF IMMUNOLOGICAL INDICATORS IN THE ORAL FLUID OF MILITARY PERSONNEL WITH CHRONIC GENERALISED PERIODONTITIS WITH CONSIDERATION OF THEIR PSYCHO-EMOTIONAL STATE

Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

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Diseases of periodontal tissues can lead to the appearance and development of complications in internal organs and systems that affect the general condition of the body and, most importantly, reduce the fighting and working capacity of military personnel.

The aim of the study – to investigate the content of immunological parameters in the oral fluid of the military personnel of the Armed Forces of Ukraine with chronic generalised periodontitis of the initial – stage II, taking into account their psycho-emotional state.

Materials and methods. The levels of certain cytokines (IL-2, IL-4 and TNF- α) and C-reactive protein (CRP) in oral fluid were measured in 94 patients with chronic generalised periodontitis (CGP) of the initial – stage II. The latex agglutination method was used to determine C-reactive protein (CRP) in oral fluid. The levels of IL-2, IL-4 and TNF- α in oral fluid were determined by enzyme-linked immunosorbent assay using a “Star Fax 303 Plus” device.

Results. As a result of laboratory studies in military personnel with initial – stage II CGP, an increase in the content of proinflammatory cytokines TNF- α – by 30.90% and IL-2 – by 57.89%, $p < 0.01$, against the background of a decrease in the content of anti-inflammatory cytokine IL-4 – by 19.27%, $p < 0.05$, compared with the data in the control group with initial – stage II CGP. There was a 62.10% increase in the level of CRP in the oral fluid in the control group compared to the data of civilians, $p < 0.01$.

Conclusions. Thus, in the military personnel of the Ukrainian Armed Forces of the main group with the initial – stage II chronic generalised periodontitis, there was a significant increase in the level of C-reactive protein, proinflammatory cytokines TNF- α and IL-2 in the oral fluid against the background of a decrease in the content of proinflammatory cytokine IL-4, compared with the results of studies in civilians with the initial – stage II chronic generalised periodontitis of the control group.

Key words: generalised periodontitis, military personnel, oral fluid, cytokines, C-reactive protein.

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Ю. Л. Бандрівський, В. О. Білан

ДОСЛІДЖЕННЯ ІМУНОЛОГІЧНИХ ПОКАЗНИКІВ У РОТОВІЙ РІДИНІ ВІЙСЬКОВОСЛУЖБОВЦІВ З ХРОНІЧНИМ ГЕНЕРАЛІЗОВАНИМ ПАРОДОНТИТОМ З УРАХУВАННЯМ ЇХНЬОГО ПСИХОЕМОЦІЙНОГО СТАНУ

Тернопільський національний медичний університет імені І. Я. Горбачевського Міністерства охорони здоров'я України, Тернопіль, Україна

У статті наведено результати лабораторного дослідження, в якому встановлено, що у військовослужбовців ЗСУ із захворюваннями тканин пародонта виявлено дисбаланс значень імунологічних параметрів у ротовій рідині, що поглиблювався з погіршенням їхнього психоемоційного стану. Так, у військовослужбовців з хронічним генералізованим пародонтитом початкового – II ступеня визначено збільшення рівнів у ротовій рідині прозапальних цитокінів ФНП- α – на 30,90% та IL-2 – на 57,89%, $p < 0,01$, на тлі зниження вмісту протизапального цитокіну IL-4 – на 19,27%, $p < 0,05$, стосовно даних у осіб порівняльної групи з хронічним генералізованим пародонтитом початкового – II ступеня. Відзначено збільшення вмісту С-реактивного білка у ротовій рідині на 62,10% порівняно з даними осіб цивільних професій порівняльної групи, $p < 0,01$.

Ключові слова: генералізований пародонтит, військовослужбовці, ротова рідина, цитокіни, С-реактивний білок.

Introduction. The worst consequence of Russia's brutal invasion of Ukraine is the great loss of life, both civilian and military. War takes from us the most precious thing – life – and provokes a series of irreversible changes in the whole body. Numerous studies have demonstrated the devastating impact of violent conflict and war on citizens, refugees and migrants from war-torn areas [1; 2]. Armed

conflict has been shown to exacerbate three groups of diseases. First, wars are associated with a significant spread of infectious diseases such as tuberculosis, polio, measles and even cholera. Second, conflicts cause mental health problems such as post-traumatic stress disorder (PTSD), depression and somatic disorders. Third, wars can increase the incidence of chronic non-communicable diseases, such as cardiovascular disease, diabetes, arthritis, asthma, dental disease and cancer [3].

Most of the research on active military personnel and military veterans has been conducted mainly in the United States and the United Kingdom. However, these studies are

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not directly related to the Russian-Ukrainian war, which is an existential conflict and a struggle for national identity. In contrast to veterans from the United States and the United Kingdom, Ukrainian veterans are returning to a country that has been devastated by the war and is still engaged in an existential struggle for its own survival. They also return to their hometowns where their family and friends are also experiencing the effects of war [4; 5].

In the context of the ongoing conflict, the formation of combat units in the Armed Forces of Ukraine takes place against the background of a high prevalence of somatic diseases, in particular dental diseases [6; 7]. The state of health of servicemen, including the oral cavity, in particular periodontal tissues, is one of the main factors determining the combat readiness and combat effectiveness of the Armed Forces of Ukraine. Diseases of periodontal tissues can lead to the emergence and development of complications from internal organs and systems, which affect the general condition of the body and, most importantly, reduce the fighting ability and performance of military personnel [8]. It is evident that the role of microflora is pivotal in the pathogenesis of periodontal tissue diseases. Nevertheless, the nature, severity and direction of the inflammatory response are largely determined by the ability of the body to resist pathogenic microflora in the oral cavity by activating cellular and humoral local and systemic factors of nonspecific and specific immune defense [9]. Neutrophils activated by a microbial pathogen in the oral cavity produce a number of cytokines, which trigger and cooperate with a number of immunocompetent cells [10]. The balance between the secretion of pro- and anti-inflammatory cytokines determines the direction of local reactions in inflammatory periodontal diseases, the organization of cell-mediated immune response and the effectiveness of anti-infection protection [11]. Disruption of the inflammatory mediator and immunoglobulin system in periodontal tissues can lead to destructive changes and negative effects on tissue repair [12].

The course of the inflammatory response is influenced by numerous organs and tissues, primarily through the intermediary metabolites. C-reactive protein (CRP) is one of the most sensitive proteins of the acute phase of inflammation, and its concentration increases rapidly during inflammation. CRP activates monocytes, the complement system, and phagocytosis, thereby initiating the early non-immune removal of Gram-negative bacteremia and tissue breakdown products [13]. Additionally, alterations in CRP synthesis are influenced by cytokines, which are secreted primarily by macrophages, but also by fibroblasts, endothelial and epithelial cells. Of the more than 20 different cytokines, interleukin 6 (IL-6) and tumour necrosis factor-alpha (TNF- α) are of particular importance in the acute phase of inflammation, as evidenced by their role in the inflammatory cascade [14].

The existing literature does not sufficiently cover the issue of periodontal tissue diseases in servicemen participating in active combat operations. The lack of such coverage makes our study timely and relevant.

The aim of the article – to study the content of immunological parameters in the oral fluid of military personnel of the Armed Forces of Ukraine with chronic generalised

periodontitis of the initial – stage II, taking into account their psycho-emotional state.

Materials and methods. The levels of certain cytokines (IL-2, IL-4 and TNF- α) and C-reactive protein (CRP) were determined in 94 patients with chronic generalised periodontitis (CGP) of the initial – stage II. The main group consisted of 78 (82.98%) military patients and the comparison group consisted of 16 (7.02%) civilian specialty patients. The study was conducted in the medical center “Zdorovya” LLC “Future Plus” in Ternopil; 54 patients (57.4%) were diagnosed with initial – stage I CGP and 40 patients (42.6%) were diagnosed with stage II CGP. The classification of M.F. Danilevsky (1994) with additions by I.S. Mashchenko (2002) was used to assess the state of periodontal tissues [15].

The study was approved by the Bioethics Committee of I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Protocol No. 77 of 18.04.2024) and was conducted in accordance with the tenets and principles of the Declaration of Helsinki for research involving human subjects, respecting patient confidentiality and obtaining all necessary consents.

Taking into account the results of our previous studies of the psycho-emotional state of servicemen of the Armed Forces of Ukraine, the patients of the main group were divided into four subgroups: subgroup I – people with a high degree of stress tolerance and low levels of reactive anxiety; subgroup II – people with a high degree of stress resistance and a low level of reactive anxiety; subgroup III – people with a threshold level of stress resistance and a moderate level of reactive anxiety; subgroup IV – people with a low degree of stress resistance and a high level of reactive anxiety. It is important to note that in military personnel with very high stress tolerance and low levels of reactive anxiety (subgroup I), the initial – stage II CGP was not objectivized. Additionally, the absence of stage II CGP was observed in military personnel with high levels of stress tolerance and low levels of reactive anxiety.

CRP in oral fluid was determined using the latex agglutination method. It consists in immobilization of antibodies to CRP on latex particles. This method was employed as an indicator of the activity of inflammatory and dystrophic-inflammatory and necrotic processes in periodontal tissues [16]. CRP latex test was performed using diagnostic kits of domestic production (State registration number 1248/2002, LLC SPC “Granum”, Kharkiv). The concentration of IL-2, IL-4 and TNF- α in the oral fluid was determined by enzyme-linked immunosorbent assay (ELISA) [17], using a Star Fax 303 Plus device with reagents from the “HumanIL-PlatinumELISA” kit, manufactured by “NovaMedline” (Germany).

Excel and Statistica (trial license) programs were used to evaluate the results, errors and statistical treatment, as well as the mathematical and statistical methods used, with special attention paid to Student’s t-test [18].

Results and discussion. The results showed (Table 1) that in the comparison group with stage I CGP, the CRP level in oral fluid was (7.15 \pm 0.28) mg/L, which was 52.86% lower than that of military patients with initial forms of CGP (main group) – (10.93 \pm 0.36) mg/L ($p < 0.01$).

Table 1

The value of C-reactive protein content in the oral fluid of patients with chronic generalised periodontitis of the initial – stage I depending on their psycho-emotional state

Indicators	Comparison group, (n=12)	Main group (military personnel)			
		Subgroup I	Subgroup II (n=2)	Subgroup III (n=28)	Subgroup IV (n=12)
CRP, mg/l	7.15±0.28	–	9.83±0.30*	10.45±0.38*	12.50±0.41*

Note: * p<0,01 – significant difference in values compared to the data in the comparison group.

At the same time, in patients of the main group, an increase in the values of the analysed parameter was noted from (9.83±0.30) mg/l in patients of subgroup II (high level of stress tolerance with a low level of reactive anxiety) to (12.50±0.41) mg/l in patients of subgroup IV (low level of stress tolerance with a high level of reactive anxiety), p<0.01.

In patients of both groups with stage II CGP, the level of CRP in the oral fluid increased and was (7.50±0.30) mg/l in the comparison group. At the same time, in patients of the main group, the CRP content in the oral fluid exceeded the data in comparison by 71.33%, p<0.01 (Table 2).

In patients with stage II CGP, the level of CRP in the oral fluid ranged from (11.59±0.40) mg/l in subgroup III (moderate level of stress tolerance at the threshold level of reactive anxiety) to (14.10±0.45) mg/l in subgroup IV (low level of stress tolerance at a high level of reactive anxiety), p<0.01.

So, the level of CRP in the oral fluid of patients of both study groups increased with the intensification of dystrophic-inflammatory processes in periodontal tissues, and in the

main group – with the deterioration of the psycho-emotional state. Generalized assessment of CRP protein content, a marker of inflammatory processes (Fig. 1) showed that the level of CRP protein in the oral fluid of the servicemen with initial – II stage CGP exceeded that in the group of comparison on average by 62.10%, p<0.01.

Analysis of the values of the cytokine profile in the oral fluid of patients with initial – stage II CGP (Table 3) showed that the level of TNF-α in the oral fluid of the comparison group was (21.15±1.86) ng/ml and did not differ significantly from subgroup II of the main group (22.86±1.88) ng/ml, p>0.05. However, in patients of subgroups III and IV, the level of TNF-α exceeded the data of comparison by 22.93%, p<0.05 and 39.24%, p<0.01, respectively.

The values of IL-2 in the oral fluid of patients in the comparison group with initial – stage I of CGP were lower than in patients in the main group: by 11.11% in subgroup II, by 22.22% in subgroup III, p>0.05, and by 61.11% in subgroup IV, p<0.05. At the same time, the level of IL-4 was the highest in patients of the comparison group (15.64±0.43) ng/ml.

Table 2

The value of C-reactive protein level in the oral fluid of patients with chronic generalised periodontitis stage II depending on their psycho-emotional state

Indicators	Comparison group (n=4)	Main group (military personnel)			
		Subgroup I	Subgroup II	Subgroup III (n=12)	Subgroup IV (n=24)
CRP, mg/l	7.50±0.30	–	–	11.59±0.40*	14.10±0.45*

Note: * p<0,01 – significant difference in values compared to the data in the comparison group.

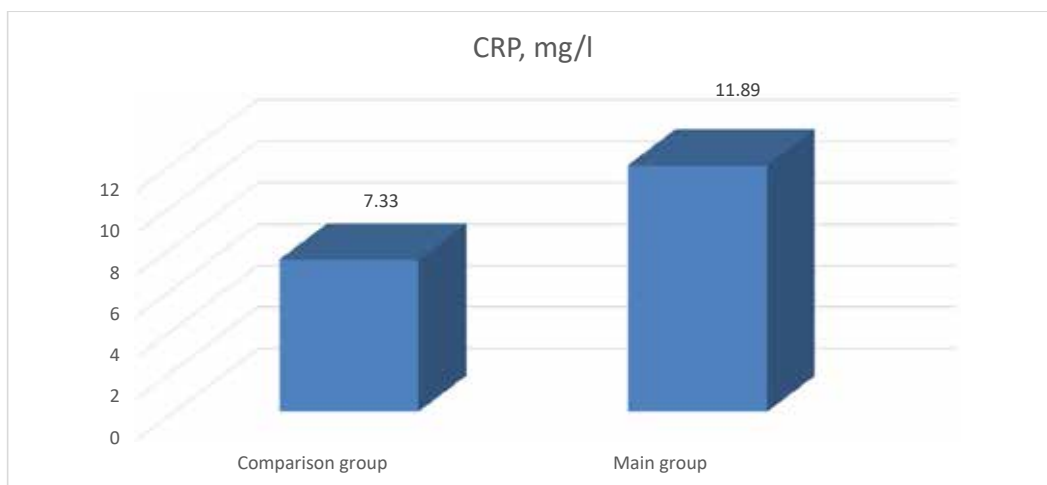


Fig. 1. Generalised assessment of CRP content in the oral fluid of patients with chronic generalised periodontitis of initial – stage II

Table 3

Cytokine levels in the oral fluid of patients with chronic generalised periodontitis of the initial – stage II group depending on their psycho-emotional state

Indicators	Comparison group, (n=12)	Main group (military personnel)			
		Subgroup I	Subgroup II (n=2)	Subgroup III (n=28)	Subgroup IV (n=12)
FNP-α (ng/ml)	21.15±1.86	–	22.86±1.88	26.00±1.95**	29.45±2.32*
IL-2 (ng/ml)	0.18±0.03	–	0.20±0.03	0.22±0.04	0.29±0.04**
IL-4 (ng/ml)	5.64±0.43	–	5.10±0.40	4.77±0.32	4.26±0.29**

Note: * p<0,01, **p<0,05 – significant difference in values compared to the data in the comparison group.

In patients of the main group, the studied index decreased as their psycho-emotional state worsened and was lower than in patients of the comparison group: by 9.58% – in subgroup II, by 15.43% – in subgroup III and by 24.47% – in subgroup IV, p<0.05.

The analysis of the cytokine profile in the oral fluid of patients with stage II CGP showed (Table 4) that in the main group the level of TNF-α in the oral fluid exceeded the data of comparison: 37.72% – in patients with moderate level of stress tolerance and threshold level of reactive anxiety (subgroup III) and 51.96% – in patients with low level of stress tolerance and high level of reactive anxiety (subgroup IV), p<0.01. The patients in the main group showed an increase in IL-2 content in oral fluid, which exceeded the data in the comparison group: by 85.0% – in subgroup III and by 105.0% – in subgroup IV, p<0.01.

At the same time, there was a decrease in the level of IL-4 in the oral fluid of the main group: by 22.0% – in subgroup III, p>0.05 and by 35.45% – in subgroup IV, p<0.01, compared with the data in the comparison group.

Thus, the imbalance of cytokine profile values was determined in the oral fluid of the patients with CGP of the initial – II stage, which deepened as the intensification of processes in the periodontium increased and, probably, intensified as the psycho-emotional state of patients worsened.

The imbalance of cytokine indices in servicemen with initial – II stage CGP was characterized (Fig. 2) by the increase of TNF-α content in oral fluid by 30.90%, p<0.01, IL-2 by 57.89%, p<0.01, against the background of IL-4 decrease by 19.27%, p<0.05.

Conclusions. Thus, in the servicemen of the Armed Forces of Ukraine in the main group with CGP of the initial –

Table 4

Cytokine levels in the oral fluid of patients with chronic generalised periodontitis stage II depending on their psycho-emotional state

Indicators	Comparison group (n=4)	Main group (military personnel)			
		Subgroup I	Subgroup II	Subgroup III (n=12)	Subgroup IV (n=24)
TNF-α (ng/ml)	24.15±1.86	–	–	33.26±2.03*	36.70±2.15*
IL-2 (ng/ml)	0.20±0.02	–	–	0.37±0.04*	0.41±0.06*
IL-4 (ng/ml)	4.64±0.43	–	–	3.62±0.35	3.00±0.31*

Note: * p<0,01 – significant difference in values compared to the data in the comparison group.

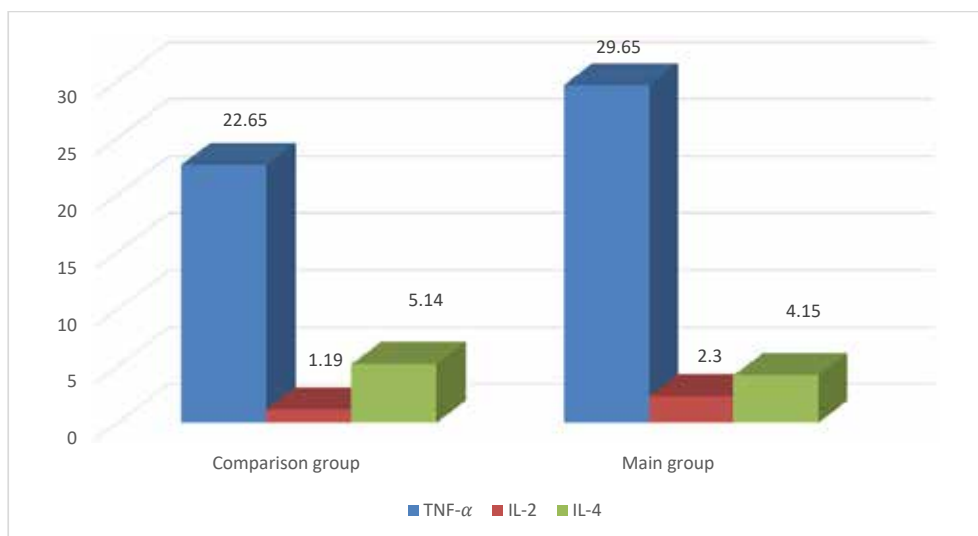


Fig. 2. Generalised assessment of the cytokine content in the oral fluid of patients with generalised periodontitis of the initial – stage II

II stage a significant increase in the level of C-reactive protein, pro-inflammatory cytokines TNF- α and IL-2 in oral fluid was observed against the background of a decrease in the content of pro-inflammatory cytokine IL-4, compared with the results

of studies in civilians with CGP of the initial – II stage of the comparison group. At the same time, the imbalance of these parameters in patients with CGP of the main group deepened as their psycho-emotional state worsened.

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Електронна адреса для листування bandrivsky@tdmu.edu.ua