

Complex Prophylaxis and Prognosis of Inflammatory Complications after Dental Implantation

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Abstract

Aim and Scope: The main task of dental implantation at the modern stage of its development remains the struggle against complications. At present, there is no generally accepted set of biomarkers and an algorithm for their use for early diagnosis of the risk of complications and monitoring of osseointegration. The objective of the study is to increase the effectiveness of dental implantation by developing a method of complex prophylaxis and prediction of development of complications in the long-term perspective after the operation. **Materials and Methods:** A method of preventing the development of complications of dental implantation by applying a complex of antioxidant vitamins and amino acids was approved and introduced. **Results and Discussion:** The significance of serum and local indices of the cytokine profile as diagnostic and prognostic parameters in patients with peri-implantitis developed after the operative intervention, was determined. It was found that patients with simultaneous installation of 5 or more implants, in spite of competent surgical work, are at higher risk of complications development in the long-term due to the presence of peculiarities of the mediator reaction with the prevalence of local inflammation processes. **Conclusion:** Perhaps a promising direction of prevention in the future will be the development of methods for targeted delivery of antioxidant substances to tissues to fill the deficit of protective components and establish an equilibrium.

Key words: Antioxidant potential, dental implantation, interleukin status, mediator response, myeloperoxidase, nitrate-nitrites, peri-implantitis

INTRODUCTION

At present, dental implantation is one of the main directions in denture prosthetics.^[1-6] It occupies an important place in esthetic correction of the face of patients of any age.

However, despite the fact that implantology has been characterized by a high level of technology development in recent years, more and more information about the risk of development of early or distant complications appears in the scientific literature.^[3,7-9] The number of

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disintegrations of implants, according to various authors, varies from 3% to 10%.^[10]

Prevention of inflammation, which largely determines the course of regeneration process or the implant rejection and consequently, the long-term prognosis of dental implantation, is of great importance for improving quality of the performed implantation.^[11] The use of modern digital methods of diagnostics - orthopantomography (OPTG), multispiral computed tomography (MSCT), cone-beam computed tomography - is highly informative but allows to actually register already developed changes in soft tissue and bones.

One of the important tasks of modern dentistry is the search for indicators that would predict the development of complications of dental implantation^[12] as well as the development of a set of preventive measures that increase the duration of implant operation and improve quality of dental care for patients.^[10]

The objective of the study is to increase the effectiveness of dental implantation by developing a method of complex prophylaxis and prediction of development of complications in the long-term perspective after the operation.

The main tasks of the research are:

1. To study the cytokine profile (interleukin [IL]-1 β , IL-6, IL-10, IL-17A, and CD401) of blood and gingival fluid of patients with peri-implantitis of varying severity developed in the long-term period after implantation (from 6 months to 3 years).
2. To reveal the change in activity of tissue myeloperoxidase and concentration of nitrate nitrites as indices of radical tissue damage of patients with peri-implantitis that developed during long-term after implantation.
3. To discover the peculiarities of mediator response at the local and systemic levels when installing a different number of implants.
4. To assess the possibility of using the studied biomarkers in predicting the development of complications.
5. To investigate the possibility of preventing the development of complications in dental implantation with the help of a complex of antioxidant vitamins and amino acids.

The necessity of determining local indices of the cytokine profile as diagnostic and prognostic criteria for effectiveness of dental implantation has been scientifically substantiated. For the first time, it was revealed that, alongside with imbalance between the activity of pro-inflammatory and anti-inflammatory mediators, the long-persistent elevated concentration of CD401 and IL-17A plays a pathogenetically significant role. For the first time, diagnostic criteria have been determined, which allow to predict violation of reparative osteogenesis processes and to carry out their prevention. For the first time, an increase in the activity of tissue myeloperoxidase in tissue samples taken from the

peri-implant area during the inflammatory process was observed against a background of a decrease in the antioxidant tissue potential. The presence of features of the mediator reaction with the prevalence of local inflammation processes in patients with a large number of simultaneously established dental implants was first established and scientifically proven (5 and more implants). It has been scientifically proven the presence of a positive effect of a complex of antioxidant amino acids and vitamins in the complex prevention of complications of dental implantation.

MATERIALS AND METHODS

During completion of work on the development of a method for early prediction and prevention of complications after dental implantation, a comprehensive assessment of factors affecting the success of the operation in 135 patients was carried out.

Clinical characteristics of studied groups of patients

Groups of patients with peri-implantitis developed in the long-term after installation of dental implants (from 6 months to 3 years or more)

A comprehensive examination of 32 patients (19 men and 13 women, that is 59.4% and 40.6%, respectively), operated in various clinics of the country and applied to the department of the CFC RCH and the KBSU KBGU for complications after dental implantation. The average age of patients was 43 years and ranged from 29 to 57 years.

Patients were divided into two groups according to severity of the peri-implantitis:

First group - 17 patients (53.1%) with moderate-sized peri-implantitis (corresponding to Class III according to classification of S. A. Jovanovic (1990) and N. Spiekermann (1991));

Second group - 15 patients (46.9%) with moderate-sized peri-implantitis (corresponding to Class III according to classification of S. A. Jovanovic (1990) and N. Spiekermann (1991)).

Before diagnosing, all patients underwent an objective examination, which included a survey, an external examination, determination of the local status, palpation, sounding, sighting, OPTG. With a peri-implantitis, X-ray pattern does not always reflect the true picture of the bone tissue state, so in doubtful cases, MSCT with the dental program was used to detail bone changes.

For treatment of developed peri-implantitis, conservative and surgical methods were used. All patients underwent professional oral hygiene with Sirona ultrasound scalers

and abrasions. Next, photodynamic therapy was applied using a laser device Sirolaser Advance (Sirona, Germany). Technical specifications: Wavelength: 980 nm; operation mode: Continuous (CW)/pulse; power: 0.5–7 W; frequency range: 1 Hz – 10 kHz. Number and duration of procedures were determined individually, depending on the severity of the inflammatory process.

With peri-implantitis of a severe degree, implant surface cleaning and osteoplasty were carried out. For operative access, the mucus-periosteal flap was casted away, or a mucosal incision was made along the projection of the pathological focus. Revision of the bone pocket was carried out by removing the peri-implantitis granulation tissue and infiltrated epithelium. The surface of the dental implant was cleaned with plastic abrasions. Disinfection of the implant surface and washing of the pocket were carried out with sodium hypochlorite 0.06%. In the future, osteoconductive material (Bio-Oss, Geistlich, granules 0.25–1 mm) and a barrier membrane (Bio-Gide Perio, Geistlich, 16*22 mm) were introduced into the bone pocket, followed by suturing the wound and applying a periodontal bandage. Patients were prescribed rinsing with solutions of antiseptics (chlorhexidine 0.05%) and antibacterial therapy.

Six patients of the second group (with severe peri-implantitis localized in the region of two and four adjacent teeth) had their implants removed due to significant bone resorption and agility of implants in three directions.

Determination of concentrations of IL-1 β , IL-6, IL-10, IL-17A, and CD401 (pg/ml) in the blood serum and the content of gingival sulcus of teeth adjacent to implants of patients with peri-implantitis was performed in the acute period of the disease. As indicators of oxidative stress of patients of this group, the level of nitrate nitrites (μ M/ ml) and myeloperoxidase (mmol/g of protein) in the acute period of the disease was investigated. The determination of antioxidant tissue activity in the Fenton reaction was also performed once in the acute period of the disease.

Groups of patients with different number of implants installed

For a follow-up implant treatment, 103 patients (57 men and 46 women, 55.3% and 44.7%, respectively) underwent a comprehensive examination at the KBSU KBGU and the department of the CFC RCH for partial secondary adentia. The average age of patients was 47 years and ranged from 28 to 66 years.

Patients were divided into three groups according to the number of implants installed:

First group - 24 patients with 1–2 implants, among them 13 men and 11 women aged from 28 to 62 years (23.3%).

Second group - 28 patients with 3–4 implants, among them 16 men and 12 women aged from 38 to 66 years (27.2%).

Third group - 51 patients with 5 and more implants, among them 28 men and 23 women aged from 39 to 61 years (49.5%).

Before deciding on the operation of dental implantation, a comprehensive examination was carried out for all patients, including:

- Collection of anamnesis (concomitant diseases, allergic anamnesis, etc.);
- General clinical examination (general blood test, general urine test, coagulogram, electrocardiogram, fluorography of chest organs, and consultations of focused specialists on indications);
- Definition of local status;
- Definition of indices of oral hygiene (the index of effectiveness of oral hygiene [patient hygiene performance] (Podshadley, Haley, 1968), simplified index simplified oral hygiene index (J. C. Green, J. R. Vermillion, 1964);
- Detection of presence of diseases of hard tissue of teeth;
- Detection of malocclusion;
- Identification of presence of teeth and roots to be removed;
- Study of periodontal tissues with determination of periodontal indices “community” periodontal index, gingival index (H. Loe, J. Silness, 1964), and papillary - marginal - attachment (in modification of C. Parma, 1960);
- Evaluation of condition of alveolar processes (width, degree of atrophy, interalveolar height in the area of lost teeth);
- Assessment of mucosa (depth of the mouth vestibulum, presence of keratinized mucosa, and the presence of strings that help to bare the neck of the implant).
- Evaluation of the temporomandibular joint dysfunction;
- X-ray examination (OPTG, MSCT with dental program).

Further on, planning stages of treatment and preparation for implantation (according to the plan) were carried out.

Definition of the treatment plan for dental implants was carried out by an orthopedic dentist together with related specialists - a dental surgeon, a dentist, orthodontist, and periodontist. The volume and sequence of preparatory measures, the type of orthopedic construction was indicated, and the number and type of implants were confirmed. Oral cavity was sanitized, surgical, orthopedic, orthodontic preparation for dental implantation was completed.

Total number of implants installed was 489 (268 for men and 221 for women, i.e., 54.8% and 45.2%, respectively). All patients were fitted with screw endosseous implants, ICX-templant system, Volksimplantat, Germany.

Determination of concentrations of IL-1 β , IL-10, and IL-17A (pg/ml) in the blood serum and content of gingival

sulcus of the teeth adjacent to implants of patients with different dental implants was performed in early post-operative period (1–3 days) and in more distant terms (2–3 months after surgery). To study the effect of the number of implants onto local mediator response of patients of this group, the level of nitrate nitrites ($\mu\text{M}/\text{ml}$) was studied in 1–3 days after implantation and 2–3 months later.

Groups of patients “immunogen,” “placebo”

To study the effectiveness of prevention of peri-implantitis of patients after dental implantation (with 5 or more implants), the complex of antioxidant vitamins and amino acids “Immunogen” (“IDI FARMACEUTICI S.p.A.” Italy, Via dei Castelli Romani, 83\85 00040 Pomezia, registration certificate No. 002279.И.380.12.2012) there were two groups of patients under our supervision:

First group – “Placebo” - 23 patients (12 men and 11 women, 52.2% and 47.8%, respectively) aged from 36 to 57 years, who received a standard package of measures for dental implantation;

Second group – “Immunogen” - 25 patients (13 men and 12 women, 52% and 48%, respectively) aged from 41 to 59 years, taking the Immunogen complex (1 capsule 2 times a day during meals).

Groups “Immunogen” and “Placebo” were formed from the third group of patients with installation of a different number of implants (5 or more implants, 51 people; 3 patients were not included in the group due to unsatisfactory oral hygiene).

In the “Placebo” Group, 8 people (34.8%) had implantation in the lower jaw, 6 people (26.1%) - in the upper jaw and 9 people (39.1%) - in both jaws. End defects made up to 61.7%, included ones – 38.3%.

In the “Immunogen” Group, 7 people (28%) had implantation in the lower jaw, 8 people (32%) - in the upper jaw and 10 people (40%) - in both jaws. End defects made up to 59.2%, included ones – 40.8%.

Groups were comparable in age, sex, somatic status; in time since loss of teeth (about 80% of patients lost their teeth 2–3 years ago).

Determination of concentrations of IL-1 β , IL-10, and IL-17A (pg/ml) in the blood serum and content of gingival sulcus of teeth adjacent to implants of patients of groups “Immunogen” and “Placebo” was performed in early post-operative period (1–3 days) and in more distant terms (2–3 months after surgery and 6 months after surgery). As indicators of oxidative stress of patients of these groups, the level of nitrate nitrites ($\mu\text{M}/\text{ml}$) was studied in 1–3 days,

2–3 months and after 6 months after implantation. Antioxidant tissue potential (%) was studied at the same time (after 1–3 days, after 2–3 months, and after 6 months after the operation).

Control group

The group consisted of 25 donors from the Republican blood transfusion station in the Nalchik City and was comparable in terms of sex and age to groups of patients (15 men and 10 women, which was 60% and 40%, respectively, the average age was 37 years and fluctuated within 26–48 years old). Donors with somatic pathology, diseases of dentition and abnormalities in blood tests were excluded from the group.

Determination of concentrations of IL-1 β , IL-6, IL-10, IL-17A, and CD-401 (pg/ml) in serum and the contents of the gingival sulcus of intact teeth, the level of nitrate nitrites ($\mu\text{M}/\text{ml}$) and myeloperoxidase (mmol/g of protein), as well as antioxidant tissue activity in the Fenton reaction among participants in this group was performed once.

Laboratory research methods

To study content of IL-1 β , IL-6, IL-10, and IL-17A in blood serum and gingival fluid, the method of solid-phase enzyme-immunoassay (Vector-Best, Russia [IL-1 β , IL-6, and IL-10]); Protein Contour, Russia (IL-17A). Total concentration of nitrate and nitrites in blood plasma of patients and healthy donors (μM) was determined spectrophotometrically using the Gris reactive (Nitrate\ Nitrite Colorimetric Assay Kit) (Cayman, USA). Determination of tissue myeloperoxidase activity was carried out by introducing a freshly prepared substrate solution into the supernatant from the tissue sample homogenate and incubating with the further addition of 10% sulfuric acid solution to terminate the reaction. Optical density of the solution was determined at a wavelength of 492 nm. Determination of antioxidant tissue activity was observed in the Fenton reaction (antioxidant activity of the tissue homogenate was judged by the parameter: $A = (A_0 - A_+) / A_0$, where A_0 is the maximum amplitude of chemiluminescence of the reaction in the absence of the test substance, A_+ is the maximum luminescence amplitude of the reaction in the presence of the homogenate).

Statistical processing of results

In mathematical processing of results of the research, following methods were used: Calculation of mean values and confidence interval calculated from n measurements. The confidence range was estimated using the Student’s test for $P < 0.05$. Statistical processing and calculation of the correlation rate were carried out using the Microsoft Excel software.

RESULTS

Study of features of the mediator response of patients with peri-implantitis at serum and local levels

It was found that among 17 patients (with peri-implantitis of moderate severity) 11 patients (64.7%) had peri-implantitis developed in the area of one implant, 6 patients (35.3%) - in the area of two implants.

9 patients (52.9%) - 6 men and 3 women - had a history of periodontal disease. 11 men (91.7% of all men) were smokers.

8 people (47.1%) - 6 men and 2 women - had a total value of the IGR-Y rate (J. C. Green, J. R. Vermillion, 1964) in the range of 3.1–4.8, which indicates a low hygiene level.

Of 15 patients, two groups (with severe peri-implantitis), 9 patients (60%) had an inflammatory process localized in the region of one implant, 5 patients (33.3%) in the area of two implants, and 1 person (6.7%) - in the area of 4 implants.

10 patients (66.7%) - 6 men and 4 women - had a history of periodontal disease. 7 men (100% of all men) were smokers. 7 people (46.7%) - 4 men and 3 women - had total value of IGR-U rate noted at the level of 3.2–5.3, which is an indicator of a low hygiene level.

By all patients, the inflammatory process in the peri-implant area developed in the distant period - from 6 months to 3 years or more after dental implantation. From the anamnesis, it was concluded that 56.3% of patients (18 people - 11 men and 7 women) underwent simultaneous installation of 5 or more implants. The majority of patients pointed to the fact of lack of proper dynamic medical control after the operation of dental implantation.

Complaints of patients with moderate peri-implantitis were usually reduced to pain in the area of the implant, swelling, hyperemia, and bleeding of the gum areas adjacent to the implant. In severe peri-implantitis, patients noted excreta discharge from bone pockets or formed fistulas, and, in most cases, impaired function of the implant due to its agility. All of the above mentioned was confirmed during determination of local status, radiography, and laboratory studies.

When serum levels of pro-inflammatory cytokines IL-1 β , IL-6, and IL-17A were studied, their concentrations were significantly increased ($P < 0.01$) [Table 1], and there is a correlation with severity of the disease ($r = 0.67$ - a moderate correlation). Anti-inflammatory immunocytokine IL-10 is the leading mediator involved in initiating reduction of protective responses. It was found that the concentration of IL-10 in serum was not significantly changed ($P > 0.01$) [Table 1].

Since immunocytokines are short-living molecules, local immunocytokine status indicators are more diagnostic and prognostically significant.^[13] A significant increase in the concentration of IL-1 β , IL-6, and IL-17A of patients with a more severe course of the pathological process was found in contents of gingival sulcus of teeth adjacent to implants [Table 2].

Unlike blood serum levels, the detected burst of pro-inflammatory cytokines occurs against the background of a low level of IL-10, which aggravates severity of the inflammatory reaction and is one of the factors of an uncontrolled local reaction leading to tissue damage due to free radicals, acute phase mediators released by activated cells in high concentrations.

Thus, there was a violation of the balance of pro-inflammatory and anti-inflammatory ILs in the gingival fluid which plays a significant pathogenetic role in a chronic process since it supports the activation of cells of innate immunity and is a risk factor for severe peri-implantitis. A high level of pro-inflammatory cytokines leads to activation of effector T-cells.

One of the groups of parameters actively studied recently as indicators of activation of macrophages and T-cells is the family of tumor necrosis factors, in particular, CD401, which supports inflammation in high concentrations and aggravates the damage of cells and tissues due to activation of T-lymphocytes.

Table 1: Concentrations of IL-1 β , IL-6, IL-10, IL-17A in blood serum of patients with peri-implantitis of different severity, pg/ml

Index	First group n=17	Second group n=15	“Control” n=25
IL-1 β	46.0 \pm 4.0 ¹	85.0 \pm 7.0 ^{1,2}	14.0 \pm 3.0
IL-6	4.5 \pm 0.5 ¹	15.0 \pm 0.8 ^{1,2}	2.5 \pm 0.5
IL-10	14.0 \pm 1.0	11.0 \pm 1.0	12.0 \pm 2.0
IL-17A	16.0 \pm 1.0 ¹	28.5 \pm 5.0 ^{1,2}	9.0 \pm 2.0

¹ $P < 0.01$ in comparison with the rate of the “control” group; ² $P < 0.01$ in comparison with the rate of the first group. IL: Interleukin

Table 2: Concentrations of IL-1 β , IL-6, IL-10, IL-17A in contents of gingival sulcus of teeth adjacent to implants of patients with peri-implantitis of different severity degrees, pg/ml

Index	Group one n=17	Second group n=15	“Control” n=25
IL-1 β	45.0 \pm 4.0 ¹	65.0 \pm 7.0 ^{1,2}	15.0 \pm 3.0
IL-6	3.5 \pm 0.5 ¹	5.0 \pm 0.8 ¹	1.5 \pm 0.5
IL-10	4.5 \pm 1.0 ¹	1.5 \pm 0.5 ^{1,2}	10.0 \pm 2.0
IL-17A	83.0 \pm 18.0 ¹	152.5 \pm 20.0 ^{1,2}	23.0 \pm 2.0

¹ $P < 0.01$ in comparison with the rate of the “control” group; ² $P < 0.01$ in comparison with the rate of the first group. IL: Interleukin

It was found out that CD401 serum rates of patients of the first group were not changed ($P > 0.01$), but at the local level, a significant excess of CD401 concentration was revealed [Table 3].

In the second group of patients with peri-implantitis, serum and local CD401 levels [Table 3] were significantly increased, which indicates the active involvement of macrophages and T-killers into the systemic response of an organism to local inflammation.

Examination of tissue indices of oxidative stress of patients with peri-implantitis

Since earlier local cytokines were more significant in studying features of mediator response of patients with peri-implantitis, then we studied the biopsy tissue as a measure of oxidative stress.

Evaluation of activity of tissue myeloperoxidase revealed a significant index increase in tissue samples taken from the peri-implant area during inflammatory process (group 1: MPO = 0.386 ± 0.07 mmol/g of protein, $P < 0.01$; group 2: MPO = 0.556 ± 0.15 mmol/g of protein) compared to healthy tissue (MPO = 0.205 ± 0.15 mmol/g of protein).

The level of nitrate nitrites in pathological bone pockets with peri-implantitis (245.0 ± 35.0 μ M/ml) is significantly higher than in the field of stable implants (14.0 ± 2.5 μ M/ml ($P < 0.01$) and gingival sulcus of natural teeth without signs of periodontitis (10.0 ± 1.5 μ M/ml), ($P < 0.01$).

However, antioxidant enzymes have an inductive nature, and, to avoid damage to own cells and tissues against at the background of increased radical formation, antiradical defense mechanisms are activated. To determine the antioxidant potential of the tissue homogenate against one of the most toxic and active oxygen radicals, effects of bioplate samples in the model system of hydroxyl radical generation (Fenton reaction) were analyzed. Tissue homogenates of patients with the Group I peri-implantitis reduced CL in 57%

of cases in the Fenton reaction, homogenates of patients with peri-implantitis of the 2nd group reduced CL in 30% of cases. Thus, contact with infectious agents in conditions of dental implantation leads to activation of tissue macrophages, as evidenced by increased rates of nitrate nitrites and myeloperoxidase. Reduced antioxidant tissue potential indicates a stage of decompensation. Prolonged violation of free-radical equilibrium with the development of antioxidant tissue deficit over time becomes a damaging factor and may be the cause of rejection of the implant.

Features of systemic and local mediator responses of patients with different amounts of dental implants

We conducted a comprehensive examination and implant treatment of 103 patients, who were divided into three groups depending on the number of implants being installed:

First group - 24 patients with 1–2 implants, among them 13 men and 11 women aged from 28 to 62 years (23.3%);

Second group - 28 patients with 2–4 implants, among them 16 men and 12 women aged from 38 to 66 years (27.2%);

Third group - 51 patients with 5 and more implants, among them 28 men and 23 women aged from 39 to 61 years (49.5%);

Main causes of tooth loss were: Caries and its complications (in 57.3% of patients), periodontitis (in 34.9%), and trauma (in 7.8%).

Among concomitant diseases of men, the pathology of digestive organs (chronic gastritis, chronic gastroduodenitis, and chronic cholecystitis) predominated in 21.1%, ones of women - in the cardiovascular system (AH 1st, IHD) - in 21.7%; women without concomitant pathology made 45.7%, men - 45.6%.

Elevated concentrations of IL-1 β and IL-17A were detected in the early period after dental implantation both in the blood serum and in gingival sulcus [Table 4].

Levels of IL-1 β and IL-17A correlated with the extent of intervention and were greatest in the third group [Table 4]. Such a rise in the concentration of pro-inflammatory immunocytokines is associated with the body's response to an operation trauma and is a normal physiological protective reaction.

In the more distant period, from 2 to 3 months after dental implantation, it was found that concentrations of IL-1 β and IL-10 in the blood serum do not differ from those ones of the "control" group ($P > 0.01$) While the study of the same parameters in the content of gingival sulcus revealed the presence of a significant difference [Table 5]. Levels of

Table 3: Concentrations of CD401 in the serum and content of gingival sulcus of teeth adjacent to implants of patients with peri-implantitis of different severity (pg/ml)

Index	Group one <i>n</i> =17	Second group <i>n</i> =15	Group "control" <i>n</i> =25
CD401 (blood serum)	96.0 \pm 4.0	155.0 \pm 17.0 ^{1,2}	95.0 \pm 15.0
CD401 (gingival fluid)	75.5 \pm 7.5 ¹	134.0 \pm 0.8 ^{1,2}	58.0 \pm 7.0

¹ $P < 0.01$ in comparison with the rate of the "control" group; ² $P < 0.01$ in comparison with the rate of the first group

IL-1 β and IL-17A are lower than those of the early period ($P < 0.01$) but significantly higher than those in the control group ($P < 0.01$) and are maximal in the third group [Table 5]. The IL-10 index has an inverse relationship and is minimal in the group of patients with a large number of implants (5 or more). When calculating the rate of pro-inflammatory and anti-inflammatory cytokines which characterizes the overall orientation of the mediator changes, a direct correlation was found with the number of implants ($r = 0.9$) installed.

Thus, 2–3 months after dental implantation, a persistent local pro-inflammatory mediator reaction was revealed in patients of groups 2 and 3 with absent clinical signs of an inflammatory reaction. In the blood serum, in the long-term observation period, no significant changes in cytokines studied were observed in any group.

In some works, it was found earlier that nitric oxide is involved into damage of alveolar portions, and its level can be an indicator of the state of post-traumatic regeneration.^[14,15] In this regard, to study the effect of the number of implants on the local mediator response further, concentrations of nitrate nitrites in tissues were studied 2–3 months after implantation.

It was found that nitrate-nitrite concentrations were significantly higher in the third group ($P < 0.01$), in the first and second groups of patients there were no differences from parameters of the “Control” group ($P > 0.01$) [Figure 1].

Thus, a mediator reaction with the prevalence of local inflammation processes in patients with a large number of simultaneously established dental implants was revealed.

These facts serve as the basis for the use of a set of measures to prevent the development of peri-implantitis, timely antibiotic therapy, prescription of antioxidants to counter oxidative stress and immunological disorders.

Evaluation of the effectiveness of complex of antioxidant vitamins and amino acids of patients with extensive implantation as a complex prophylaxis of complications

Since it was previously revealed that a local pro-inflammatory mediator reaction has been sustained for a long time after dental implantation (5 or more implants) for 2–3 months after absence of clinical signs of inflammation, the effect of the “Immunogen” complex of antioxidant vitamins and amino acids (IDI Farmaceutici, Italy) onto local indices of cytokines and redox-inflammation was researched; we observed two groups of patients with simultaneous installation of 5 or more implants:

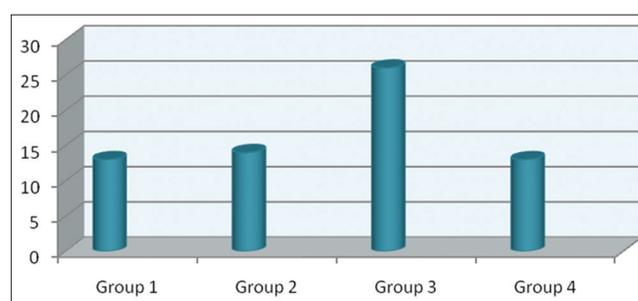


Figure 1: The concentration of nitrate nitrites ($\mu\text{M}/\text{ml}$) in gingival sulcus of teeth adjacent to the implant at a remote study period (2–3 months after installation) in different patient groups

Table 4: Concentrations of IL-1 β , IL-10, IL-17A in contents of gingival sulcus of teeth adjacent to an implant in the early period of the study (1–3 days after implantation) (pg/ml)

Index	Group one $n=24$	Second group $n=28$	Third group $n=51$	“Control” $n=25$
IL-1 β	28.0 \pm 2.0	35.0 \pm 3.0 ^{1,2}	56.0 \pm 7.0 ^{1,2,3}	15.0 \pm 3.0
IL-10	10.5 \pm 1.0	3.5 \pm 0.5 ^{1,2}	2.0 \pm 0.5 ^{1,2,3}	10.0 \pm 2.0
IL-1 β /IL-10	1.8 \pm 0.2	10.60 \pm 0.7 ^{1,2}	28.0 \pm 1.2 ^{1,2,3}	1.5 \pm 0.3
IL-17A	35.0 \pm 11.01	38.5 \pm 10.0 ¹	76.0 \pm 12.0 ^{1,2,3}	10.0 \pm 1.0

¹ $P < 0.01$ in comparison with the rate of the “control” group; ² $P < 0.01$ in comparison with the rate of the first group; ³ $P < 0.01$ in comparison with the rate of the second group. IL: Interleukin

Table 5: Concentrations of IL-1 β , IL-10, IL-17A in contents of gingival sulcus of teeth adjacent to an implant in the long-term period of the study (2–3 months after implantation) (pg/ml)

Index	Group one $n=24$	Second group $n=28$	Third group $n=51$	“Control” $n=25$
IL-1 β	18.0 \pm 2.0	25.0 \pm 3.0 ^{1,2}	36.0 \pm 7.0 ^{1,2,3}	15.0 \pm 3.0
IL-10	9.5 \pm 1.0	4.5 \pm 0.5 ^{1,2}	2.5 \pm 0.5 ^{1,2,3}	10.0 \pm 2.0
IL-1 β /IL-10	1.8 \pm 0.2	5.6 \pm 0.7 ^{1,2}	14.4 \pm 1.2 ^{1,2,3}	1.5 \pm 0.3
IL-17A	15.0 \pm 11.0	16.5 \pm 10.0	58.0 \pm 12.0 ^{1,2,3}	10.0 \pm 1.0

¹ $P < 0.01$ in comparison with the rate of the “control” group; ² $P < 0.01$ in comparison with the rate of the first group; ³ $P < 0.01$ in comparison with the rate of the second group. IL: Interleukin

First group “Placebo” - 23 patients (12 men and 11 women, 52.2% and 47.8%, respectively) aged from 36 to 57 years, who received a standard package of measures for dental implantation;

Second group “Immunogen” - 25 patients (13 men and 12 women) aged from 41 to 59 years, who received an “Immunogen” complex.

Complex therapy of patients after the operation on dental implantation consisted of:

- antibacterial therapy (Amoxiclav, 625 mg, 2 times a day, for 5–7 days);
- anti-inflammatory therapy (mouthwash 0.05% with an aqueous solution of chlorhexidine, Metrogl denta on the seam line if necessary (hyperthermia and pronounced edema) - parenteral administration of anti-inflammatory medication (Metrogl, 100 ml, intravenously every 8 h for 3 days);
- Anti-edematous therapy (cold locally, “Traumeel C);”
- Administration of painkilling medication (at 1st day).

After 1st days of therapy, the condition of patients of both groups was characterized as satisfactory; there were no complaints; on the background of primary epithelization of post-operative wounds on the 10th day, seams were removed.

During the control examination of the implantation zone after 4 weeks, there were no deviations from the normal course of the postoperative period; the groups are comparable in all parameters. Since the 5th week in the group “Immunogen,” taking of the complex of antioxidant vitamins and amino acids inside has been started from 1 capsule during meals 2 times a day, taking continues for 8 weeks.

Since correlation coefficients of mediators from different groups are more indicative for evaluation of equilibrium state in the system, the ratio of mediators from different groups was studied; the ratio of IL-1 β /IL-10 in contents of gingival sulcus of teeth adjacent to implants at different observation times was studied [Figure 2]. Against the background of complex therapy with the application of “Immunogen” medication, a reliable difference was found after 3 months, and normalization of the cytokines ratio after 6 months.

When studying the concentration of nitrate nitrites which are indicators of NO-synthase activity, it was found out that there are no long-persistent high concentrations that are risk factors for tissue damage due to free radical oxidation ($P > 0.01$) in the Immunogen group (unlike the placebo group) [Figure 3].

When studying the antioxidant potential of tissues against the background of therapy with the application of “Immunogen” medication, an increase in the percentage of tissue biopsies

showing antioxidant properties in the Fenton reaction was revealed [Figure 4].

Thus, when a complex of antioxidant vitamins and amino acids is involved at patients with extensive implantation, the imbalance of pro-inflammatory and anti-inflammatory mediators is not observed after 3 months, nitrate-nitrite rates in tissues do not increase, and the antioxidant tissue potential significantly increases ($P < 0.05$). In the period of 6 months, tissue indices normalize.

On examination, during first 3 months, as well as during examination and radiography, before setting of gingiva formers (4 months and 6 months after the operation), the condition of gingival mucosa and the bone tissue state corresponded to normal in patients of both groups,

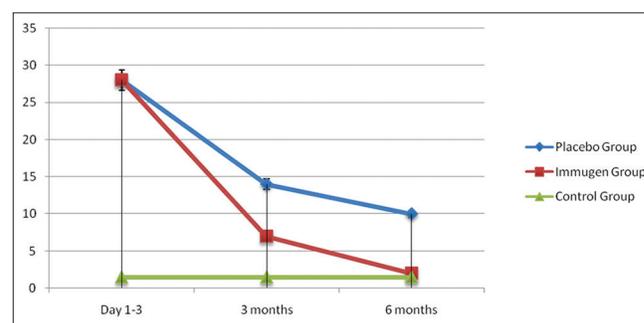


Figure 2: The ratio of interleukin (IL)-1 β /IL-10 in contents of gingival sulcus of teeth adjacent to implants at different observation times (pg/ml)

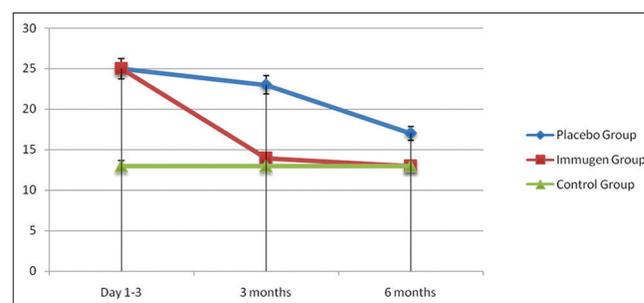


Figure 3: Concentration of NO₂-NO₃ in contents of the gingival sulcus of teeth adjacent to implants at different times of observation (pg/ml)

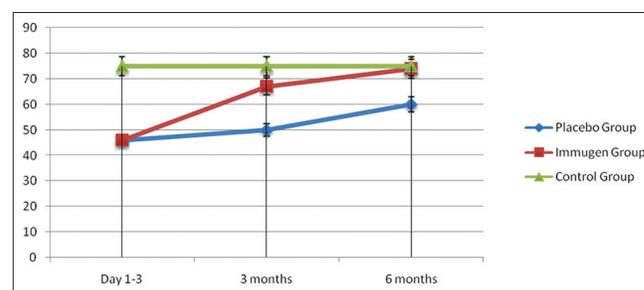


Figure 4: Percentage of tissue homogenates exhibiting antioxidant properties in the Fenton reaction (%) at different observation times

which indicates limited diagnostic significance of clinical methods of research, therefore, to fully understand processes of osseointegration, it is necessary to take into account laboratory immunological and biochemical indicators (cytokine profile and free radical indicators of tissues).

A follow-up examination 1.5 years after surgery revealed signs of peri-implantitis in 3 patients of the placebo group; patients of the “Immunogen” group have stable implants, no complaints, no inflammation. Thus, in the evaluation of clinical results of the application of “Immunogen,” the absence of distant complications of an inflammatory nature was noted. This complex of antioxidant vitamins and amino acids resists damage caused by free radicals at the cellular level and promotes the acceleration of repair of damaged tissues, thereby being an effective component of complex therapy in preventing complications of dental implantation.

Patients with the simultaneous installation of 5 or more dental implants are advised to take a complex of antioxidant vitamins and amino acids to prevent the development of complications (2 capsules 2 times a day during meals for 30–60 days).

CONCLUSIONS

Patients with peri-implantitis which developed in the remote period after the installation, significantly increased serum concentrations of IL-1 β , IL-6, and IL-17A ($P < 0.01$). Anti-inflammatory IL-10 in serum has not significantly changed. In contrast to serum levels, the detected burst of pro-inflammatory cytokines in the content of gingival sulcus occurs against the background of a low level of IL-10, which aggravates the severity of the inflammatory response.

The serum (by $163.0 \pm 17.0\%$) and local (by $231.0 \pm 24.0\%$) CD401 levels were significantly increased in the group of patients with severe fever, indicating the active involvement of macrophages and T-killers into the systemic response of organism onto local inflammation.

In tissue samples taken from the peri-implant area during the inflammatory process, an increase in activity of tissue myeloperoxidase ($P < 0.01$) was revealed against a background of a decreased tissue antioxidant potential.

In patients with extensive implantation, a pro-inflammatory mediator reaction with significantly increased concentrations of pro-inflammatory ILs and nitrate nitrites ($P < 0.01$) was detected in 3–6 months after implantation.

Local study of the correlation of pro-inflammatory (IL-1 β , IL-6, and IL-17A) and anti-inflammatory

(IL-10) immunocytokines reliably reflects the dynamics of osseointegration processes and can be used to predict the development of complications of dental implantation.

Admission of a complex of antioxidant vitamins and amino acids to patients with extensive implantation (5 or more implants) helps to eliminate the identified imbalance of pro-oxidant and antioxidant systems, thereby reducing the risk of complications in the long term.

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