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IMMUNOLOGICAL AND MICROBIOLOGICAL ASPECTS OF THE EFFECTIVENESS OF ORAL HYGIENE FOR PATIENTS WITH PERIODONTAL INFLAMMATORY DISEASES

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Abstract. *The research objective consisted in studying the local factors of protection of an oral cavity on the basis of balance definition between proinflammatory (INf - γ, TNF - α, IL-8) and anti-inflammatory (IL-4) cytokines with the degree of colonization of Candida in patients with chronic generalized parodontitis. On the basis of the results of this study, it has been established that patients with chronic parodontitis who have a mild and moderate severity level of disease, a high degree colonization of periodontal pockets with Candida tropicalis correlates with a substantial increase in proinflammatory cytokines (TNF - α, IL-8), concentration fall of INF - γ and the increase in anti-inflammatory cytokines of IL-4 level.*

Key words: *Fungous microflora of an oral cavity, parodontitum, cytokines*

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1. INTRODUCTION

Currently, the system of preventive medical care which is dealing with the questions of dental disease treatment, particularly periodontal diseases, is becoming increasingly important. Many authors in their publications noted that one of the main reasons for initiation and progression of periodontal diseases was the lack of full, regular and extensive oral hygiene [1]. Basically, in the development of periodontitis, there is a complex occurrence in the oral cavity with pathological shifts, associated with immunological and microbiological changes in the periodontal tissues [2,3]. With a long-term course of chronic inflammatory processes in the oral cavity and a wasteful long-term treatment of patients with periodontal pathology using synthetic antibiotics and local antiseptics, an imbalance develops between different types of fungal-bacterial associations, which are accompanied with the emergence of oral cavity dismicrobiocenosis [4]. The prevalence of the active colonization of yeast-like fungi of Candida type in periodontal pockets of patients with chronic periodontitis is in 40% to 70% of cases [5,6]. At the moment, the actual question remains about the impact of personal oral cavity hygiene products that contain antiseptics to the composition of the microbiota and the local immune status of the oral cavity.

Aim of the study is to show the improvement of the hygienic condition of oral cavity in patients with chronic generalized periodontitis using the adjusted health-promoting types of toothpaste under the control of microbiological and immunological diagnostic methods.

2. METHODS

This study involved 330 patients with periodontal pathology – 170 patients in the preliminary epidemiological survey group, 160 in the final study group, and 30 patients with clinically healthy periodontium. Initially, an epidemiological study was carried out which consisted of 170 patients aged 20 to 50 years with periodontal diseases without somatic pathology to determine the prevalence of yeast-like fungi of Candida type. Then the final group of 190 patients who had chronic generalized periodontitis of mild (CGPM1) and moderate (CGPM2) severity was formed to conduct a comprehensive examination, including clinical methods and index assessment of periodontal tissue (papillary-marginal-alveolar index (PMA %), periodontal index (PI) (Russel), bleeding index (IR) (Muhlleman), index of treatment needs (CPITN), hygiene index (OHI-S) (Greene-Vermillion)); microbiological methods (gingival fluid tap (GFT) and the periodontal pocket (PP), followed by the seeding of the material in bacteriological laboratory with sectoral method on 5% blood or chocolate agar identifying isolated strains of microorganisms using bacteriological analyzer mini API (France) Biomerieux firm); culture methods (mycological-clinical material taking was carried out with the mucous membrane of the gingival sulcus (S), the periodontal pocket (PP) and mucosal membrane of tongue, followed by the seeding of the material on the selective medium Candiselect 4

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Bio-rad (France) for the detection and counting of Candida colonies: albicans, tropicalis, glabrata, krusei); and immunological studies (determining the level of pro-inflammatory (INF-γ, TNF-α, IL-8) and anti-inflammatory (IL-4) cytokines in the papillary blood and contents of a PC by enzyme-linked immunosorbent assay using reagent kits ZAO "Vector-Best" (Novosibirsk) photometer vertical scanning ANTHOS 2010 (Austria)). Depending on the ongoing treatment, patients were assigned to the treatment group (40 patients with CGPM1 and 40 patients with CGPM2 aged between 20 and 50 years, male sex, undergoing a comprehensive examination and pathogenetically-based treatment for an improved scheme, supplemented by the inclusion in the general and local regimen of "Bifidumbacterin forte" eubiotics (Partner, Russia), as well as personal hygiene products – they used a toothpaste based on plant components "Parodontax" (GlaxoSmithKline)), and a control group with the same number of patients, who were treated according to traditional methods, and as a tool for daily oral care they used a medical and prophylactic toothpaste "Blend-a-med complete tender mint". The control group consisted of 30 men with clinically healthy periodontium, who were 20 to 50 years of age. X-ray examination (orthopantomography) was performed on 150 patients with periodontitis before treatment and 6 and 12 months after treatment.

3. Results

Epidemiological survey showed a high percentage of occurrence of fungal microflora patients with chronic generalized periodontitis of a high – 93.75% – and high degree of colony-formation in the contents of the PP – 8.8 ± 0.2 CFU/1ml. Patients with CGPM2 – 90% – 6 ± 0.9 CFU/1ml and those with CGPM1 were 78.6% of the cases with PP moderate bacterial content equal to 4 ± 0.9 CFU/1ml. Studying of specific representative yeast-like fungi patients with periodontal pathology of a high degree of colony-formation and the detection percentage of 70.3% is typical for C. tropicalis colonies. The performed examination of the original clinical status indicates a significant index increase in patients with CGPM1: the OHI-S 2.7 ± 0.24; CPITN – 1.6 ± 0.08; PI – 0.7 ± 0.08; bleeding index (IR) – 1.6 ± 0.1; PMA – 24.6 ± 1.3 ($p < 0.001$) and patients with CGPM2: the OHI-S 4.9 ± 0.7; CPITN – 3.35 ± 0.1; PI – 3.5 ± 0.26; IR – 2.3 ± 0.12; PMA – 57.8 ± 1.3 ($p < 0.001$). Microbiological and culture (mycological) survey found that patients with CGPM1 had a high and moderate degree of PP bacterial contamination with biotope Gram-positive cocci: Streptococcus sanguis (52.5%) – 5.3 ± 0.5; Streptococcus mitis (35%) – 5.7 ± 0.5; Staphylococcus hominis (52.5%) – 5.7 ± 0.5; Streptococcus salivarius – (52.5%) – 5.3 ± 0.5; and yeast-like fungi S.tropicalis genus - (64%) – 4 ± 0.9. Patients with CGPM2 – Streptococcus sanguis (70%) – 7.2 ± 0.7; Streptococcus mitis (70%) – 7.6 ± 0.5; Staphylococcus hominis (70%) – 7.2 ± 0.5; Streptococcus salivarius – (70%) – 5.2 ± 0.4 and S.tropicalis – (75%) – 6.6 ± 1, but the quantitative (%) and qualitative indicators (CFU/1 ml) in this group of patients were significantly ($p < 0.05$)– 1.5-2 times – higher than in patients with CGPM1. Thuswise, along with a high prevalence of 52-70% of PC biotope patients with CGPM1 and CGPM2, gram-positive cocci revealed a high degree of bacterial contamination and prevalence (64% -75%) of fungal microflora. The study indicates that, in patients with intact periodontium, in 46.6% of cases, there is a GF bacterial contamination with C.tropicalis colonies, which is 2 ± 0.9 CFU/1ml. The frequency of this type of fungal flora increases, on the one hand, with the increasing of disease severity and, in patients with CGPM1, it is equal to 64% - 4 ± 0.9 CFU/1ml and, on the other hand, 75% - 6.6 ± 1 CFU/1 ml in patients with CGPM2, with severe immunological changes in periodontitis, promoting and maintaining a prolonged chronic inflammation in periodontal tissue complex.

The immunological status indicates a significantly low rate concentration of INF-γ and a fairly high level of TNF-α, IL-8, IL-4 in this group of patients compared with healthy individuals ($p < 0.01$). The findings suggest an important role of studied proinflammatory cytokines in the pathogenesis of inflammatory periodontal diseases. The mycological research result found that with the application of an improved method of complex treatment of patients with CGPM1 and CGPM2, after 6 months, a significant decrease in bacterial contamination of PP with S.tropicalis to 1.7 ± 0.9 ($p < 0.01$) can be seen, and up to 2 5 ± 1.3 CFU / 1 ml ($p < 0.05$) which represents 32.5% and 60% occurrence of this type of flora in the PP biotope; after 12 months, S.tropicalis colonies could not be identified. In patients from the comparison group, after 6 months a significant decrease of C. tropicalis colony formation to 2,0 ± 1 and 4,8 ± 0,7 CFU/1 ml ($p < 0.01$), respectively, could be seen representing 50% and 100% of cases, and after 12 months, again a moderate bacterial contamination of PP to 4.0 ± 0.7 CFU/1 ml ($p < 0.01$) was revealed in 100% of patients with CGPM1 and an active colony-formation of yeast-like fungi of Candida type in PP with an increase rate in patients with CGPM2 to 6.3 ± 1.2 CFU / 1 ml – in 100% of cases. It indicates that the restoration of the original oral microbial landscape is achieved after 1 year in contrast to a traditional therapy background. For efficacy evaluation of applied and improved treatment method, the variation of proinflammatory (INF-γ, TNF-α, IL-8) and anti-inflammatory (IL-4) cytokines in the papillary blood and the content of the PP patients with CGPM1 and CGPM2 was studied. We found that, after the treatment of CGPM1 and CGPM2 with the improved method, parameters INF-γ in papillary blood and contents of the PP after 6 and 12 months were significantly higher, up to 2 times ($p < 0.01$), than in patients treated with the traditional method. TNF-α concentration in the contents of the PP patients with CGPM1 and CGPM2 after 12 months of treatment with the refined and traditional method is significantly decreased – 17 times, compared with the baseline ($p < 0.01$). TNF-α level in the peripheral gum blood in the treatment group was significantly lower (7 times) than the concentration with TNF-α before and after treatment in control group patients ($p < 0.01$). In the treatment group, after 6 and 12 months a significant (2-time larger) decrease of IL-4 level in the papillary blood and in the PP content ($p < 0.01$) was observed, unlike in the control group. According to the research, we found that the level of IL-8 in the papillary blood and contents of the PP in the study

group at 6 and 12 months were significantly (2-times) lower in comparison with the control group ($p < 0.01$).

4. Conclusions

Using "Bifidumbacterin forte" eubiotics and "Parodontax" toothpaste in the complex therapeutic measures of patients with CGPM1 and CGPM2 a significant (4-times higher) reduction of the bacterial contamination of PP by S.tropicalis colonies ($p < 0.01$) was possible, due to the antagonistic action against pathogenic and opportunistic pathogens, which correlates with a significant increase in pro-inflammatory cytokines (TNF-α, IL-8), a decrease in the concentration of INF-γ, and increasing levels of anti-inflammatory cytokine IL-4.

Oral hygiene products containing antiseptic (chlorhexidine, triclosan) should not be recommended as means of hygiene for daily long-term use, in order to avoid the problems with oral cavity microbiocenosis. Such hygiene products should be properly administered to patients with inflammatory periodontal diseases during the attack period, for no longer than 2-4 weeks, followed by a break of two to three months for the restoration of the local microbial background.

Chronic use of these agents in low doses, insufficient to achieve an antibacterial effect, may result in antibiotic-resistant strains of microorganisms. A dynamic study of the changes in clinical and laboratory parameters on the complex and improved traditional methods of therapy allows establishing more efficient treatment results of CGPM1 40% and CGPM2 by 37%, thereby increasing the quality of the treatment process in patients with inflammatory periodontal diseases.

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