EFFECTIVENESS OF PROBIOTIC THERAPY IN THE TREATMENT AND PREVENTION OF PERIMUCOSITIS: A CLINICAL, IMMUNOLOGICAL, AND MICROBIOLOGICAL STUDY

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Abstract

The widespread application of intraosseous dental implants has significantly enhanced modern dental rehabilitation; however, peri-implant inflammatory complications such as perimucositis and peri-implantitis remain a major clinical challenge. This study evaluated the effectiveness of a probiotic preparation (Enteflavin 4g, Pharmaluce) as an adjunct to basic therapy in the treatment and prevention of perimucositis. Forty-two patients with 3–4 mm peri-implant probing depths were divided into two groups: a control group receiving basic therapy alone and a main group receiving basic therapy plus a 30-day probiotic regimen. Clinical indices (plaque index, gingival index, bleeding on probing, pocket depth), functional parameters of oral fluid, cytokine profiles (IL-1β, IL-4, TNF-α), and microbiological data were assessed before, and at 1 and 6 months after treatment. The main group demonstrated significantly better clinical outcomes, including reduced inflammatory indices, improved salivary gland function, and more pronounced normalization of cytokine status, particularly a marked decrease in IL-1β levels. Microbiological analysis showed a nearly twofold reduction in key periodontopathogens (A.a., P.g., P.i., F.n., T.d.) in the probiotic group compared to controls. The findings indicate that adjunctive probiotic therapy enhances the effectiveness of conventional perimucositis treatment by modulating oral microbiota and inflammatory responses, reducing disease severity, and lowering recurrence risk. Incorporating probiotics into peri-implant maintenance protocols may improve long-term implant success.

Keywords: *Perimucositis; Peri-implantitis; Dental implants; Probiotics; Oral microbiota; Cytokines; IL-1β; TNF-α; Periodontal pathogens; Salivary diagnostics; Inflammation; Enteflavin.*

1. Introduction

The widespread use of intraosseous dental implants is one of the most distinctive features and achievements of modern practical and scientific dentistry. Comprehensive implantation treatment for improving dental orthopedic care using dental implants is aimed at timely elimination of negative changes in the parotid tissues and normalization of the body's overall reactivity. Removable and non-removable dentures using implants are highly effective, and in some cases, the only treatment method for dentofacial system defects [1, 2].

However, with the development of destructive generalized forms of periodontopathies at these stages of restorative treatment, the likelihood of serious complications in the form of perimucositis and periimplantitis, up to the loss of the implants themselves, significantly increases [3 - 6]. Over the past decade, interest in alternative strategies in dentistry has increased, such as the use of probiotics for the prevention and treatment of caries, periodontal diseases, halitosis, and other oral diseases [7 - 10]. The purpose of the study is to determine the effectiveness of using a probiotic in the treatment and prevention of perimucositis based on control clinical, immunological, and microbiological studies.

2. Material and methods

To fulfill the assigned task, 42 patients with perimucositis with a depth of 3-4 mm were selected after prosthetics with non-removable structures on dental implants. The effectiveness of treatment was assessed based on control clinical, immunological, and microbiological studies conducted directly before and 1 and 6 months after treatment. The study fully complied with the ethical standards of the Committee for Human Experiments of the 1975 Helsinki Declaration and its revised version in 2000. The main and control groups of patients with perimucositis were formed: the main group - 21 patients who underwent basic therapy + probiotic; the control group, 21 patients - basic therapy.

Basic therapy in both groups included the sanation of peri-implantal pathological foci: professional hygiene was carried out using the PIEZON Master 600 ultrasound device and the Perio Flow (EMS) air-abrasive device, Greysi's curette (HuFriedy), special polishing brushes and pastes, as well as regular individual oral hygiene monitoring. The probiotic Enteflavin 4g, Pharmaluce (L. reuteri DSM 26866, L. rhamnosus DSM 21690, L. bulgaricus DSM21690, Bifidobacterium animalis ssp. lactis DSM 17741) was included in the standard course of basic therapy once a day for 30 days in the main group.

The criteria for assessing the condition of periodontal tissues were the dental coating index (Silness & Loe, 1964), gingival index (Loe & Silness, 1964), bleeding during probing (Ainamo & Vau, 1975), and the depth of the pocket using the PCP UNC 15 (Hu Friedy) periodontal probe directly before and 1 and 6 months after treatment.

Material collection for microbiological studies and subsequent cultivation were carried out with a set of nutrient media: for aerobic and facultative-anaerobic bacteria - chocolate agar; for anaerobic bacteria - thioglycol nutrient agar with a 5% suspension of sheep erythrocytes; for fungi - Saburo agar.

During biochemical studies, the levels of cytokines IL-1β, IL-4, and TNF-α were determined using solid-phase enzyme-linked immunosorbent assay. The method of solid-phase enzyme-linked immunosorbent assay (ELISA) is based on the specific binding of antibodies to antigens. Determination of cytokine concentration was carried out using a calibration curve using a computer program. The amount of cytokines was expressed in picograms per milliliter (pg/ml).

Statistical processing of the obtained data was carried out using standard methods using software: Microsoft Excel and Statistica 6.0. Student's t-criterion and Mann-Whitney's U-criterion were used to test statistical hypotheses. The measure of linear relationship between the parameters was determined using Pearson's correlation coefficient. For all criteria and tests, the critical significance level was taken as 5%, i.e., the null hypothesis was rejected at $p<0.05$.

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3. Results and discussion

In the initial and later periods of clinical trials after the course of basic therapy, the average values of the dental coating index, gingival index, bleeding during probing, and the depth of pocket probing in patients of the main group were significantly lower than in the control group, and the condition of the periodontal tissues was determined as "absence of the inflammatory process" (Table. 1). This was manifested in a more pronounced and reduced intensity of perimucositis development and weakening of its clinical manifestations when using a probiotic compared to the traditional management of individuals with dental prostheses on implants alone.

From the perspective of determining the course and severity of pathological processes in the organs and tissues of the oral cavity, it is very important to use indicators of the functional state of salivary glands and oral fluid [11, 12]. Studying the quantitative and qualitative indicators of oral fluid status has certain diagnostic and prognostic significance.

Table 1. Clinical parameters before and after treatment

Clinical parameters	Groups	Before treatment	After 1 month	After 6 months
Plaque Index, points	Control (n=21)	2.28 ± 0.021	0.97 ± 0.016	1.25 ± 0.017
	Main (n=21)	2.32 ± 0.023 (p>0.05)	0.51 ± 0.019 (p<0.001) p1 < 0.001	0.67 ± 0.017 (p<0.001)
Gingival Index, points	Control (n=21)	2.06 ± 0.024	1.33 ± 0.017	1.57 ± 0.035
	Main (n=21)	2.04 ± 0.026 (p>0.05)	0.63 ± 0.018 (p<0.001) p1 < 0.001	0.71 ± 0.033 (p<0.001)
Bleeding on probing, %	Control (n=21)	82.1 ± 0.87	26.1 ± 0.36	17.6 ± 0.29
	Main (n=21)	82.4 ± 0.75 (p>0.05)	21.3 ± 0.40 (p<0.001) p1 < 0.001	12.2 ± 0.30 (p<0.001)
Probing pocket depth, mm	Control (n=21)	3.75 ± 0.051	3.21 ± 0.042	2.98 ± 0.036
	Main (n=21)	3.86 ± 0.057 (p>0.05)	2.98 ± 0.045 (p<0.001) p1 < 0.001	2.65 ± 0.054 (p<0.001)

Note:p — statistical significance relative to baseline (before treatment), p1 — statistical significance relative to the control group.

When determining the initial values of saliva secretion rate in the examined patients of both groups, it was established that this indicator was significantly lower than the norm of 0.58±0.013 and 0.57±0.015 mg/min, respectively, in the control and main groups. In these

same groups of patients, saliva "acidification" was detected before treatment, with an average pH value of 6.81±0.065 and 6.83±0.086 (Table. 2).

After 1 and 6 months of treatment, an increase in the functional activity of the salivary glands and the rate of saliva secretion was observed. In the control group, after traditional treatment, a significant increase in the salivation rate was also established, but more pronounced than in the main group. In patients of both groups, who had indicators within negative values before treatment, individual values of saliva secretion rate continued to increase in the subsequent stages of observation.

The pathogenesis of inflammatory diseases in the peri-implantation zone includes a number of immune reactions initiated by microorganisms of the oral mucosa and leading to the sharp release and accumulation of pro-inflammatory cytokines in the oral fluid. During the implementation of comprehensive therapeutic and preventive measures with the introduction of probiotic agents as supporting therapy in patients of the main group, a more pronounced normalization of the cytokine status was noted compared to patients of the control group: the content of IL-1β in saliva before and after the course of basic therapy in the group of patients receiving probiotics was 671.9±1.59 and 105.4±0.78 ng/ml, respectively (Table. 3).

Table 2. Effect of treatment on salivation rate and saliva pH

Observation period	Salivation rate, mg/min		pH	
	Control (n=21)	Main (n=21)	Control (n=21)	Main (n=21)
Before treatment	0.58 ± 0.013	0.57 ± 0.015	6.81 ± 0.065	6.83 ± 0.086
1 month	0.82 ± 0.007 *(p<0.001)*	0.68 ± 0.010 *(p<0.001)* *p1 < 0.001*	6.75 ± 0.009 *(p>0.05)*	6.89 ± 0.026 *(p>0.05)* *p1 < 0.001*
6 months	0.64 ± 0.014 *(p<0.05)*	0.65 ± 0.010 *(p<0.001)* *p1 > 0.05*	6.79 ± 0.010 *(p>0.05)*	6.98 ± 0.017 *(p>0.05)* *p1 < 0.001*

Note:p *— statistical significance relative to baseline (before treatment),* ***p1*** *— statistical significance relative to the control group.*

It should be noted that during rehabilitation measures with the switching of pro-inflammatory cytokine synthesis to anti-inflammatory ones, a significant number of patients noted a decrease in the inflammatory process in the soft tissues around the implant before the normalization of the cytokine status. With the preservation of the pathological process, a functional imbalance is still observed in this system, i.e., the cytokine system, being a very important pathogenetic link of the inflammatory processes occurring in the mouth, largely determines the course and degree of their severity. The use of a probiotic as a companion to basic dental therapy allows for a significant and timely reduction in the severity of perimucositis and the risk of its recurrence, thereby carrying out planned treatment in full.

Table 3. Cytokine status of the oral cavity in patients of the main group before and after treatment

Immunological markers	Before treatment	After 1 month	After 6 months

IL-1β, ng/ml	671.9 ± 1.59	105.4 ± 0.78 *(p<0.001)*	120.9 ± 2.10 *(p<0.001)*
TNF-α, ng/ml	212.3 ± 2.80	52.4 ± 1.17 *(p<0.001)*	31.5 ± 0.40 *(p<0.001)*
IL-4, ng/ml	43.2 ± 0.61	98.2 ± 0.87 *(p<0.001)*	83.6 ± 0.66 *(p<0.001)*

Note: p — statistical significance relative to pre-treatment (baseline) values.

Thus, the significant decrease in the value of the anti-inflammatory interleukin - IL-4 and negative shifts in the overall cytokine balance, identified in the main group after treatment of perimucositis, indicated the normalization of oxidative stress in the examined patients with subsequent correction of the pathological process in the soft tissues of the periodontium. Detection of enzymes in saliva has diagnostic value, and a decrease in their level is an unfavorable prognostic sign. The immunoglobulin imbalance we discovered in oral fluid is, most likely, a risk factor for the occurrence of a pathological process in oral tissues, including the peri-implant zone [12, 13]. It should be noted that cytokine imbalance with pronounced hyperproduction of pro-inflammatory cytokines can be an important pathogenetic mechanism for the development of inflammatory and destructive diseases of oral organs and tissues. Clinical studies in the control group of patients with perimucositis also revealed that the development of pathological changes in the peri-implantation zone is accompanied by an increase in the number of pro-inflammatory cytokines - IL-1β, IL-6, and TNF-α in unstimulated saliva (Table. 4). Regarding the quantitative content of pro- and anti-inflammatory cytokines in saliva, their concentration changed to a lesser extent at all stages of biochemical studies compared to the data of the main group.

It should be noted that the detection of staphylococci, streptococci, pathogenic actinomycetes, and yeast fungi in oral biopsies, with their pronounced ability to adhere, plays an important role in the pathogenesis of inflammatory and destructive diseases in the maxillofacial region, as well as in the area of implants [14 - 16]. According to microbiological studies, the drug based on the probiotic Enteflavin 4g, Pharmaluce, significantly reduces the detection of periodontal pathogens after complex treatment of mucositis (Table. 5). Thus, in the presence of perimucositis, the detection of Prevotella intermedia (P.i.), Treponema denticola (T.d.), Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Fusobacterium nucleatum (F.n.) in the main and control groups was 19.0, 38.1, 23.8, 28.6, 14.3% and 23.8, 38.1, 28.6, 28.6, 19.0%, respectively. After a course of treatment using traditional methods and a probiotic, the detection of the above-mentioned periodontopathogens in the groups decreased by almost 2 times and amounted to in the main group: A.a. 9.5%, P.g. 14.3%, P.i. 9.5%, F.n. 14.3%, T.d. 4.8%, which is lower compared to the situation after treatment, which developed in the control group, where the values for the studied microorganisms were 14.3, 28.6, 19.0, 19.0 and 9.5%.

According to microbiological studies, the effectiveness of the probiotic agent is almost 2 times higher compared to traditional methods of treating mucositis, and in the samples taken from the main group where the probiotic was used, representatives of the normoflora and conditionally pathogenic microorganisms prevailed more often. This, to a certain extent, confirms the specificity of the action of probiotic agents in terms of preventing dysbacteriosis and positively affecting the normal oral microflora.

Thus, the use of a probiotic as a companion to basic dental therapy allows for a significant and timely reduction in the severity of perimucositis. Installation of prosthetic structures on dental implants requires constant monitoring of the condition of their surrounding soft and hard tissues with timely diagnosis, prediction of the situation, and the application of effective therapeutic and preventive measures for the complete correction of emerging pathological changes.

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