ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Probiotics and Oral Health.

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ABSTRACT

The aim of this study was to evaluate the effects of probiotic lozenges as an adjunct to scaling and root planing (SRP) in chronic periodontitis patients. Thirty chronic periodontitis patients divided in examined group (SRP+ probiotic, n=15) and control group (SRP, n=15), were monitored clinically (plaque index(PI), the gingival index(GI), the probing pocket depth(PD), clinical attachment level(CAL) and microbiological parameters were recorded on day 0, and 1 month after therapy. The Prolife® lozenges containing: Bacillus coagulans (Lactobacillus sporogenes), Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacterium bifidum (≥ 2,1 x 109), were used two times a day for 15 days. PI and GI were significantly reduced in both groups ($p < 0.05$) after the treatment. PD in examined group of 4,93± 0,7mm decreased to 3,96±0,8 mm (p>0,05), versus mean PD of 5,2 ± 0,7 mm in control group that was equally after the treatment (p>0,05). CAL gains of 4,2±1,3 mm to 3,86±1,3mm in the examined group versus 4,36±1,2 mm to 4,2± 1,2 (p>0,05) in control group. Microbiological examination demonstrated decreased of quantum of above 100 bacterial colonies of anaerobes and facultative anaerobes, from 66,7% to 33,3% in the examined group, according to 53,3% to 46,7% in control group. Despite data indicate an effect of probiotics on the oral microbiota and a more limited effect on clinical periodontal outcome measures, it can be recommended as a useful adjunct to SRP in chronic periodontitis patients.

Keywords: Lactobacillus, periodontitis, probiotics, scaling and root planning.

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ISSN: 0975-8585

INTRODUCTION

Several clinical studies have already demonstrated the effectiveness of certain probiotics in the treatment of systemic and infectious diseases such as acute diarrhea and Crohn's disease. Other studies have suggested potential applications in the treatment of cardiovascular disease, urogenital infections, oropharyngeal infections and cancers (1-3).

The vast majority of probiotic bacteria belong to the genera Lactobacillus, Bifidobacterium, Propionibacterium and Streptococcus. Probiotics may also prove useful in addressing problems arising from the excessive use of antibiotics, specifically the appearance of bacterial resistance.

Physiologically, oral cavity is connected to the whole body and by this, they influenced each others continuously by and influences general health. Because the oral microbiota is at least as complex as the gastro-intestinal or vaginal microbiota and dental biofilms are considered to be difficult therapeutic targets (Socransky&Haffajee), the encouraging effects of probiotics in different fields of healthcare have resulted recently in the introduction of probiotics for oral healthcare. Today, several clinical studies on the effects of probiotics in different fields of oral pathology have been published such as: halitosis, oral candidiasis, and tooth decay, but they have had only limited study (4,5).

Healthy gingiva is associated with a supragingival biofilm consisted of a few (1–20) layers of oral streptococci, Gram-positive rods and a very few Gram-negative cocci, in contrast to gingivitis which is associated with a more organized dental plaque of 100–300 layers, with anaerobic Gram-negative rods and filaments, and periodontitis which is connected with several species belonging to 'red' and 'orange' complexes (*Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola,* and *Prevotella intermedia* and *Fusobacterium nucleatum*)(6).

Periodontitis is a multifactorial disease that involves the hard and soft tissue, with microbial colonization, and inflammatory responses. It's well known that the main pathogens associated with periodontitis are: Porfiromonas gingivalis, Treponema denticola, Tannerella forsythia and Agregatibacter actinomycetemcomitans. There are a several efforts to change the treatment of periodontitis and probiotics might be a promising therapy (7).

Despite the presence of these microorganisms, the deficit of good bacteria, is also thought to play an important role in their pathogenesis. Thus colonization of certain oral streptococci (*S. sanguinis)*, might be one factor offering protection against periodontitis. Some beneficial bacteria can inhibit some pathogens through metabolic antagonism or by directly inactivating them, posses both antimicrobial as well as anti-inflammatory properties (8). In this context, the administration of probiotics, could be the new therapy in the prevention and treatment of periodontal diseases.

The aim of this study was to the evaluate the microbiological and clinical effects of probiotic lozenge as an adjunct to scaling and root planning (SRP) at chronic periodontitis patients.

MATERIAL AND METHODS

30 patients mean age 36,2 ± 9,5 years, with diagnosed chronic periodontitis (clinically and radiographic evidence of periodontal pockets (5–7 mm), were included in the study. None of them, had ongoing antibiotic treatment or had any systemic disease. Patients who were pregnant, lactating, smokers, alcoholic, or who had undergone any surgical or non-surgical therapy within 6 months prior to the beginning of the study were not included. All the patients assigned informed consent for inclusion before they participated in the study. The study protocol was in accordance with the local ethical guidelines and in accordance with the Helsinki Declaration of Human Rights and approved by the local ethics committee.

Patients were divided in two groups: probiotic group (n=15) ; scaling and root planning (SRP + Prolife® probiotics), and control group (n=15) where SRP was the unique therapy. SRP in all patients was achieved by treating four quadrants for 3 days, using ultrasonic (Kavo Soniflex® 2000N Scaler; frequency 6000 Hz; amplitude 160 µm) and hand instruments (Universal Gracey Curettes, Zotas). Patients were instructed to perform regular oral hygiene, i.e. twice daily, using Bass technique for 3 min.

ISSN: 0975-8585

The Prolife®JGL (Zeta pharmaceutica) lozenge containing Bacillus coagulans (Lactobacillus sporogenes), Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus, Bifidobacterium bifidum (≥ 2,1 x 109), were used two times a day for 15 days. The lozenge were administered to the patients from day 3 after the SRP and continued until day 18. The patients were instructed to take suck lozenge by sucking in the morning and one at night, after brushing their teeth.

The clinical parameters (plaque index(PI), the gingival index(GI), the probing pocket depth(PD), clinical attachment level (CAL) and the microbiological parameters were recorded on day 0, and 1 month after therapy, from the same sites. All parameters were assessed according to the guide lines of the University Dental Clinical Centre "St. Pantelejmon", Skopje, Department of periodontology and Ethics Committee.

After recording the clinical parameters, subgingival plaque samples were collected from the bottom of periodontal pocket of the selected teeth. Subgingival pooled plaque was isolated with sterile cotton rolls (standard size 35), during 20 seconds, and the supragingival plaque was removed using cotton rolls and air dried. After collecting, they were put in sterile vacutainer K2E 5,4mg for microbiological analyze.

Samples were pot in the following mediums: blood agar (Columbia agar +5% sheep blood, Oxoid, UK) for cultivation *of Gram+ and Gram- aerobic bacteria,* Schaedler +5%sheep blood, Oxoid, UK) for *Gram+ and Gram- anaerobes and facultative anaerobes. Each microbiological sample was pot at the same time in the both mediums.*

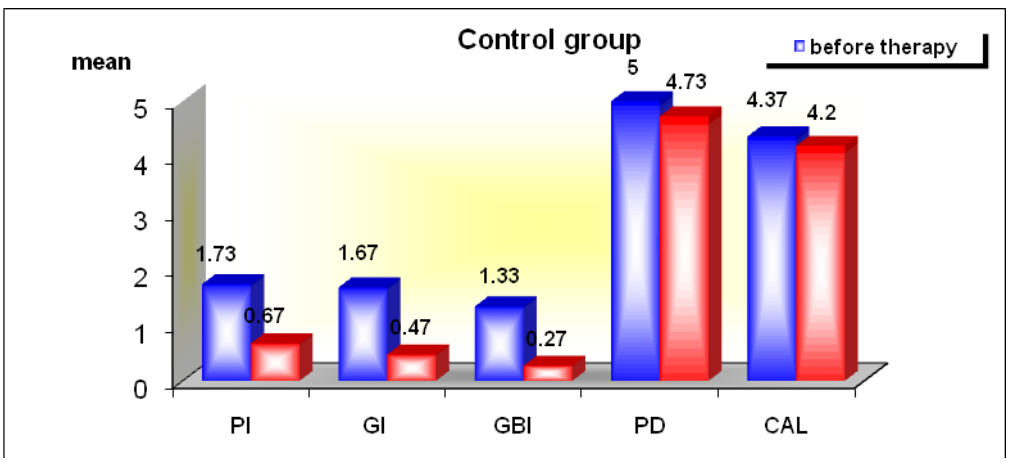
Anaerobic plates with AnaeroGen sachet were placed in a sealed jar, to realize anaerobic condition. Where an AnaeroGen sachet is placed in a sealed jar, the atmospheric oxygen in the jar is rapidly absorbed with the simultaneous generation of carbon dioxide. The oxygen level is reduced in the jar to, 1% within 30 min, and the level of carbon dioxide is between 9 to 13% . After the incubation period of 24 and 48 hours, the plates were removed and examined for the presence of anaerobes.

All clinical and microbiologic data collected were subjected to statistical analysis. Results are expressed as mean±SD and proportions as percentages. For clinical parameters, intra-group comparisons were made by paired *t*-test and inter-group comparison by unpaired *t*-test. For microbiological parameters, non-parametric methods were used for analysis since microbes were non-normally distributed; the Wilcoxon's signed rank test was used for intra-group comparison and the Mann–Whitney test for inter-group comparison. For all tests a *p*-value of less than 0.05 was considered statistically significant.

RESULTS

At the following figures (fig.1, fig.2, fig.3), is presented the mean and median value for the plaque index (PI), gingival inflammation index (GI), gingival bleeding index (GBI), the probing pocket depth(PD), and clinical attachment level (CAL) at probiotic and control group before and after therapy.

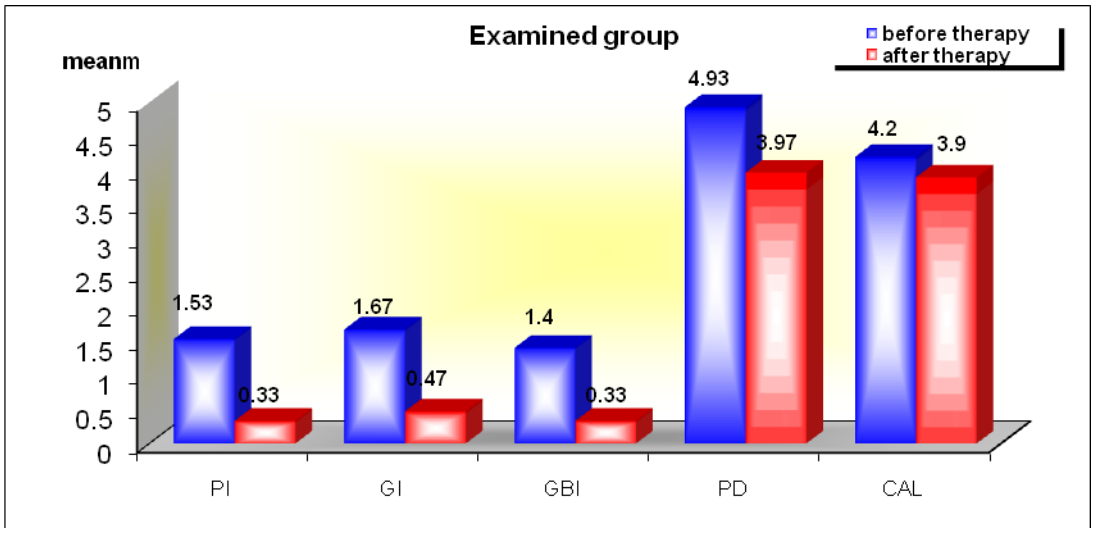
Fig 1: Findings of mean value for examined clinical parameters before and after therapy at probiotic group



ISSN: 0975-8585

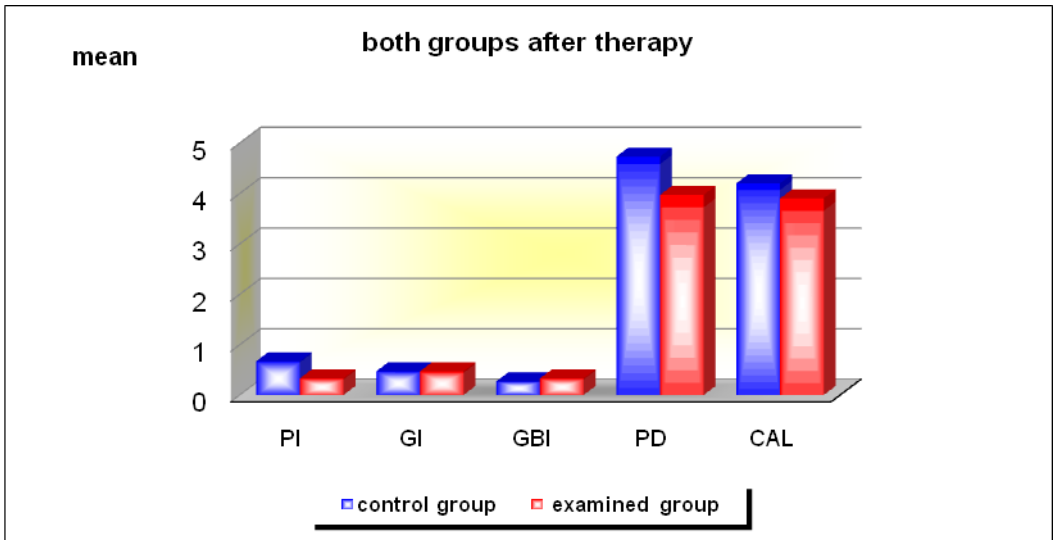
Fig.1 is showing statistical differences for PI (med 2,0 p=0,0006, GI (med 2,0 p=0,00065), GBI (med 1,0 p=0,00098), PD (4,93±0,86 p=0,003) and CAL (4,2±0,94 p=0,044), before and after therapy at probiotic group.

Fig 2: Findings of mean value for examined clinical parameters before and after therapy at SRP group



The results from control group , presents statistical differences before and after therapy for PI (med 2,0 p=0,00098), GI (med 2,0 p=0,0009), GBI (med 1,0 p=0,006), and PD (5,0±0,92 p=0,006). The mean value for CAL decreased after the conservative treatment, but unsignificant (4,37±0,85 vs 4,2±0,73 p=0,17), (Fig.2).

Fig 3: Comparison of clinical parameters at 1 months between the examined groups after therapy

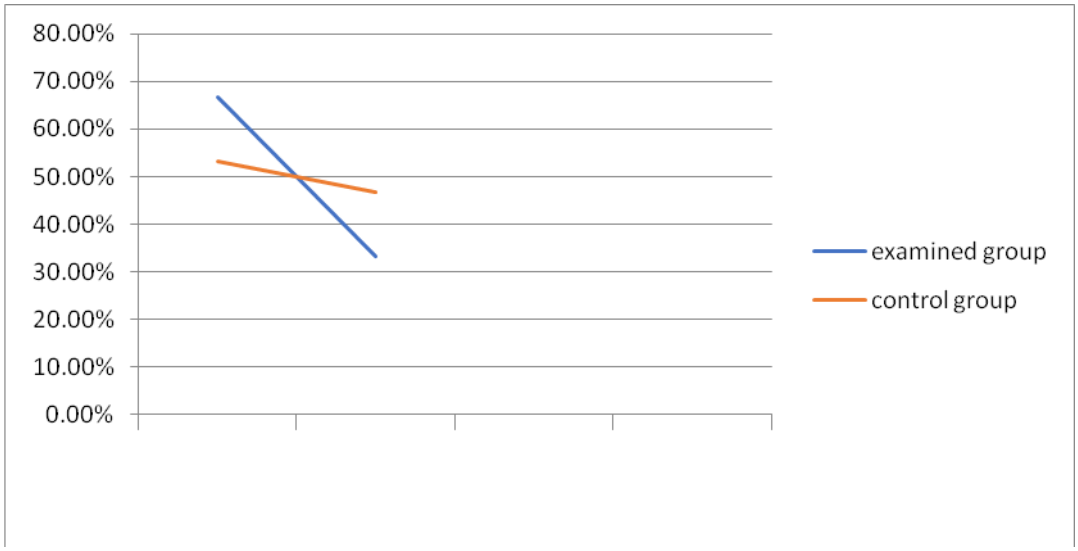


The results after treatment from the both groups (Fig.3) have unsignificant differences for PI (p=0,15 NS), GBI (p=0,76) and for CAL (p=0,32). There was significant difference only for PD (p=0,045). The mean value for PD after treatmen was less significant in the probiotic group (3,97±1,06 vs 4,73±0,94).

Microbiological examination demonstrated decreased of quantum of above 100 bacterial colonies of anaerobes and facultative anaerobes, from 66,7% to 33,3% in the examined group, according to 53,3% to 46,7% in control group (Fig.4).

ISSN: 0975-8585

Fig 4: Quantum of above 100 bacterial colonies of anaerobes and facultative anaerobes bacteria



DISCUSSION

The oral cavity is a complex environment with bacterial biofilms posing as the primary therapeutic target in periodontal disease. This complex aggregate of bacteria is supported and protected by a very dense layer of EPS (exopolysaccharide) making this environment resistant to mechanical forces and antibiotics. Although current modalities of periodontal therapy aim at eliminating all plaque microorganisms, the Specific Plaque Hypothesis states that only certain microorganisms found in dental plaque are responsible for periodontal disease (1).

It's well known that in the same time the host and microbes, are the key factors for the development of the periodontal disease. The present of the pathogenic microbes, the absense of the good microbes and the sensitivity of the host, are the main ethiological factors for the periodontal disease. The count of periopathogens is decreased after the curettage, but this is only temporary, even in combination with antiseptic and antibiotic because of recolonization at the treated surfaces. Some good bacteria possess antimicrobic and anti-inflammatory characteristic, so they could have a positive effect in periodontal treatment. It's well known that good bacteria have the possibility to perfectly adapting in human oral cavity and could play a role in the oral ecological balance (2).

It is well known that the use of doxycycline effectively reduse the A. actinomycetemcomitans counts, but in the same time it had a negative effect on the Lactobacilli counts and may also lead to the development of drug resistance (9). The need of probiotics use, appeared because of the increased use of antibiotics and resistant microorganism. Althout, the benefit of the probiotics use for the oral pathology is still limited in a small studies.

Recently, various studies have reported lactic acid inhibition of oral bacteria suggesting a promising role in combating periodontal diseases (3).

Probiotics are living and beneficial microbes, which when administrated in adequate amounts, they can improved the general health by destroying only the pathogenic microbes. Oral administration of probiotics could also be of some benefit for oral health, by preventing the growth of harmful microbiota or by modulating mucosal immunity in the oral cavity.

Treatment of periodontal diseases in recent years has moved towards an antibiotic/antimicrobial model of disease management. Probiotics might be a promising area of research in the treatment of periodontitis.

Perdigon et al. (10) have also documented the antimicrobial properties of probiotics. Probiotic bacteria can produce a range of products that demonstrate antimicrobial properties namely hydrogen peroxide, lactic acid and bacteriocins. Bacteriocin, is a proteinaceous toxin produced by bacteria to inhibit the growth of similar or closely related bacterial strain. A bacteriocin from *Lactobacillus paracasei* is lethal for *Porphyromonas gingivalis*, a primary periodontal pathogen by changing the cell envelope of the pathogen. *Streptococcus salivarius* is another bacterial species which has been studied regarding its bacteriocin production, and as a native species in the mouth, may be considered as a candidate for an oral probiotic.

Probiotic microorganisms act by different mechanism: affecting the microbiota, modulating immunological parameters, epithelial permeability and bacterial translocation, or by providing bioactive or regulatory metabolites (11). They can also suppress the endogenous pathogens or prevent the super infection with exogenous pathogens, as protecting us through the promotion of a beneficial host response (12).

Perdigon et al. (10) reported increased phagocytic capacity of macrophages when challenged with *Lactobaccilus acidophilus* and *Lactobacillus casei.* It is also known that probiotics can regulate the expression of phagocytosis receptors in the neutrophils of healthy individuals.

According to results from our study, clinical indexes: PI (med 2,0 p=0,0006), GI (med 2,0 p=0,00065), GBI (med 1,0 p=0,00098), PD (4,93±0,86 p=0,003) and CAL (4,2±0,94 p=0,044), were significantly reduced after therapy in probiotic group (Tab.1).

The same results have reported Vivekanada et al.(13) for decreased GI, and PI. Krasse et al.(14) found that intake of L. reuteri for a period of 14 days led to the establishment of the strain in the oral cavity and significant reduction of plaque in patients with moderate to severe gingivitis.

Volozhin et al.(15) has shown that a collagenous periodontal dressing containing L.casei 37, can significantly reduce the number of periodontal pathogens and extend remission periods up to 10 -12 months. This might be due to the inhibitory effect of probiotics on the growth of pathogens thus altering the composition of oral biofilm.

Tablets containing *L.salivarius* WB21, has been shown to decrease gingival pocket depth, particularly in high-risk groups such as smokers, and also affect the number of periodontopathogens in plaque (16). According to Narva et al. (17), during the fermentation process in milk, Lactobacillus helveticus produces short peptides that act on osteoblasts and increase their activity in bone formation. These bioactive peptides could thereby contribute in reducing bone resorption associated with periodontitis. Recently, an *in vitro* study (18) done on *Lactobacillus helveticus* demonstrated release of short peptide stimulate osteoblast to promote bone formation, thus proposing important role in repair of periodontal bone destruction.

The results from control group (SRP) demonstrated statistical differences before and after therapy for PI (med 2,0 p=0,00098), GI (med 2,0 p=0,0009), GBI (med 1,0 p=0,006), and PD (5,0±0,92 p=0,006). The mean value for CAL decreased after the conservative treatment, but these changes were not significant either over time of period (4,37±0,85 vs 4,2±0,73 p=0,17), (Tab.2). Inter-group comparison of PI(p=0,15), GBI(p=0,76) and CAL(p=0,32) revealed no statistical significance except for PD (p=0,045) in moderate pockets for the test group (SRP+ probiotic). The mean value for PD after the therapy is less in test group (3,97±1,06 vs 4,73±0,94).

For period of 30 days, all clinical parameters were significant decreased, althout the value for PD was more decreased in group with probiotic. According to our results, there wasn't any significant differences between the both treatments (SRP versus SRP + probiotics), as we can concluded that both therapies were efficient and satisfied, althout we can recommended the use of probiotics together with conservative treatment.

In the present study, the obtained results were in accordance with the findings of Penala et al. (19), *and* Vivekananda *et al.*(13).These results are in contrary to the findings of Teughels *et al.*(20), where they have achieved a significant PD and CAL in moderate and deep pockets, after adjunctive use of probiotics in lozenge form for 3 months. This might be due to the fact that the probiotics in their study were delivered throughout the study period (3 months), whereas in the present study, pastilles were given for only 1 month.

The possible reasons for this can be attributed to the different studies, form of probiotics, their delivery and duration of time.

The reduction of pro-inflammatory cytokines in GCF may be proof of principle for the probiotic approach combating inflammation in the oral cavity (21).

Most authors concluded that the use of oral probiotics was associated with an improvement in oral health, including a significantly reduced level of cariogenic and periodontal pathogens and a lower crevicular fluid volume and cytokine concentration (22).

Recently Shimazaki et al.(23) reported that people especially not-smokers who have consumed yogurt, have decreased value for PI and CAL, versus patients who have not consumed. Althout lactic acid bacteria are responsible for this benefit, it's necessary for further longitudinal studies, to clarify the connection between probiotics and periodontal health. The another results demonstrated that bio-yogurt and the probiotics that it contains are capable of inhibiting specific periodontal pathogens but have no effect on the periodontal protective bacteria (24).

The use of probiotic chewing gum containing *L. reuteri* ATCC 55730 and ATCC PTA 5289 also decreased levels of pro-inflammatory cytokines in GCF, and the use of *L. brevis* decreased MMP (collagenase) activity and other inflammatory markers in saliva (19).

Teughels *et al.*(20) reported that the subgingival application of a bacterial mixture including *Streptococcus sanguis, S. salivarius,* and *Streptococcus mitis* after scaling and root planing significantly suppressed the re-colonization of *Porphyromona gulae* (canine *P. gingivalis)* and *P. intermedia* in a beagle dog model.

Microbiological analyses showed that the quantum of above 100 bacterial colonies for the anaerobes and facultative anaerobes in the probiotic group, from 66, 7% during the second appointment, decreased to 33,3%. Compared to the probiotic group, the quantum of above 100 bacterial colonies for the anaerobes and facultative anaerobes in the control group during the second appointment, decreased from 53,3% to 46,7%. The result revealed significant decrease the quantum of bacterial colonies in probiotic group than the control group. Apart that, the qualitative data have shown that on the anaerobic agar the following bacterial genera were isolated in the highest proportions: Peptostreptococcus, Peptococcus, Streptococcus viridians, Bacteroides and Prevotella.

The particular strains used in the present study (*Lactobacillus)* are proven to be antagonistic to *P. gingivalis,* by producing antimicrobial substances such as reuterin (*L. reuteri*) and eliminate them from their binding sites (25). These results are in accordance with the observations of Iwamoto *et al.*(26) where they have used *L. salivarius* and *L. reuteri* probiotics, respectively.

Most of the currently available data indicate an effect of probiotics on the oral microbiota and a more limited effect on clinical periodontal outcome measures. A review of these studies indicated a limited effect of probiotics on periodontal parameters when compared to the studies reporting microbiological outcomes.

In contrast to the obtained effects of SRP alone on the clinical parameters, we found no significant effects of SRP alone on the microbiological parameters. However, this phenomenon has also been reported by others. Sbordone et al. (27) evaluated the recolonization patterns of the subgingival microflora of eight adult periodontitis patients after a single session of SRP. Their results indicate that a single session of SRP is clearly insufficient to maintain a healthy subgingival microflora. Mombelli et al. (28) conducted a study to determine the topographic distribution of *Aa* in patients with adult periodontitis before and after mechanical periodontal treatment (repeated oral hygiene instructions and systematic deep SRP) and found that *Aa* was present in 40% of the samples taken before and in 23% of the samples taken after treatment.

Probiotic therapy could also have an unlike effect, because of acidogenicity of lactobacilli and bifidobacteria. Tsubura et al.(29) revealed that one *L. salivarius* strain is able to induce caries in an animal model, and another is able to make a biofilm model more cariogenic.

The use of probiotics is still unsure, because most studies have been fairly short, long-term colonization by probiotic bacteria is seem to be unlikely, and probiotic products are used widely without control.

CONCLUSION

Within the limitations of the study, the present investigation showed that the adjunctive use of probiotics offers clinical benefit in terms of pocket depth reduction and reduced gingival parameters.

Despite the results that revealed decrease of oral bacteria, less more than clinical parameters, we belived that probiotic pastiles can be used as adjuncet therapy at periodontal disease, especially in patients that have contraindication for antibiotic use and in subjects at a high risk of periodontal disease.

Future extended and long term researches of probiotics, will allow better understanding the concept of probiotics and their effect on oral health, and answer many question, which are still actual.

ACKNOWLEDGMENT

We would like to express our gratitude to laboratory technician Nadica Ristovska, employed at the Institute of Microbiology and Parasitology, Medical Faculty in Skopje, for performing standard microbiology techniques of the samples as soon as they arrived at the Institute.

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