Study of local immunity of oral cavity in oral fluid of military personnel of the Armed Forces of Ukraine with chronic catarrhal gingivitis depending on their psycho-emotional state

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Study of local immunity of oral cavity in oral fluid of military personnel of the Armed Forces of Ukraine with chronic catarrhal gingivitis depending on their psycho-emotional state

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ABSTRACT

Purpose. To analyse the changes in local immunity in the oral fluid of servicemen of the Armed Forces of Ukraine with chronic catarrhal gingivitis depending on their psycho-emotional state.

Material and methods. The study of local oral immunity in oral fluid was conducted in 22 military patients of the Armed Forces of Ukraine with chronic catarrhal gingivitis (main group), in 11 patients of civilian specialties with chronic catarrhal gingivitis (comparison group), and in 16 dentally healthy individuals (control group). Additionally, military patients in the main group were divided into 4 subgroups depending on their psychoemotional state. The determination of sIgA in the oral fluid was performed by the method of Mancini et al. The photoelectrocolorimetric method was used to measure lysozyme activity in the oral fluids of patients. The content of cortisol in the oral fluid of patients in the study groups was determined by a competitive enzyme-linked immunosorbent assay.

Results. As a result of the laboratory studies, it was found that in patients with chronic catarrhal gingivitis, military personnel of the Armed Forces of Ukraine had a 1.2-fold decrease in the content of sIgA in the oral fluid ($p<0.01$) and lysozyme activity ($p<0.05$), against the background of an increase in cortisol levels by 61.18 % ($p<0.01$) compared to the data in the comparison group.

Conclusion. Thus, summing up the data of laboratory studies, it can be stated that patients of the main group (military personnel of the Armed Forces of Ukraine) with chronic catarrhal gingivitis have a more pronounced weakening of local oral immunity, which is manifested by a decrease in sIgA levels and lysozyme activity, compared with similar data in the comparison group (patients of civilian professions) with chronic catarrhal gingivitis. At the same time, the imbalance of immunological parameters deepened with the deterioration of the psychoemotional state of patients in the main group, which was confirmed by an increase in the level of cortisol in the oral fluid.

Keywords: chronic catarrhal gingivitis, military personnel, psycho-emotional state, local oral immunity, oral fluid

INTRODUCTION

In today's challenging circumstances, namely during wartime, the formation of combat units of the Armed Forces of Ukraine is taking place against a backdrop of a high prevalence of somatic diseases, in particular the dentition. The state of health of servicemen, including the state of their oral cavity and periodontal tissues, is the foundation of the combat readiness and combat capability of the Armed Forces of Ukraine. Chronic catarrhal gingivitis is one of the most prevalent periodontal diseases, characterised by prolonged inflammation of the gums without deep tissue involvement [1,2]. It could lead to a decline in overall health Systemic factors, including hormonal changes, stress, smoking, and general health, play a significant role in the pathogenesis of chronic catarrhal gingivitis [3,4]. Those in the military, who frequently encounter elevated levels of stress and psycho-emotional strain, are at an increased risk of developing this disease [5,6]. It is established that stress can have a deleterious impact on the immune system, thereby reducing its capacity to safeguard the body from infection [7]. One of the primary defence mechanisms within the oral cavity is local immunity, which is provided by secretory immunoglobulin A (sIgA) and other components, including lysozyme [8,9]. A reduction in secretory immunoglobulin A (sIgA) and lysozyme activity may indicate a decline in local immunity, thereby increasing the susceptibility to pathogens [10]. The investigation of local immunity in military personnel with chronic catarrhal gingivitis, contingent on their psycho-emotional state, enables the establishment of a correlation between stress and the emergence of inflammatory conditions affecting the oral cavity [11].

MATERIALS AND METHODS

The study was based on the results of laboratory examinations of local oral immunity in 22 military personnel of the Armed Forces of Ukraine with chronic catarrhal gingivitis (the main

group), 11 civilian patients with chronic catarrhal gingivitis (the comparison group), and 16 dentally healthy individuals (the control group). In consideration of the findings from our preceding studies [12] (psycho-emotional state determination among military personnel of the Armed Forces of Ukraine), the patients in the main group were classified into four subgroups based on their psycho-emotional state: Group I – demonstrated exceptional stress resilience with minimal reactive anxiety; Group II – showed a high level of stress resilience accompanied by low reactive anxiety; Group III – had a borderline level of stress resilience with moderate reactive anxiety; Group IV – presented a low level of stress resilience with elevated reactive anxiety [13].

The *secretory immunoglobulin A (sIgA) levels in oral fluid* was detected by the method of Mancini et. al. [14]. This method is based on the fractionation of the proteins of the substrate under study with organic solvents and buffer solutions. The formation of protein-buffer complexes alters the photoelectric density of the medium, enabling the photoelectrocolourimeter to display indicators that characterise the Ig content.

Determination of lysozyme activity in oral fluid. Oral fluid was obtained from patients on an empty stomach, 10 minutes after rinsing the mouth with 5ml of saline solution. The samples were centrifuged at 3000 rpm for 15 minutes, and then the supernatant was collected. To ascertain the activity of lysozyme in the oral fluid, a wash of the daily agar culture of *Micrococcus lysodeikticus* (1/15 with phosphate buffer at pH 6.2) was prepared. The resulting micrococcal suspension was standardised using a CFC-2 photoelectrocolourimeter with a green light filter, resulting in an optical density of 0.66. For the purposes of the study, 0.1 ml of diluted oral fluid, 0.4 ml of 1/15 phosphate buffer at pH 6.2, and 2.0 ml of the standardised micrococcal suspension were added to the test tubes. Three control tubes were filled with 0.5 ml of 1/15 M phosphate buffer at pH 6.2 and 2.0 ml of micrococcal suspension. The tubes were incubated in a thermostat at 37°C for 30 minutes, after which the optical density of the contents of the control and test tubes was measured using a CFC-2 photoelectrocolorimeter with a green light filter at a wavelength of 520 nm. In accordance with the calibration tables, taking into account the degree of preliminary dilution of the oral fluid samples, the concentration of lysozyme was determined in mg/ml [15].

Determination of cortisol content in oral fluid. The concentration of cortisol in the oral fluid of patients enrolled in the study was determined by competitive enzyme-linked immunosorbent assay (ELISA) [16] using an ELISA kit from Diametra (Italy).

The studies were conducted in accordance with the provisions and principles set forth in the Declaration of Helsinki for Research Involving Human Subjects, with particular attention paid to the protection of patient confidentiality and the acquisition of informed consent.

Data analysis was performed on a personal computer utilizing licensed software, including Microsoft Excel 2021 and Statistica.

RESULTS AND DISCUSSION

In order to investigate the state of local immunity and local immune response, the levels of sIgA and lysozyme activity in the patients of the study groups were examined. According to the data shown in Figure 1, the level of sIgA in the oral fluid of the control group was 290.0±11.30 mg/ml. At the same time, in patients with chronic catarrhal gingivitis of both study groups (main and comparison), the level of sIgA was significantly lower than in the control group: by 28.10 % in the comparison group and by 37.73 % in patients-military personnel of the main group, $p<0.01$, $p_1<0.05$.

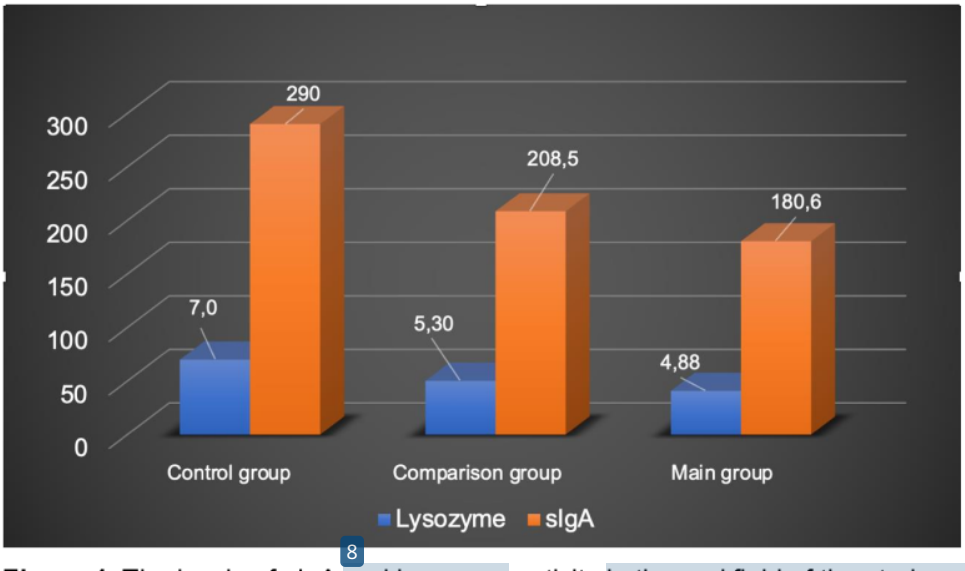

Figure 1. The levels of sIgA and lysozyme activity in the oral fluid of the study groups

The activity of lysozyme in the oral fluid of healthy subjects in the control group was 7.0±0.18 ng/ml and was higher than in patients with chronic catarrhal gingivitis: 24.29 % in the control group and 30.19 % in the main group, $p<0.01$, $p_1>0.05$.

The study of sIgA content and lysozyme activity in the oral fluid of patients in the main group according to their psychoemotional state showed (Table 1) that in patients with chronic catarrhal gingivitis with very high and high resistance to stress and low level of reactive anxiety (subgroup I-II), the content of sIgA ranged from 196.25±9.20 mg/ml to 184.13±9.15 mg/ml, $p_1>0.05$, and did not differ significantly from the values in the control group (208.50±9.26 mg/ml). At the same time, in patients-military personnel with chronic catarrhal gingivitis at

threshold and low resistance to stress with high level of reactive anxiety (subgroups III-IV), the content of sIgA in oral fluid was significantly higher than in the control group: by 17.51 % in subgroup III, p<0.05, and by 18.47 % in subgroup IV, p<0.01.

Table 1. Values of sIgA content and lysozyme activity in oral fluid of patients with chronic catarrhal gingivitis of the main group according to their psychoemotional state

Research groups	Indicators	
	sIgA, mg/ml	Lysozyme, ng/ml
Comparison group, (n=11)	208,50±9,26	5,30±0,16
I subgroup, (n=3)	196,25±9,20	5,10±0,16
II subgroup, (n=3)	184,13±9,15	4,92±0,15
III subgroup, (n=9)	172,0±9,00**	4,83±0,14**
IV subgroup, (n=7)	170,0±8,80*	4,70±0,13**

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

The dynamics of lysozyme levels in the oral fluid of military personnel with chronic catarrhal gingivitis was characterised by a decrease in the data of the studied parameter with the deterioration of their psychoemotional state: from 5.10±0.16 ng/ml in patients of the first subgroup to 4.92±0.15 ng/ml in the second subgroup, which did not differ statistically from the data of patients of the comparison group (5.30±0.16 ng/ml), p>0.05. At the same time, in patients of subgroups III and IV, the data of the studied parameter were 8.87 % and 11.33 % lower than in the comparison group, p<0.05.

The study found that military personnel with chronic catarrhal gingivitis had significantly elevated levels of the stress hormone cortisol in their oral fluid (Figure 2).

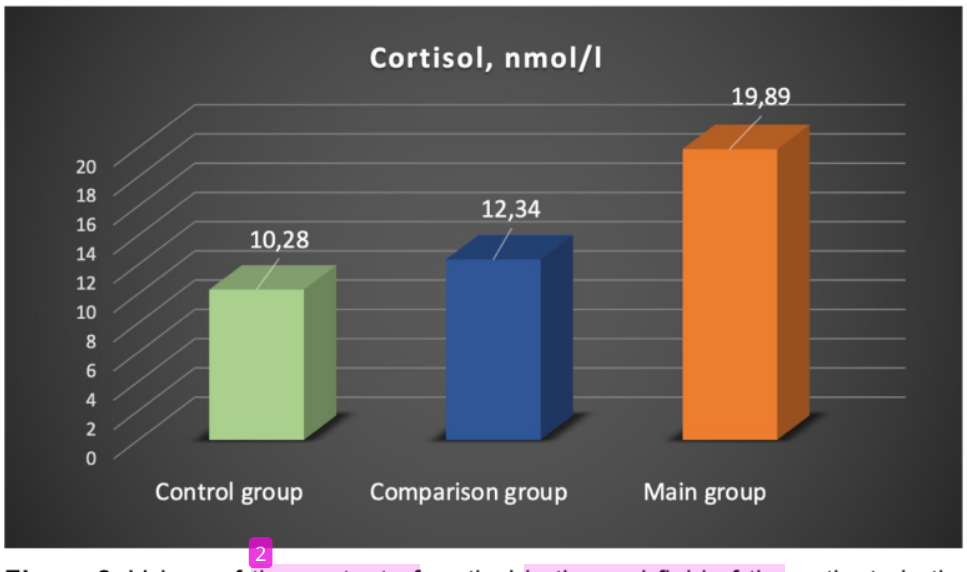

Figure 2. Values of the content of cortisol in the oral fluid of the patients in the study groups

Thus, it was found that in somatically and dentally healthy persons of the control group the content of cortisol in the oral fluid was 10.28±1.23 nmol/l, which was lower than in the data: by 20.0 % in civilian subjects with chronic catarrhal gingivitis (comparison group), $p<0.05$, and by 93.48 % in patients- military personnel of the Armed Forces of Ukraine (main group) with chronic catarrhal gingivitis, $p<0.01$. At the same time, the level of cortisol in oral fluid in patients of the main group with chronic catarrhal gingivitis exceeded the same values in patients of the control group by 61.18 %, $p_1<0.01$.

Analysis of the values of oral fluid cortisol in patients in the main group according to their psychoemotional state showed (Table 2) that only in patients with very high stress resistance and low reactive anxiety the content of oral fluid cortisol did not differ significantly from the values in the comparison group (13.28±2.87 nmol/l vs. 12.34±1.56 nmol/l, $p_2>0.05$).

Table 2. Cortisol content in oral fluid of patients-military personnel of the Armed Forces of Ukraine (main group) with chronic catarrhal gingivitis in relation to their psycho-emotional state

Research groups	Indicator
	Cortisol, nmol/l
Comparison group, (n=11)	12,34±1,56
I subgroup, (n=3)	13,28±2,87
II subgroup, (n=3)	15,50±2,92 ††
III subgroup, (n=9)	22,05±3,08 ••, †

IV subgroup, (n=7)	28,71±3,24 •, *, †

Notes:
•p<0,01; ••p<0,05 – significant difference in values in relation to the data of subgroup I;
*p_1<0,01 – significant difference in values in relation to the data of subgroup II.
†p_2<0,01; ††p<0,05 – significant difference in values in relation to the data of comparison group.

In patients of the main group with high stress resistance and low reactive anxiety (subgroup II), the content of cortisol in oral fluid exceeded the data by 25.60 %, p, p_2<0.05, and increased significantly in patients of the third and fourth subgroups: by 78.68 %, p_2<0.01, p_1<0.05, and by 132.66%, p, p_1, p_2<0.01, respectively.

DISCUSSION

The study of local oral immunity in military personnel with chronic catarrhal gingivitis revealed a significant correlation between the immunity parameters in the oral fluid and the psycho-emotional state of the subjects. In particular, it was found that increased levels of cortisol, which is a marker of stress [17], are accompanied by a decrease in the level of secretory immunoglobulin A (sIgA) and a decrease in the activity of lysozyme in the oral fluid. This suggests that stress has a detrimental effect on local oral immunity [18]. A reduction in secretory immunoglobulin A (sIgA), the primary component of local immunity, renders the oral mucosa more susceptible to pathogens, which may contribute to the progression of gingivitis and other dystrophic-inflammatory diseases of the oral cavity [19]. Furthermore, a reduction in lysozyme activity, which is responsible for the destruction of bacterial cell walls, can additionally impair local immunity [20]. It is important to note that the results confirm the relationship between psycho-emotional state and oral health, which indicates the necessity for an integrated approach to the treatment and prevention of gingivitis in military personnel.

Comparable findings have been reported in research conducted by prominent scientists. [21-23]. In particular, similar changes were recorded by the research team [24] in 2023, who also noted the impact of psycho-emotional stress on the immune parameters of the oral cavity. The results confirm that increased cortisol levels under stress cause suppression of local immunity, which may be one of the factors in the development and exacerbation of chronic catarrhal gingivitis [25]. In light of these findings, it is important to consider the psycho-emotional state of patients when developing strategies for the prevention and treatment of oral diseases. A study by [26] in 2022 also found a correlation between cortisol levels and a decrease in sIgA, confirming the relationship between stress and the state of local immunity. In general, these data indicate the need for an integrated treatment approach, including psycho-emotional support, for patients with chronic catarrhal gingivitis.

Further research will be conducted to examine the influence of anti-stress interventions on cortisol levels and indicators of local immunity in oral fluid, as well as to identify potential avenues for correcting immune disorders through the regulation of the psycho-emotional state. This may prove to be a significant factor in the development of new strategies for the prevention and treatment of chronic catarrhal gingivitis in military patients with a low degree of stress resistance and high levels of reactive anxiety. Therefore, the findings of this study underscore the necessity for a multidisciplinary approach to oral health, encompassing both dental and psychological elements.

CONCLUSION

Thus, summing up the data of laboratory studies, it can be stated that patients of the main group (military personnel of the Armed Forces of Ukraine) with chronic catarrhal gingivitis have a more pronounced weakening of local oral immunity, which is manifested by a decrease in sIgA levels and lysozyme activity, compared with similar data in the comparison group (patients of civilian professions) with chronic catarrhal gingivitis. At the same time, the imbalance of immunological parameters deepened with the deterioration of the psychoemotional state of patients in the main group, which was confirmed by an increase in the level of cortisol in the oral fluid.

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