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Memory of  
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## ORIGINAL ARTICLE

# CHANGES IN IMMUNOLOGICAL INDICATORS UNDER THE INFLUENCE OF TREATMENT OF GENERALIZED PERIODONTITIS IN PATIENTS WITH MANIFESTATIONS OF PSYCHOEMOTIONAL STRESS

DOI: 10.36740/WLek202206118

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**ABSTRACT**

**The aim:** The aim of investigation was to determine the effect of the proposed treatment of patients with generalized periodontitis and psychoemotional stress on changes in the immune system of patients.

**Materials and methods:** The clinical and laboratory investigations were conducted to revealed the effectiveness of the proposal complex of adrenoblockers in complex treatment of generalized periodontitis in 40 patients with psychoemotional stress. To assess the effectiveness of the treatment, the level of cortisol in saliva was determined. 40 patients with chronic course of generalized periodontitis I-II grade were divided into two subgroups: main and control. For the treatment of main subgroup patients used a modified enhanced complex of adrenoblockers to suppress the manifestations of psychoemotional stress.

**Results:** The effectiveness of usage of the proposed complex of adrenoblockers were revealed after treatment of main group patients. The complex of adrenoblockers lead to stabilization of the generalized periodontitis in patients with psychoemotional stress. Such combination of adrenoblockers oppresses main symptoms of psychoemotional stress in with generalized periodontitis as evidenced by the decrease of cortisol levels in the patients saliva.

**Conclusions:** As a result of researches the obtained data testify to achievement of stabilization of pathological process in a periodontium. All this together demonstrates the beneficial effect of the proposed complex of adrenoblockers on the dystrophic-inflammatory process in the periodontium. Decreased levels of cortisol in saliva indicate a decrease in the manifestations of psychoemotional stress in patients with generalized periodontitis.

**KEY WORDS:** generalized periodontitis, psychoemotional stress, cortisol levels in the saliva

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**INTRODUCTION**

Periodontitis is one of the manifestations of chronic inflammation. Many variable risk factors, such as smoking, contribute to increased systemic markers of inflammation through a variety of biological mechanisms [1-3]. The ability of psychological stress to promote the development and progression of the inflammatory response of the periodontal disease has been shown [4]. This interaction is most evident in the adaptive capacity of overload during psychological stress [5].

In the case of the development of chronic psychoemotional stress, there is an increase in circulating neutrophils and functional immunosuppression [6]. Given that chronic psychoemotional stress leads to dysregulation of the immune system with increased levels of cytokines and other pro-inflammatory mediators [7], this may be the cause or progression of periodontal disease under stress.

For the treatment of patients with generalized periodontitis in conditions of psycho-emotional stress was proposed a complex of medicines: Zoxone (0.002 g once a day), Nicergoline (0.005 g three times a day), Sibazone (0.005 g

once a day) [8, 9]. Our previous experimental (biochemical and pathohistological) studies showed a positive influence of the adrenoblocker complex on the development of experimental periodontal lesions [10, 11].

**THE AIM**

The aim of investigation was to determine the effect of the proposed treatment of patients with generalized periodontitis and psychoemotional stress on changes in the immune system of patients.

**MATERIALS AND METHODS**

The clinical and laboratory investigations were conducted to revealed the effectiveness of the proposal complex of adrenoblockers in complex treatment of generalized periodontitis in 40 patients with psychoemotional stress. To assess the effectiveness of the treatment, the level of cortisol in saliva was determined. 40 patients with chronic course of generalized periodontitis I-II grade were divided into two subgroups: main and control.

In the complex treatment of patients of the main subgroup used a modified enhanced complex of adrenoblockers to suppress the manifestations of psychological stress.

For the treatment of main subgroup patients used a modified enhanced complex of adrenoblockers to suppress the manifestations of psychoemotional stress.

Patients in the control subgroup were treated similarly to patients in the main subgroup, but without the use of a modified complex of adrenoblockers. The level of psychological stress was assessed using questionnaires (DASS-21; Spielberger-Khanin) [12]. All patients underwent thorough scaling and root planning of the subgingival surfaces of the tooth roots, so-called SRP therapy. The presence of gingival inflammation was assessed using the Schiller-Pisarev test (1962) and the PMA index according to C. Parma (1961) [13]. The state of oral hygiene was assessed using the OHI-S index (1964) [14] and the O'Leary index [15], the level of gingival bleeding was assessed by the bleeding index PBI (HR Mühlemann, S. Son, 1971) [16]. In general, the condition of the periodontium was assessed using the periodontal index PI by A.L. Russel [16]. The diagnosis of periodontal disease was established according to the classification of NF Danilevsky [14].

Cortisol content was determined in the oral fluid samples. The patients were asked to abstain from taking meals and drinks to hours before taking saliva for test. The oral fluid was collected in the morning on an empty stomach in special Eppendorf tubes.

After the samples were transported to the laboratory, they were centrifuged at 3000 rotations per minute during 5 minutes. In case of necessity saliva samples could be kept at the temperature of 20 °C. 20 µl were taken from saliva and put into the sample's cup. Cortisol content was determined in nmol/L by means of RP Elecsys set (Roche Diagnostics, USA) in the analyzer Cobas e 411 [17, 18].

Venous blood (taken in the morning on an empty stomach) was used as the material to examine immunologic parameters. The blood was in the amount of 5 ml in Vacuum Tubes EDTA.K3 (whole blood). The blood was examined during 2 hours after it had been taken. Lymphocytes were immunophenotyped by means of immunofluorescence analysis using flow laser cytometer BD FACS Canto II (Becton Dickinson, USA).

The results obtained were statistically processed in the packet "STATISTICA 6.1" applying parametric and non-parametric methods [19].

## RESULTS

In our previous investigation [20] was determine possible relations between the level of psychological stress in the organism of a patient with generalized periodontitis, and evaluate the parameters in the immune system cellular part. The study was conducted on the group of 40 patients of a young age. They were distributed into the following 4 subgroups. The first subgroup included 10 clinically healthy individuals without stress signs and healthy periodontal tissues. The second subgroup included 10 patients with gen-

eralized periodontitis and without stress manifestation. The third subgroup included 10 individuals with psychological stress signs without vivid lesions of the periodontal tissue. And the fourth subgroup included 10 patients suffering from generalized periodontitis and psychological stress signs [20].

The patients from the first (control) subgroup did not present stress signs, common somatic diseases, and had clinically healthy periodontal tissues. Cortisol content in the oral fluid was  $14,67 \pm 2,71$  nmol/L. This level was accepted as a norm in case of comparison with parameters of patients from other subgroups.

The second subgroup included patients suffering from I degree generalized chronic periodontitis. The patients from the second subgroup did not present psychoemotional stress signs either. Cortisol content in the oral fluid was  $24,33 \pm 5,47$  nmol/L.

The third subgroup included patients with psychoemotional stress signs and practically unnoticeable lesions of the periodontal tissues. Cortisol content in the oral fluid was  $29,33 \pm 2,55$  nmol/L.

The fourth subgroup included patients suffering from I degree generalized chronic periodontitis and with psychoemotional stress signs. Cortisol content in the oral fluid was  $41,67 \pm 5,67$  nmol/L (Fig. 1).

The parameters of the lymphocyte subpopulation content CD3+, CD4+, CD8+ in patients from different subgroups (first and fourth) differs statistically. Detection of the parameters of the lymphocyte subpopulation content: CD3+, CD4+, CD8+ in this category of patients, demonstrated decrease of their absolute amount. For example, the ratio of CD4+/CD8+ lymphocytes in patients of the fourth subgroup was the lowest, which indicates a decrease in the immune resistance of the patient's organism.

In the complex treatment of patients of the main subgroup used a modified enhanced complex of adrenoblockers to suppress the manifestations of psychological stress. Patients in the control subgroup were treated similarly to patients in the main subgroup, but without the use of a modified complex of adrenoblockers.

After treatment, patients noted an improvement in the condition of periodontal tissues, reduction of gingival bleeding and pain in gums. The color of the gingiva was pale pink, it was dense without swelling. After the treatment there was an improvement in hygienic level in patients of both subgroups. The hygienic index OHI-S improved 3.28 times from  $1.54 \pm 0.14$  points to  $0.47 \pm 0.06$  points. In patients of the main subgroup with the first degree of generalized periodontitis, the hygiene index OHI-S decreased 3.18 times from  $1.57 \pm 0.09$  points to  $0.44 \pm 0.05$  points, in patients with the second degree of generalized periodontitis, it decreased by 3.49 times from  $1.62 \pm 0.16$  to  $0.46 \pm 0.05$  points (Table I). This indicated a good hygienic condition of the oral cavity. In patients of the control subgroup with stage I disease, the index OHI-S decreased by 2.32 times from  $1.72 \pm 0.16$  points to  $0.78 \pm 0.08$  points,

It is possible to estimate a hygienic condition of an oral cavity as a whole by means of the O'Leary index. After



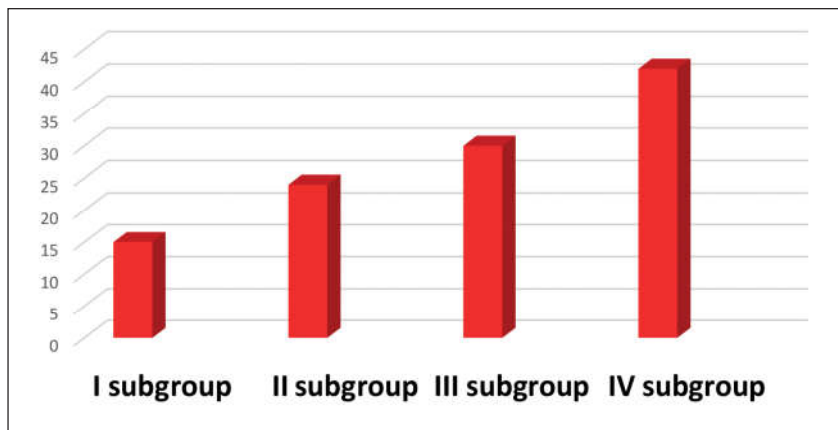


Fig. 1. Graphic representation of cortisol content in oral fluid (nmol/L)

Table I. Indicators of periodontal tissue status in patients with generalized periodontitis with manifestations of psychoemotional stress (M ± m)

Indicators	Timing of the survey	I grade		II grade	
		Study subgroups			
		Main	Control	Main	Control
OHI-S, points	Before treatment	1,57±0,09	1,72±0,16	1,62±0,16	1,82±0,17
	After treatment	0,44±0,05*	0,78±0,08	0,46±0,05*	0,78±0,08
O`Leary index (%)	Before treatment	68,5±5,4	66,4±5,3	75,4±5,8	74,8±5,7
	After treatment	10,4±0,92*	13,7±0,86	11,7±0,95*	15,7±1,2
PMA, %	Before treatment	62,5±2,5	64,1±2,3	65,2±2,5	69,3±2,8
	After treatment	8,2±0,72*	9,8±0,8	9,1±0,7*	12,3±0,9
BOP, points	Before treatment	2,58±0,18	2,51±0,19	2,75±0,18	2,76±0,18
	After treatment	0,54±0,05*	0,78±0,08	0,64±0,05*	0,89±0,08
PI, points	Before treatment	1,97±0,12	2,05±0,11	2,15±0,12	2,19±0,11
	After treatment	1,69±0,11	1,87±0,11	1,81±0,13	2,03±0,12

Note: \* - the data are significantly (p < 0.05) different from the comparison subgroup data.

a comprehensive treatment, the value of the periodontal index O`Leary decreased, which indicated an improvement in the hygienic condition of the oral cavity. In patients of the main subgroup, the periodontal index O`Leary decreased 6.62 times from 70.67±5.7% to 10.67±0.96%. In the case of the first degree of the disease, the value of the O`Leary index decreased 6.45 times from 68.5±5.4% to 10.4±0.92%, in the case of the second degree - it decreased 6.46 times from 75.4±5.8% to 11.7±0.95% (Table 1). In the case of grade I disease in the control subgroup, the value of the periodontal index O`Leary was reduced 6.54 times from 66.4±5.3% to 13.7±0.86%, and in the case of grade II - it was reduced in 5.82 times from 74.8±5.7% to 15.7±1.2%.

Clinically, the improvement of the hygienic state of the oral cavity led to a decrease in the manifestations of inflammation in the gums, as evidenced by the Schiller-Pisarev test. It was negative in 42 (84.0%) patients of the main subgroup: in 26 out of 30 (86.67%) patients with grade I and in 16 out of 20 (80.00%) patients with grade II generalized periodontitis. In numerical terms, it decreased to 1.15±0.09 points (before treatment 2.38±0.25 points) - a decrease of 2.07 times.

The decrease of inflammation in the periodontium was evidenced by changes in the PMA index (Table 1). In pa-

tients with stage I generalized periodontitis, the value of the PMA index decreased to 8.2±0.72% (7.57 times); in patients with grade II PMA index decreased to 9.1±0.7% (7.25 times). After complex treatment, its values decreased in patients of the main subgroup in 7.70 times from 64.25±2.45% and amounted to 8.7±0.78%. In the control subgroup, the value of the PMA index decreased 6.44 times from 67.35±3.51% to 10.46±0.98%; in the first degree of the disease - up to 9.8±0.8%, in the second degree - up to 12.3±0.9%) in 6.54 times and 5.63 times, respectively).

After treatment, the state of oral hygiene improved and the manifestations of inflammation decreased. One of the main indicators of inflammation in the periodontium (gums) is the bleeding on probing index (BOP). Its value in patients of the main subgroup decreased from 2.66±0.19 points to 0.61±0.11 points (4.36 times); in patients with grade I BOP index decreased to 0.54±0.05 points (4.78 times), in the case of the second degree - up to 0.64±0.05 points (4.29 times). In patients of the control subgroup, the bleeding index BOP decreased from 2.76±0.18 points to 0.89±0.08 points (a decrease of 3.1 times).

The periodontal index (PI) can be used to assess the degree of periodontal damage due to the dystrophic-in-

flammatory process. The complex treatment of generalized periodontitis stabilizes the pathological process in the periodontium and the value of the periodontal index does not increase. In patients of the main subgroup, the value of the PI index, which before treatment was  $2.06 \pm 0.13$  after treatment was  $1.88 \pm 0.11$  points (a decrease of 1.16 times). In patients with grade I generalized periodontitis, the value of the PI index was  $1.69 \pm 0.11$  points ( $1.97 \pm 0.12$  points before treatment, a decrease of 1.17 times). In patients with grade II, the PI index was  $1.81 \pm 0.13$  points ( $2.15 \pm 0.12$  points before treatment, a decrease of 1.19 times). In the control group, the periodontal index of PI decreased from  $2.16 \pm 0.14$  to  $1.98 \pm 0.11$  (decrease by 1.09 times):

The clinical investigation results are presented in table. I.

After treatment, the content of cortisol in saliva decreased from  $41,67 \pm 5,67$  nmol/L to almost the normal level -  $19,35 \pm 1,17$  nmol/L (Fig. 2). This indicated a favorable effect during treatment of the adreoblockers complex on the state of psychoemotional stress in patients with generalized periodontitis.

The ratio of CD4+/CD8+ lymphocytes in patients after the treatment rose almost to the level of the norm -  $1,84 \pm 0,18$  (before treatment  $1,71 \pm 0,17$ ), which indicates at satisfactory level of their immune resistance of the patients organism.

## DISCUSSIONS

In clinical investigations D.C. Peruzzo et al. (2007) reported that most studies found a positive association between psychoemotional stress, cortisol levels, and periodontal disease [21, 22]. Manifestations of stress lead to an increase in biomarkers, including cortisol, increased levels of proinflammatory cytokines and blood pressure [23, 24]. High levels of cortisol due to stress adversely affect periodontal tissues.

Our study revealed significant levels of cortisol in saliva in patients with generalized periodontitis and manifestations of psychoemotional stress. In this clinical situation, medical correction of the manifestations of psychoemotional stress is required in the complex treatment of patients with generalized periodontitis. The proposed complex of adreoblockers can reduce the manifestations of psychoemotional stress in patients, as evidenced by the decrease in cortisol levels in the saliva of patients. After treatment the positive changes of the PMA, OHI-S, BOP, PI indices were revealed. Such combination of adreoblockers oppresses main symptoms of psychoemotional stress in with generalized periodontitis as evidenced by the decrease of cortisol levels in the patients saliva.

## CONCLUSIONS

Patients with chronic periodontitis who are under stress have elevated levels of IL-6 and IL-1 $\beta$  in the gingival fluid. Similarly, patients with aggressive forms of periodontitis have elevated serum levels of IL-6 and IL-1 $\beta$ . In contrast, another study failed to find any correlation between IL-6

levels. IL-1 $\beta$  and cortisol in the peripheral blood of patients with aggressive periodontitis.

The condition of the periodontium is associated with changes in the concentration of corticosteroids of the adrenal glands, which change the reactions of oral tissues to bacterial toxins. There is a correlation between psychoemotional stress, periodontal disease (chronic periodontitis) and cortisol levels in the oral fluid, which increases when patients have a state of psychological stress. High levels of cortisol due to stress have a negative effect on periodontal tissues.

As a result of researches the obtained data testify to achievement of stabilization of pathological process in a periodontium. All this together demonstrates the beneficial effect of the proposed complex of adreoblockers on the dystrophic-inflammatory process in the periodontium. All this together demonstrates the beneficial effect of the proposed complex of adreoblockers on the dystrophic-inflammatory process in the periodontium. Decreased levels of cortisol in saliva indicate a decrease in the manifestations of psychoemotional stress in patients with generalized periodontitis.

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#### Conflict of interest:

*The Authors declare no conflict of interest.*

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