http://dx.doi.org/10.35630/2199-885X/2021/11/2/24

IMMUNOLOGICAL ASSESSMENT OF THE STRESS RESPONSE IN PATIENTS WITH INFLAMMATORY POSTPROTHETIC COMPLICATIONS

Received 11 March 2021;
Received in revised form 25 April 2021;
Accepted 3 May 2021

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ABSTRACT — Post-prosthetic complications during dental implantation are accompanied by pain symptoms leading to disorders of the psychoemotional state. All this influences the behavior of patients. In addition, psychoemotional stress is often a factor of provocation and persistence of the complications. The presence of a stress state in the identified pathology, as well as the influence of various therapies on the treatment of post-prosthetic complications, is reflected in the dynamics of changes in the concentrations of both catecholamines (epinephrine, norepinephrine) and glucocorticoids (cortisol) — hormones of the medulla and the adrenal cortex. THE AIM: to conduct an immunological analysis of the stress response in patients with post-prosthetic complications during dental implantation. MATERIALS AND METHODS: The study was performed in 120 patients with post-prosthetic complications during dental implantation before and during treatment: Group I (control) — 30 patients treated with the conventional therapy; group II — 30 patients treated with ozone therapy in addition to the conventional therapy; group III — 30 patients treated with transcranial electrical stimulation in addition to the conventional therapy; Group IV — 30 patients treated with a combination of conventional therapy, ozone therapy and transcranial electrical stimulation. The concentration of epinephrine, norepinephrine, cortisol, alkaline phosphatase, and the Garkavi index were evaluated. RESULTS: The change in these indicators after the treatment indicates the normalization of the level of the studied enzymes — markers of bone homeostasis, which is confirmed by an improvement in the clinical picture in the oral cavity. CONCLUSION: changes in immunological parameters objectively reflect the psychoemotional state of patients. The nature of changes in the hormonal stress response to the treatment of post-prosthetic complications indicates the effectiveness of the therapeutic regimens used, and, as a result, a decrease in both pain symptoms and psychoemotional stress.

KEYWORDS — epinephrine, norepinephrine, inflammation, implants, treatment.

RELEVANCE

As a response to the impact of such aggravating factors as pain, blood loss [7], mechanical tissue damage [1–5], hypoxia [6], increased free radical oxidation [8, 9], a non-specific neuroendocrine reaction appears [10]. Consequently, the influence of different types of therapeutic approaches for the treatment of post-prosthetic complications is reflected in the dynamics of changes in serum concentrations of both catecholamines (epinephrine, norepinephrine) [11] and glucocorticoids (cortisol) — hormones of the medulla and the adrenal cortex [12]. Determining the level of cortisol is an important indicator in the diagnosis of a stressful situation. Increased cortisol levels are observed with a decrease in psychomotor activity, sleep disorders, and depression. The reference values of cortisol in the blood serum are 200–700 nmol/l at 8.00 a.m. and 55–250 nmol/l at 20.00 pm. The difference between the concentrations in the morning and in the evening should be at least 100 nmol/l.

The biochemical catalyst for stress is epinephrine, which is released by the adrenal glands. It activates glycogenolysis in muscle cells, rises heart rate and blood pressure, dilates blood vessels of heart muscles and stimulates the production of ACTH and glucocorticoids. The level of adrenaline in the blood depends on the tone of the sympathetic system. Its content in the blood plasma should not exceed 112–658 pg/ml.

The mediator of the sympathetic nervous system is the hormone of the adrenal medulla — norepinephrine. A decrease in the level of the ratio of norepinephrine to adrenaline in the urine of patients may indicate their depressive states. In severe depression, the level of the norepinephrine metabolic product - 3-methoxy-4hydroxyphenylglycol is often reduced, and the amount of the serotonin derivative — 5-hydroxyindolacetic acid is reduced in the cerebrospinal fluid of these patients. An increase in the daily excretion of epinephrine and a decrease in the release of norepinephrine indicate a depressive phase of manic-depressive psychosis, and a manic state is characterized by a multiple increase in the excretion of norepinephrine. Both in mania and in depression, the blood content of adrenaline in patients is increased.

A decrease in the level of norepinephrine leads to an increase in the level of cortisol, and a deficiency of serotonin, which is accompanied by a disruption in regulation of cortisol secretion. The lack of both leads to disruption of circadian rhythms in the secretion of cortisol. The reference values of the content of norepinephrine in the blood plasma should not exceed 10 pg/ml.

The aim of our study was to evaluate the stress response of patients with inflammatory postprothetic complications against the background of various pharmacotherapy methods according to immunological parameters.

MATERIALS AND METHODS

The study included the diagnosis and follow-up of 120 patients aged 18–44 years (WHO average age) with post-prosthetic complications during dental implantation before and during treatment. The patients were randomized into groups according to the treatment regimen: Group I (control) — 30 patients who were treated with conventional treatment; group II — 30 patients who were treated with ozone therapy in addition to conventional treatment.; Group III — 30 patients, in the complex treatment of which, in addition to traditional therapy, transcranial electrostimulation was performed; group IV — 30 patients, whose complex treatment included a combination of traditional therapy, ozone therapy and transcranial electrostimulation.

Based on the general and biochemical blood analysis, the indicators that allow us to assess the stressful situation in the study groups (cortisol concentration, alkaline phosphatase, Garkavi index, epinephrine, norepinephrine) were determined.

General and biochemical blood tests were performed using a laboratory test. The leukocyte formula, the concentration of glucose, creatinine, total protein and other indicators were studied. The type of adaptive response (stress, quiet activation, re-activation) was determined by the percentage of lymphocytes in the peripheral blood, taking into account age. The Garkavi index was used as a basis.

Garkavi index = lymphocytes (%) / segmental neutrophils (%);

In healthy adults, this indicator is within the values of 0.3–0.5.

To determine the alkaline phosphatase (ALP) in the blood serum, a spectrophotometric method was used using reagents from the company "Human" (Germany) and a calibration curve constructed from a standard solution of p-nitrophenol. The method is based on the following reaction:

alkaline phosphatase

p-nitrophenyl phosphate + H_2O – " p-nitrophenol+ phosphate

The measurement was carried out at a temperature of 37° C and a wavelength of Hg 405 nm (400–420 nm). The average change in optical density per minute (AA/min) was calculated. The activity of the alkaline phosphatase was calculated by the formula

E / l = AA/min x 2757

Blood samples of the patients in the volume of 5 ml were obtained from 6.00 to 10.00 a.m. on an empty stomach (Fig. 1).

On the eve of the examination, patients were advised to refrain from overeating, eating salty, spicy, fatty foods, exclude alcohol, and do not smoke an hour before the examination. The biomaterial was collected before undergoing instrumental examination and physiotherapy. Venous blood obtained without anticoagulants in a centrifuge glass tube is settled in it at room temperature, then centrifuged for 10 minutes at 2500 rpm.

Repeated blood tests to monitor the dynamics of indicators were carried out under the same conditions (time, food regime) and in the same laboratory, since the blood sampling algorithm, study methodology and reference values (norms) may differ significantly in different medical institutions.

The results were evaluated on day 3, 7, 14, and 1 month later. Two-factor analysis of variance (RANOVA) with the Newman-Keuls post-hawk test was performed; * — $p<0.05$; ** — $p<0.01$;*** — $p<0.001$.

The clinical trial was approved by the Regional Ethics Committee, Protocol No. 2115/1-2019 of April 19, 2019.

RESULTS

Before the start of therapy, no statistically significant differences in the studied parameters were found between the groups. At the same time, for all certain hormones, a statistically significant decrease was observed as a result of therapy. The study revealed a relative increase in the concentrations of hormones (epinephrine, norepinephrine, cortisol) before the start of therapy, which reflects the overall clinical picture of the stress response. At the same time, there were no statistically significant differences between the groups before the start of therapy. The content of epinephrine varied in the range of 127.047±19.4024 pg/ml, norepinephrine 381.3607±40.2137 pg/ml, cortisol 430.62±40.275 nmol/L.

When determining the content of epinephrine in all groups, there was a decrease in its concentration at a more intense rate following the combined method of treatment (Fig. 2).

The decrease in the concentration of epinephrine in the blood serum occurred up to the 5th control point (1 month). At the same time, the treatment regimen combined with ozone therapy showed a significantly greater decrease (59.348 vs 73.438; p<0.01), but for groups III and IV, the differences were even higher (59.348 vs 58.348 and 55.406, respectively, p<0.001). At the same time, in the nearest follow-up period (up to 14 days), the lowest values were typical for groups II and IV (p<0.01 and p<0.001, respectively). For norepinephrine, similar dynamics were observed, while the differences with the comparison group (conventional therapy) were less pronounced. At the same time, the dynamics of the greatest decrease in the concentration of norepinephrine in the immediate follow-up period (3–30 days) was shown for the treatment regimen combined with ozone therapy (3, 14, 30 days — p<0.01; 7 days — p<0.001), similar values were found for group IV (3, 7, 14, 30 days — p<0.01). The dynamics of changes in concentrations characteristic of epinephrine and norepinephrine and the revealed tendency to decrease in the near-term follow-up were also shown for changes in serum cortisol concentrations, regardless of therapeutic regimens. And by day 14 of the therapy they were significantly lower relative to the start of therapy and 3 days (p<0.05). This was followed by a slight increase in these parameters. The inter-group differences for cortisol were similar to those for norepinephrine, but the lowest values were observed at the 4th control point for all groups. The dynamics of the greatest decrease in cortisol concentration in the nearest follow-up period (3–30 days) was observed in group IV (7, 14, 30 days — p<0.01; 3 days — p<0.001). Similar values were found for group II (3, 7, 14, 30 days — p<0.01). An increase in the Garkavi index which reflects the relationship between the humoral and cellular components of immunity, an assessment of the stress state, as well as an assessment of the adaptive responses of training and activation, was found in all study groups, whereas no significant differences were found between the groups. The average value was 0.687±0.05. In the course of all types of therapy normalization of the index was achieved by 14 days. A stable decrease in the index in all groups during the first 2 weeks indicates a decrease in the active inflammatory process. Thus, the nature of changes in the hormonal stress response to the treatment of post-prosthetic complications indicates the effectiveness of the therapeutic regimens used, and, as a result, a decrease in both pain symptoms and psychoemotional stress. In the near term of observation, there is a significant decrease in the concentrations of the studied hormones (catecholamines and corticosteroids), normalization of the values of the Garkavi index (Table 1–4).

Fig. 1. *Venous blood sampling*

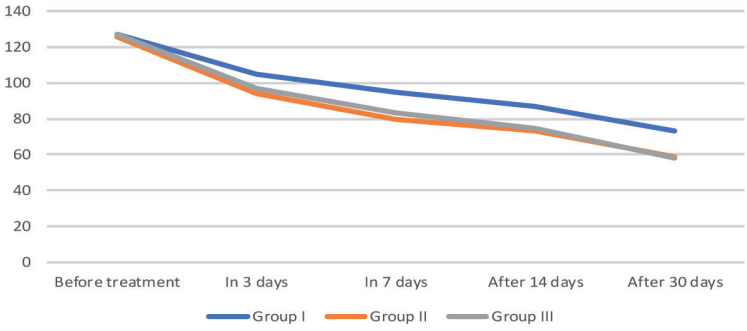

Fig. 2. *Dynamics of changes in the concentration of epinephrine during 1 month of observation*

The concentration of epinephrine in group I significantly decreased until 1 month after the start of therapy.

Evaluating the Garkavi index, a stable decrease in the index was revealed in all groups for 2 weeks which indicates a decrease in the active inflammatory process. Normal values of the index were maintained on day 30.

The parameters of epinephrine concentration in group II (in the complex treatment, ozone therapy was additionally used), group III (additional use of transcranial electrical stimulation) and in group IV (a combination of methods of groups 1–3) behaved similarly to the first, but the decrease to 1 month from the start of therapy was more pronounced.

Thus, the analysis of the dynamics of the content of stress hormones in the blood serum showed a characteristic and significant decrease in the concentrations of hormones up to 1 month, followed by a slight increase in concentrations. These trends indicate the effectiveness of the therapeutic regimens used, and as a result, a decrease in both pain symptoms and psychoemotional stress. And taking into account the relatively

Table 1. *Immunological examination of blood in patients of group I*

Signs/ deadlines	Cortisol		SCHF		Index Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	420,400000	38,760000	118,00000	10,75200	0,687400	0,05000	127,35840	20,14200	381,348600	40,354800
3 days	362,286300	30,698040	122,59390	8,75520	0,561200	0,03470	105,34580	16,35400	362,286300	20,252820
7 days	266,626500	26,680250	122,56790	6,32016	0,523040	0,03280	94,86240	16,43800	266,626500	22,801480
14 days	215,480000	19,364800	133,25590	9,21600	0,437300	0,03760	86,96423	15,34600	215,480000	18,258790
1 month	29,881800	20,482120	145,99580	9,02880	0,450300	0,05380	73,43840	13,24580	219,881800	17,504430

Table 2. *Immunological examination of blood in patients of group II*

Signs/ deadlines	Cortisol		SCHF		Ind.Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	435,240000	42,180000	117,000	8,96000	0,686900	0,057400	126,78210	19,823760	382,103500	39,246800
3 days	340,027200	30,742150	126,677	11,85030	0,527028	0,0346653	93,81634	15,079810	343,927200	19,546140
7 days	245,058900	26,718590	127,644	9,59310	0,4804876	0,0327572	80,35800	15,348600	244,058900	22,005870
14 days	194,078500	15,395500	141,248	9,21600	0,399419	0,035524	73,34680	15,103530	196,078500	17,621690
1 month	198,124800	20,511550	148,918	10,72170	0,4114996	0,0537462	59,34861	13,036520	200,124800	16,893650

Table 3. *Immunological examination of blood in patients of group III*

Signs/ deadlines	Cortisol		SCHF		Ind.Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	429,090000	40,740000	120,000	10,2400	0,689800	0,054600	127,146300	19,632050	380,904600	40,348030
3 days	343,209000	26,046490	127,594	11,2860	0,526056	0,03283619	97,346800	16,914630	345,209000	16,588630
7 days	247,097700	22,244850	128,568	12,0960	0,5045652	0,04050112	83,346000	15,346800	249,097700	18,676170
14 days	196,486000	14,215370	142,256	9,8503	0,4018549	0,0450433	75,348600	14,957470	198,486000	14,955360
1 month	199,642800	18,380180	149,996	9,2160	0,4154934	0,05091029	58,348120	12,910440	202,642800	14,337480

Table 4. *Immunological examination of blood in patients of group IV*

Signs/ deadlines	Cortisol		SCHF		Ind.Garkovi		Adrenaline rush		Norepinephrine	
	M	SD	M	SD	M	SD	M	SD	M	SD
Before treatment	437,750000	39,420000	119,000000	12,80000	0,687045	0,052400	126,907500	18,001710	381,086200	40,905370
3 days	336,507200	30,423910	131,421800	12,09600	0,5170252	0,02936557	94,354860	15,509960	342,119200	18,921110
7 days	242,522000	26,442000	132,425000	9,21600	0,4854849	0,03622036	76,365860	15,124030	245,351900	21,302180
14 days	192,069400	15,236130	144,463700	9,59310	0,3994168	0,04028245	69,648560	13,715330	195,750400	17,058190
1 month	196,073800	20,299220	154,495900	9,59310	0,4111898	0,04552932	55,406310	11,838300	199,851300	16,353440

maximum decrease in the concentrations of the presented hormones for group IV, combining traditional therapy with ozone therapy and transcranial electrical stimulation, for which there were large inter-group differences relative to traditional therapy both in the near and long-term follow-up periods, it can be argued

that this scheme was most effective in the treatment of post-prosthetic complications.

An increase in the content of alkaline phosphatase up to 1 month and a further retention of the values in the plateau phase may indicate that the use of therapy, especially combined with ozone therapy and transcranial electrical stimulation, activates the processes of connective tissue synthesis.

The results of the obtained values indicate a high level of psychological stress in groups of patients with different therapy for post-prosthetic complications of dental implantation at different time intervals. Based on the data obtained, it can be argued that the changes in the concentrations of epinephrine, norepinephrine, alkaline phosphatase, and cortisol detected at the beginning of therapy fully fit into the picture of the immune response under psychophysiological stress and objectively reflect the changes in the psychophysiological state of patients with postprothetic complications.

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