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IMMUNOLOGICAL INDICATORS IN PATIENTS WITH GENERALIZED PARODONTITIS AND PSYCHOEMOTIONAL STRESS

Abstract. Psychological stress of patients promotes development of periodontal disease. It should be considered in a comprehensive treatment of patients with periodontal disease. Objective: to 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. Material and methods. The study was conducted on the group of 40 young patients. They were divided into the following 4 subgroups, 10 people each. The first group: clinically healthy individuals with no manifestations of stress with clinically healthy periodontal tissues. The second group: patients with generalized periodontitis without manifestations of stress. The third group: persons with manifestations of psychological stress without visible lesions of the periodontal tissues. The fourth one: patients with generalized periodontitis and manifestations of psychological stress. The level of psychological stress was assessed using questionnaires. All patients with generalized periodontitis were subjected to a thorough clinical examination of the oral cavity. Cortisol content was determined in the oral fluid samples using the RP Elecsys kit (Roche Diagnostics, USA). Immunophenotyping of lymphocytes was performed in venous blood of patients. Results. The lowest level of cortisol in saliva was found in patients without clinical signs of the disease - 14.67 ± 2.71 nmol/l. The presence of periodontal disease slightly increases the level of cortisol to 24.33 ± 5.47 nmol/l. In case of psychoemotional stress, the level of cortisol increases to 29.33 ± 2.55 nmol/l. When psychoemotional stress is combined with generalized periodontitis, the level of cortisol increases to 41.67 ± 5.67 nmol/l. Determination of the parameters of subpopulation composition of lymphocytes: CD3+, CD4+, CD8+ in this category of patients showed a decrease in their absolute number in patients with periodontal disease (generalized periodontitis - 2nd subgroup), psychoemotional stress (3rd subgroup) and in case of combination of periodontal disease and psychoemotional stress (4th subgroup). Most of these changes are pronounced in patients of the 4th subgroup in case of combination of periodontal disease and psychoemotional stress. Determination of the parameters of subpopulation composition of lymphocytes: CD3+, CD4+, CD8+ in this category of patients showed a decrease in their absolute number in patients with periodontal disease (generalized periodontitis - 2nd subgroup), psychoemotional stress (3rd subgroup) and in case of combination of periodontal disease and psychoemotional stress (4th subgroup). Most of these changes are expressed in patients of the 4th subgroup in case of combination of periodontal disease and psychoemotional stress. Conclusions. The conducted research enables to confirm a certain relationship between the manifestations of psychoemotional stress, periodontal disease (generalized periodontitis) and cellular immunity. Such a condition requires appropriate medical treatment of this group of patients with generalized periodontitis and manifestations of psychoemotional stress.

Key words: generalized periodontitis, psychoemotional stress, cortisol, cellular immunity.

Interrelations between opportunistic microorganisms of the biofilm and patient's (host's) organism are important in pathogenesis of generalized periodontitis [13]. Immune response of the host's organism is essential in these interrelations [5]. Many various factors influence on them, namely, general somatic diseases, social-economic condition of the patient, smoking and psychological stress. Psychological stress, in particular, is an

important factor of development of many pathological conditions including periodontal diseases [9, 14, 17]. Available stress results in decrease of immune resistance and development of clinical important disorders of immune response [3, 8].

One of objective parameters determining availability of psychological stress and reflects the level of immune protection is cortisol content in the

oral fluid [2, 11]. Detection of cortisol level in the oral fluid enables to find certain relations between the level of stress and periodontal diseases.

Changes occurring in the parameters of the functional activity of the immune system cellular part are an important factor of psycho-emotional stressors [1, 5]. They are affected by stress parameters, state of the anti-stress system, determining peculiarities of immunologic response, which is valuable for the selection of therapeutic tactics of a patient with generalized periodontitis.

Objective: to 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.

Materials and methods. 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 generalized 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 group included 10 patients suffering from generalized periodontitis and psychological stress signs.

The level of psychological stress was assessed using questionnaires (DASS-21; Spielberg-Hanin's scale). [12, 16]. The examined patients filled in Spielberg-Hanin's questionnaire. It helps to determine personal and situational anxiety. The answers were estimated according to the keys, the total score was calculated considering all the scales separately (situational anxiety scale and individual anxiety scale).

During examination of the oral cavity the color and consistency of the mucous membrane of the vestibule, its depth, condition and height of frenula attachment were evaluated. The state of the mucous membrane of the cheeks, soft and hard palates, tongue and oral fundus was determined as well. The gums were examined from the vestibular and oral sides. The color of the gums, availability of lack of swelling, consistency, and relief of the gingival margin were determined. Availability, localization and intensity of inflammatory process were determined by means of Pisarev-Schiller's test

[6, 7]. Special attention was paid to dental deposits: their condition, consistency, amount and localization. To find dental deposits (dental plaque) diagnostic dyes were used. Oral hygiene was evaluated by means of Green-Vermillion index (1964). PMA index was used to determine the degree of gingival inflammation [6, 7].

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 mcl 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 Cobase 411 [4, 15].

Venous blood was used as the material to examine immunologic parameters. The blood was taken in the morning on an empty stomach 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) after an automatized preparation of the whole blood samples by BD FACS Sample Prep Assistant II (Becton Dickinson, USA). An absolute (10^9 cells/L) and relative (%) content of certain parameters of the lymphocyte subpopulation content were determined: CD3+, CD4+, CD8+. The ratio CD4+/CD8+ (T-helpers/T-suppressors) was further determined.

The results obtained were statistically processed in the packet "STATISTICA 6.1" applying parametric and non-parametric methods. Correct distribution of signs according to every variation series, mean values according to every signs and their standard errors and deviations were estimated [10].

Results. The patients from the first (control) subgroup did not present stress signs, common somatic diseases, and had clinically healthy periodontal tissues. Their level of oral hygiene was close to excellent, and hygiene index was on an average 0,15. Inflammation of gums was practically lacking. Yellowish color was found in certain parts of

the gums only. PMA index was on an average 4,7%. Cortisol content in the oral fluid was 14,67±2,71 nmol/L. This level was accepted as a norm in case of comparison with parameters of patients from other groups.

The second subgroup included patients suffering from I degree generalized chronic periodontitis. The patients from the second subgroup did not present psycho-emotional stress signs either. Their level of oral hygiene was close to good, and hygiene index was on an average 1,33. Cortisol content in the oral fluid was 24,33±5,47 nmol/L.

The third subgroup included patients with psycho-emotional stress signs and practically unnoticeable lesions of the periodontal tissues. Inflammation of gums was practically absent, only yellowish color was found in certain parts of the gums. Cortisol content in the oral fluid was 29,33±2,55 nmol/L.

The fourth subgroup included patients suffering

from I degree generalized chronic periodontitis and with psycho-emotional stress signs. Their level of oral hygiene was close to good, and hygiene index was on an average 1,57. Inflammation of gums was not considerable. OMA index was on an average 38,6%. Cortisol content in the oral fluid was 41,67±5,67 nmol/L.

Therefore, association of psycho-emotional stress with periodontal diseases, namely, generalized periodontitis, results in the most considerable increase of cortisol level in the oral fluid – up to 41,67±5,67 nmol/L. Its content differs statistically reliably ($p < 0,05$) from cortisol level of patients in other subgroups.

The parameters of the lymphocyte subpopulation content CD3+, CD4+, CD8+ in patients from different groups are presented in Table 1. The parameters of the first group were used as control values for the parameters obtained in patients from other groups.

Table 1

Content of lymphocyte subpopulations in the examined patients (M ± m)

Parameters		I subgroup	II subgroup	III subgroup	IV subgroup
CD3+	%	37,4±3,7	37,9±3,8	40,3±4,1	41,2±4,2
	10 ⁹ kl/L	0,65±0,21	0,62±0,23	0,59±0,23	0,52±0,23
CD4+	%	12,4±1,3	10,9±3,3	9,17±3,21	8,67±3,21
	10 ⁹ kl/L	0,17±0,07	0,15±0,06	0,13±0,06	0,12±0,05
CD8+	%	5.3±1,7	5.2±1,7	2,52±2,12	1,30±1,15
	10 ⁹ kl/L	0,08±0,07	0,08±0,07	0,07±0,07	0,07±0,07
CD4+/CD8+		1.95±0,17	1,86±0,17	1,86±0,17	1.71±0,17

Detection of the parameters of the lymphocyte subpopulation content: CD3+, CD4+, CD8+ in patients from the second subgroup (suffering from I degree generalized chronic periodontitis) showed decrease of their absolute amount (Table 1). An absolute amount of CD3+ lymphocytes, for example, statistically 4,62% decreased from 0,65±0,21 (10⁹ kl/L) to 0,62±0,23 (10⁹ kl/L). An absolute amount of CD4+ lymphocytes, for example, statistically 11,76% decreased from 0,17±0,07 (10⁹ kl/L) to 0,15±0,06 (10⁹ kl/L). An absolute amount of CD8+ lymphocytes remained on the level similar to that of the control group - 0,08±0,07 (10⁹ kl/L). Parameter CD4+/CD8+ (T-helper/T-suppressor) unreliably 3,08% decreased from 1.95±0,17 to 1,86±0,17.

The fourth group included patients suffering from I degree generalized chronic periodontitis and

with psycho-emotional stress signs. Detection of the parameters of the lymphocyte subpopulation content: CD3+, CD4+, CD8+ in this category of patients, demonstrated decrease of their absolute amount (Table 1). An absolute amount of CD3+ lymphocytes, for example, statistically unreliably 20,00% decreased from 0,65±0,21 (10⁹ kl/L) to 0,52±0,23 (10⁹ kl/L). An absolute amount of CD4+ lymphocytes statistically unreliably 29,41% decreased from 0,17±0,07 (10⁹ kl/L) to 0,12±0,05 (10⁹ kl/L). An absolute amount of CD8+ 12,5% decreased from 0,08±0,07 (10⁹ kl/L) to 0,07±0,07 (10⁹ kl/L). The parameter CD4+/CD8+ (T-helpers/T-suppressors) 12,31% unreliably decreased from 1.95±0,17 to 1,71±0,17.

Conclusions. The conducted investigation enables to confirm certain interrelations between psycho-emotional stress signs and periodontal

diseases – generalized periodontitis. It should be noted that cortisol level in the oral fluid is more affected by psychological stress available than pathological process in the periodontal tissue. Certain interrelations between psycho-emotional stress signs, periodontal diseases (generalized periodontitis) and cellular links of immunity are confirmed.

Therefore, the conducted investigation enables to suggest a negative effect of psycho-emotional stress on the periodontal tissue. This condition requires an appropriate pharmacological treatment of this group of patients suffering from generalized periodontitis with psycho-emotional stress signs.

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