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A Randomized Controlled Trial to Evaluate the Effectiveness of Synbiotic Mouthwash vs Probiotic Mouthwash as an Adjunct to Scaling in Patients with Plaque induced gingivitis along with Fixed Orthodontic Appliances: A Clinical and Microbiological Study

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Abstract

Background: Patients with fixed orthodontic appliances benefit with the use of adjunctive therapy such as mouthwash along with mechanical tooth brushing. Chlorhexidine, although being the gold standard has associated side-effects, limiting its long-term use. The current study aims at providing a suitable alternative oral-hygiene adjunct for long-term usage. The goal of the study is to evaluate clinically and microbiologically the effectiveness of Synbiotic mouthwash vs Probiotic mouthwash as an adjunct to scaling in patients with fixed Orthodontic appliances having plaque-induced gingivitis.

Methods: Sample comprised of 80 patients with fixed orthodontic appliances having plaqueinduced gingivitis. Patients were divided equally into 4 groups. All patients used either probioticmouthwash(control group) or synbiotic mouthwash(experimental group) or 0.12% chlorhexidine mouthwash(Positive control) or warm saline rinse(negative control) for 30 days. Dental prophylaxis was performed before the procedure. The plaque, gingival and papilla bleeding indices were used. Presence or absence of stains was evaluated; the number of colony forming units (CFU's) were assessed, at baseline and 1 month post-op.

Results: The Intragroup results showed statistically significant improvement ($p<0.05$) in all the groups except the negative control. The Intergroup analysis revealed no statistically significant differences in plaque ($p=0.1496$), gingival ($p=0.1468$) and papilla bleeding indices ($p=0.1688$). No staining was observed in any of the groups. The control and experimental groups showed Significant increase in the no of CFU's; the positive control showed significant reduction in the no of CFU's whereas the negative control showed no change. In terms of adverse effects, four patients in the positive control group reported a bad taste.

Conclusions: The study revealed that both the experimental and study group ie., synbiotic and probiotic groups respectively showed significant improvement in

clinical parameters within a month, which were comparable to the chlorhexidine group. After co-relating the clinical and microbiological analysis, it can be concluded that Probiotic and Synbiotic achieve clinical result similar to chlorhexidine merely by altering the composition of microflora with minimal sideeffects.

Summary

Oral cavity is a home to a plethora of microbes, with most being commensals and few being pathogens. When dysbiosis occurs, this balance is disrupted and disease sets in. Using antimicrobials to totally abolish this micro flora is not always necessary. Synbiotic and probiotics on the contrary, alter the bacterial ecology and show improvement in clinical parameters comparable to antimicrobials such as chlorhexidine. This alternative bacteria replacement therapy suppresses pathogenic micro flora by promoting the growth of more beneficial commensals. It is thus beneficial to the host naturally with limited side-effects and hence can be used as an alternative adjunctive therapy.

Key words: Synbiotic, Probiotic, Chlorhexidine, bacteria replacement therapy, plaque induced gingivitis, orthodontic treatment, mouthwash.

Introduction

Orthodontics aims at establishing functional occlusion with improved esthetics and phonetics. However,orthodontic treatment usually requires usage of complex arch-wires, bands and brackets. These, not justact as plaque retentive areas, but also make self-cleaning and oral hygiene maintenance difficult,subsequently resulting in inflammation. Also, positive effects of orthodontic treatment may bethreatened if adequate and regular oral hygiene is not practiced.As noted by De Paola et al.,1 mechanical oral hygiene methods of plaque removal require time,motivation, and manual dexterity. Although home care practices are effective, they are neitherpracticed precisely nor consistently. These limitations necessitate the use of other suitable adjuncts.2Chemotherapeutic agents can play a crucial role as adjuncts of mechanical plaque-control methods.These antimicrobial agents include metal salts (tin fluoride, zinc, or copper); essential oils; phenols(triclosan); fluorides (sodium fluoride or stannous fluoride); bisbiguanides(chlorhexidine); quaternaryammonium compounds (chloride cetylpyridium); sanguinarine; and oxygenating agents among others. Chlorhexidine is considered the gold standard agent for its clinical efficacy in chemical plaque control. But with the reported side effects, its long term use is questionable.3 However with improved knowledge and better understanding of human body at bio-molecular levels,there has been a paradigm shift from nonspecific to specific approach. Newer treatment optionspropose altering ecology of niches, in order to modify pathological plaque to a biofilm ofcommensalisms.4 “Probiotic therapy” or ‘bacterial replacement therapy’ is one such alternative. Probiotics are live microorganisms administered in adequate amounts with beneficial health effects on the host.5 Not all bacteria are bad. Unlike antibiotics, probiotics repopulate the beneficial bacteria which can help kill pathogenic bacteria and fight against infection. Probiotic species bacteria are generally regarded as safe because they can reside in the human body causing no harm and on the other hand are also important for promoting health. They play a crucial role in halting, altering or delaying periodontal diseases and have great potential in arena of Periodontics in terms of plaque modification, altering anaerobic bacteria colonization, improvement of pocket depth and clinical attachment loss.6To improve the survival of probiotics, Prebiotics were introduced. Prebiotics are non-digestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of or a limited number of

bacterial species already established, and thus in effect improve host health. It includesinulin, fructo-oligosaccharides, galacto-oligosaccharides and lactulose.6 They are beneficial to probioticsby acting as a fertilizer.7 High potential is attributed to the simultaneous use of probiotic and prebioticalso called as 'Synbiotic'. Synbiotic means a mixture of probiotics and Prebiotics affecting the host by improving the survival of live microbial dietary supplements in gastrointestinal tract of host.8 "Synbiotic" essentially means synergism, and this term should be reserved for products in which the prebiotic compounds selectively favor probiotics.9 It appears that the rationale to use synbiotics, is based on observations showing the improvement of survival of the probiotic bacteria du ring the passage through the upper intestinal tract.9 This clinical and microbiological study aims to assess the clinical efficacy of Synbiotic mouthwash and probiotic mouthwash as against the positive control 0.12% chlorhexidine mouthwash and warm saline rinse as the negative control in patients with fixed orthodontic appliances with plaque induced gingivitis over a 30-day period. Other objectives of this clinical investigation were to determine the effect of the different mouthwashes on the total colony counts when cultured.

Materials and Methods

Study Population: A Total Of 80 Patients (38 Male And 42 Female, Aged 15-28 Years) Were Included In The study. The Study Was Conducted By The Department Of Periodontology, Nair Hospital Dental College, Mumbai, India From October 2018 To January 2019. Ethical Clearance Was Obtained From Institutional ethics Committee. The Trail Was Duly Registered With The Clinical Trial Registry, India (Ctri Regno. Ctri/2018/10/016100)The Inclusion Criteria For The Study Were:

1. Patients with fixed orthodontic appliances with a minimum of 20 teeth (≥5 teeth per quadrant)
2. Patients classified as stage II and stage III gingivitis based upon the gingival score given by Loe and Silness 1963.
3. Patient with clinical bleeding on probing present.

The exclusion criteria for the study were:

1. Allergy or hypersensitivity to chlorhexidine or related compounds
2. Use of antibiotics within last 3 months
3. Periodontal pockets >4mm
4. Systemic diseases
5. Pregnant/ lactating women
6. Smokers
7. History of undergoing nonsurgical and surgical periodontal therapy in the last 6 months.

Study Design

A randomized, parallel group clinical study was conducted on 80 systemically healthy patients with fixed orthodontic appliances and plaque induced gingivitis. The clinical parameters were recorded in a case history proforma. The subjects were assessed for plaque and gingival inflammation by recording the Gingival index(GI) (Loe and Silness 1963)10, Papilla bleeding index (PBI)(Muhlemann 1977)11, Bonded bracket Plaque Index (BBPI)(Kilicoglu et al)12 and presence or absence of stains. The total colony forming units were determined using aerobic and anaerobic culture. Samples were collected first thing in the morning. Patients were advised not to eat/drink/rinse 30 min before sample collection. For aerobic culture, 10 ml of normal saline was given to the patients to be swished in the oral cavity for 30s and then collected in a labeled sterile container. For anaerobic culture sub gingival plaque samples were collected from premolar-molar area using sterile Gracey curettes, after isolating the area with sterile cotton rolls. The obtained

samples were immediately transferred to the microbiological laboratory where they were serially diluted and plated on Blood agar and MacConkey agar plates. Following an incubation period of 24h at 37oC, the total colony forming units (CFU's) were assessed using colony counter by the Microbiologist. Thorough dental prophylaxis was performed. Tooth brushing (Charter's Technique) was demonstrated to the patients. No other oral hygiene aid/adjunct was provided. The patients were randomly divided using computer assisted randomization into 4 groups consisting of 20 patients each as under:

Group A: Synbiotic mouthwash (Biofibe probiotic with prebiotic, Shrey Neutraceutics Pvt ltd+10 ml water)

Group B: Probiotic mouthwash (Wonderpro Probiotic, Lifezen +10 ml water)

Group C: Chlorhexidine Mouthwash 0.12% with ADS (Hexidine EP, ICPA)

Group D: Warm Saline mouth rinse

An informed written consent was obtained from each patient included in the study.

The patients in Group A were given Biofibe sachets (1g containing Lactobacillus acidophius, Lactobacillus rhamnosus, Bifid bacterium longum, Lactobacillus sporogenes, Saccharomyces boulardii, Llactobacillusparacasei, inulin and fructooligosaccharides)The patients in Group B were given Wonderpro sachets (1g containing Lactobacillus acidophius, Lactobacillus rhamnosus, Bifid bacterium longum, Lactobacillus sporogenes, Saccharomyces boulardii)The patients were demonstrated and instructed to prepare the experimental probiotic and Synbiotic mouth wash by mixing together the contents of the provided sachet and 10 ml of water. Emphasis was made to explain to the patient that the solution had to be stirred thoroughly until all the contents were completely dissolved in the distilled water. The formulation had to be prepared and rinsed immediately once prepared and could not be stored. All the four groups were advised to rinse their mouths with the respective mouthwashes prescribed to them for 30 days without any dilution for 1 min twice daily half an hour after brushing. They were advised not to eat anything for half an hour after using the mouthwash. The clinical parameters of BBPI, GI, and PBI recorded at baseline were repeated one month post-op.

Data Analysis

Descriptive and inferential statistical analyses were carried out. Results on continuous measurements were presented on Mean ± SD and results on categorical measurement were presented in number (%). Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant. Chi square analysis was used to find the significance of study parameters on categorical scale. Student t tests (two tailed, paired) was used to find the significance of study parameters on continuous scale within the group at different time intervals. Analysis of variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis). Further post hoc analysis was carried out if the values of ANOVA test were significant. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables etc.

Results

The mean baseline BBPI value for synbiotic mouthrinse was 1.93±0.39; probiotic mouthrinse was 1.95±0.43; Chlorhexidine mouthwash was 1.93±0.39 and warm saline rinse was 1.96±0.42. The mean BBPI value 1 month post-op for synbiotic mouthrinse was 0.32±0.14; probiotic mouthrinse was 0.38±0.22; Chlorhexidine mouthwash was 0.45±0.24 and warm saline rinse was 1.00±0.34. The

degree of increment of mean PI scores was more pronounced for the negative control(warm saline) rinse as compared to Synbiotic, Probiotic and chlorhexidine rinses. The intra group analysis were statistically significant with $p<0.05$, whereas the Turkey 's post hoc intergroup analysis between Synbiotic, probiotic and chlorhexidine mouth rinses were statistically insignificant. [Table 1A and 1B] The mean baseline GI value for Synbiotic mouth rinse was 1.89±0.34; probiotic mouth rinse was 2.06±0.51; Chlorhexidine mouthwash was 1.85±0.48 and warm saline rinse was 1.95±0.30. The mean BBPI value 1 month post-op for Synbiotic mouth rinse was 0.29±0.10; probiotic mouth rinse was 0.57±0.26; Chlorhexidine mouthwash was 0.38±0.22 and warm saline rinse was 1.00±0.11. In comparison to the baseline data, there was a significant decrease in mean GI scores of Synbiotic probiotic and chlorhexidine rinses as compared to the negative control rinses, whereas that between Synbiotic, probiotic and chlorhexidine it was statistically insignificant. [Table 2A and 2B] The mean baseline PBI value for Synbiotic mouth rinse was 1.96±0.52; probiotic mouth rinse was

1.89±0.53; Chlorhexidine mouthwash was 1.79±0.39 and warm saline rinse was 1.95±0.48. The mean BBPI value 1 month post-op for Synbiotic mouth rinse was 0.25±0.16; probiotic mouth rinse was 0.24±0.14; Chlorhexidine mouthwash was 0.63±0.14 and warm saline rinse was 1.09±0.21. In comparison to the baseline data, there was a significant decrease in mean GI scores of Synbiotic probiotic and chlorhexidine rinses as compared to the negative control rinses, whereas that between Synbiotic, probiotic and chlorhexidine it was statistically insignificant. [Table 3A and 3B] No stains were observed clinically in any of the groups. The total aerobic colony forming units significantly increased one month post-op in Synbiotic and probiotic groups. The colonies were, however beyond the countable range. Anaerobic culture showed no difference in the total olony counts. The positive control group, on the other hand showed significant decrease in the no of aerobic and anaerobic colonies. The negative control did not show significant improvement on the total colony counts.

Discussion

Chemotherapeutic agents have been used increasingly as an adjunct to mechanical plaque control. They are intended to supplement mechanical oral hygiene aids, and not to replace them. Chlorhexidine is a gold standard chemotherapeutic adjunct which becomes indispensible when periodontal disease sets in. However, long-term use of chlorhexidine often is associated with a number of side effects. These side effects are brown discoloration of the teeth and tongue, oral mucosal erosion, and taste perturbation. And thus need arises to look for an alternative antiplaque agent.13 Antimicrobial mouth rinses such as chlorhexidine act nonspecifically on the oral micro flora resulting in decrease in number of both commensals and pathogens. In contrast, probiotics utilize a specific approach through administration of beneficial bacteria to promote a healthy balance of microorganisms in the mouth.14Probiotic species bacteria are generally regarded as safe because they can reside in the human body causing no harm and on the other hand are also important for promoting health. They play a crucial role in halting, altering or delaying periodontal diseases and have great potential in arena of Periodontics in terms of plaque modification, altering anaerobic bacteria colonization, improvement of pocket depth and clinical attachment loss. Probiotic species mostly belong to the genera lactobacilli and bifid bacterium. To be able to exert its properties in the oral cavity, it is essential for the probiotic microorganisms to resist the oral environmental conditions and be able to successfully colonize and inhibit oral

pathogens.15 Probioticscan be used as a single strain or a consortium of multiple strains. A consortium of multiple strains ismore effective than a single strain.Synbiotics, as mentioned earlier, are a synergistic combination of probiotics and prebiotics.FAO/WHO defines prebiotics as a non-viable food component that confer health benefit(s) on the host associated with modulation of the microbiota. Prebiotics act as fertilizer for probiotics. Synbiotics were developed to overcome possible survival difficulties for probiotics.16The exact mechanism by which probiotics act is still unknown. numerous mechanisms have been proposed including prevention of adhesion of pathogens to host tissues, stimulation, and modulation of the mucosal immune system, e.g., by reducing production of pro-inflammatory cytokines through actions on NFkB pathways, increasing production of anti-inflammatory cytokines such as interleukin-10(IL-10, and host defense peptides such as beta-defense 2, enhancing immunoglobulin A defenses, and influencing dendritic cell maturation. Killing or inhibition of growth of pathogens through production of bacteriocins or other products, such as acid or peroxide, which are antagonistic toward pathogenicbacteria has also been reported.17 It is a common consensus that the oral biofilm in association with aerobic bacteria is the main etiological factor in development of periodontal disease.18 Few experimental studies have explored the use of probiotics in periodontal disease. Krasse et al.19 did astudy in patients with moderate to severe gingivitis who were given one of the two different L.reuteriformulations (LR-1 or LR-2) at a dose of 2 × 10 (8) CFU/day, or a corresponding placebo. L.reuteri was efficacious in reducing both gingivitis and plaque in patients with moderate to severe gingivitis. Noordin and Kamin14 conducted a trial among 90 school children and assigned them into placebo, chlorhexidine, and probiotic groups; and plaque scores were recorded at baseline (0 day), on15th day (after 14 days of intervention), and 3 weeks (after discontinuation of intervention). Probioticmouthrinse was more effective for inhibition of dental plaque accumulation after 14 days ofintervention and also after 3 weeks of discontinuation of intervention. Harini and Anegundi13 evaluatedclinically the efficacy of a probiotic and chlorhexidine mouthrinses on plaque and gingival accumulation in children for 14 days and concluded that the probiotic mouth rinse was found effective in reducing plaque accumulation and gingival inflammation. Teughels et al.20 in a randomized placebo-controlled clinical trial evaluated the effects of L. reuteri - containing probiotic lozenges and placebos as an adjunct to SRP in 30 patients with chronic periodontitis, monitored clinically, and microbiologically at baseline, 3,6, 9, and 12 weeks after therapy. Significant improvement in all clinical parameters reduced P.gingivalis levels, more pocket depth reduction and attachment gain in moderate and deep pockets was observed in the SRP + probiotic group. Purunaik et al.21 aimed to investigate the efficacy of Probiotic (1 gpowder of 1.25 billion freeze dried combination, a mixture of L. acidophilus, L. rhamnosus, B. longum, and S. boulardii), 0.2% of chlorhexidine and placebo mouthrinses in reducing plaque and gingivitis among 90 school children aged 15–16 years. It was found that both probiotic and chlorhexidine mouth rinses were able to significantly reduce plaque and gingival levels after 14 days. Our results indicate that Synbiotic and probiotics could be useful as an adjunct in oral hygiene maintenance especially in subjects at a high risk of developing periodontal disease like the study population. The advantages of using Synbiotic and probiotic mouth rinse are that as it contains friendly commensals, there is no issue of antibiotic resistance, and there are no known/proven toxicities caused due to their use. Very few

studies till date have studied the basic/initial treatment for periodontal patients in terms of SRP and use of probiotic mouthwash in the reduction of clinical parameters of gingivitis in India. There is no clinical study till date which evaluates the efficacy of Synbiotic mouth rinse as against Probiotic mouth rinse and the Gold standard-Chlorhexidine gluconate. A maiden attempt was made in this randomized, parallel clinical trial to evaluate the benefits of scaling and Synbiotic sachet in the treatment of chronic gingivitis and to compare it with scaling and probiotic sachet and with chlorhexidine, which has been regarded as the "gold" standard in dentistry for the prevention of plaque and gingivitis. In three out of the four groups namely Synbiotic, Probiotic and chlorhexidine, BBPI, GI and PBIwere significantly reduced within each group over a period of 30 days. The fourth group namely warm saline rinse did show some reduction in the clinical parameters, which was however not statistically significant. Also the total colony counts in the Synbiotic and probiotic group showed a significant increase, which after correlation with clinical parameters, implies increase in the colonization of 'beneficial bacteria' or 'commensals'. Chlorhexidine on the other hand, showed a significant decrease inthe total colony counts indicating a 'anti' microbial effect on both pathogens and commensals. Warm saline rinse group, had the least effect on the total colony counts. In the present study, using a negative (saline) and a positive control (chlorhexidine), we wearable to state that the Synbiotic mouthwash has shown a good potential as an antiplaque agent and its effectiveness in reducing the plaque accumulation and gingival inflammation is comparable to probioticmouthwash and chlorhexidine. Considering the local side effects of chlorhexidine including brown staining, taste disturbance, enhanced supra gingival calculus formation, and less commonly desquamation of the oral mucosa. Synbiotic and probiotic mouth rinse seems a very effective and economical alternative for patients with periodontal disease The results of our study showed a significant reduction of plaque and gingival status and were in accordance with the above-mentioned studies suggesting that combinations of probiotics strains may have synergistic adhesion effect. Though these strains tested maintained the oro-microbiological balance, their action in the oral cavity is dubious as oral mucosa is not their innate habitat. Furthermore, there is also a need to evaluate whether these lactobacilli strains are momentary or stable oral colonizers. However, it seems plausible that prolonged administration of probiotic preparations may have a preventive role against the development of plaque and gingivitis. The present study had few major limitations. As no Synbiotic and probiotic mouth rinse is commercially available, fresh preparations were needed for every use which was to be used immediately once prepared and could not be stored. Thus a proper vehicle is needed for delivering these beneficial bacteria so as to improve the patient compliance. Likewise, it would be interesting to learn about the additional effects of Synbiotic and Probiotics when the patient is instructed to ingest rather than expectorate the mouth rinse. This was not possible in the current study, since it was a comparative trial evaluating four mouth rinses. The plaque accumulating ability of stainless steel a ceramic brackets differ, which could be a confounding factor. Microbiology did reveal some interesting results. The added effect of synergistic combination of Synbiotic over Probiotics is still unclear. Hence, more precise microbiological tests are needed to evaluate and delineate the appropriate alteration of micro flora. Longitudinal studies involving probiotics and further microbiological evaluation are also essential when prescribing them in place of antiseptics and antimicrobials.

Conclusion

Experimental evidence shows that the micro biome is needed for the health of the host and that alterations in the ecological equilibrium of microbes can lead to disease. Therefore, it is logical to expect that the use of microbes that are members of the micro biome might help us restore balance.22 thus the emergence of Synbiotic and Probiotics appears to be a boon for treatment of oral and systemic diseases. In the present study, the Synbiotic mouth rinse tested was as effective as probiotic mouth rinse and the positive control, Chlorhexidine mouth rinse in reducing plaque accumulation and gingival inflammation. Also the increase in the total beneficial colony counts by Synbiotic and Probiotics seems intriguing. Therefore, Synbiotic and probiotic mouth rinse have a potential therapeutic value, and further long-term studies are recommended to determine its efficacy.

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Abbreviations: GI**:** gingival index, BBPI: bonded bracket plaque index, PBI: papilla bleeding index, CFU: colony forming units.

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Legends Table

Table 1A: Comparison of the Bonded Bracket Plaque Index (BBPI) values in terms of {Mean (SD)} at different time intervals among all the groups using ANOVA test

Group	N	Mean		Std. deviation		P value
		(baseline)	(1 month post-op)	(Baseline)	(1 month post-op)	
Synbiotic	20	1.9270	0.3220	0.38761	0.14047	<0.001**
Probiotic	20	1.9455	0.3805	0.43375	0.22988	<0.001**
Chlorhexidine	20	1.9295	0.4505	0.39420	0.24215	<0.001**
Warm saline rinse	20	1.9635	1.000	0.42250	0.19682	<0.001**
Total	80	1.9414	0.5383	0.40238	0.33903	

(p<0.05 - Significant*, p< 0.001 - Highly significant**SD: Standard Deviation)

Table 1B: Comparison of the **Bonded Bracket Plaque Index (BBPI)** values in terms of {Mean(SD)} at different time intervals among all the groups **Tukey's post hoc analysis**

	Synbiotic	Probiotic	Chlorhexidine	Normal saline
Synbiotic	-	0.806	0.208	<0.001**
Probiotic	0.806	-	0.706	<0.001**
Chlorhexidine	0.202	-	0.706	<0.001**
Warm saline rinse	<0.001**	<0.001**	<0.001**	-

(p<0.05 - Significant*, p< 0.001 - Highly significant** SD: Standard Deviation)

Table 2A: Comparison of the **Gingival Index (GI)** values in terms of {Mean(SD)} at different time intervals among all the groups using ANOVA test

Group	N	Mean		Std. deviation		P value
		(baseline)	(1 month post-op)	(Baseline)	(1 month post-op)	
Synbiotic	20	1.8870	0.2960	0.34339	0.10007	<0.001**
Probiotic	20	2.0555	0.5700	0.51639	0.26428	<0.001**
Chlorhexidine	20	1.8520	0.3840	0.48157	0.22486	<0.001**
Warm saline rinse	20	1.9515	1.0070	0.30301	0.11649	<0.001**
Total	80	1.9365	0.5643	0.42005	0.33273	
(p<0.05 - Significant*, p< 0.001 - Highly significant** SD: Standard Deviation)						

Table 2B: Comparison of the **Gingival Index (GI)** values in terms of {Mean(SD)} at different time intervals among all the groups using **Tukey's post hoc analysis**

	Synbiotic	Probiotic	Chlorhexidine	Normal saline
Synbiotic	-	0.014	0.462	<0.001**
Probiotic	0.442	-	0.014	<0.001**
Chlorhexidine	0.462	0.014	-	<0.001**
Warm saline rinse	<0.001**	<0.001**	<0.001**	-

(p<0.05 - Significant*, p< 0.001 - Highly significant**, SD: Standard Deviation)

Table 3A: Comparison of the **Papilla Bleeding Index (PBI)** values in terms of {Mean(SD)} at different time intervals among all the groups using ANOVA test

Group	N	Mean		Std. deviation		P value
		(baseline)	(1 month post-op)	(Baseline)	(1 month post-op)	
Synbiotic	20	1.9615	0.2525	0.51639	0.15637	<0.001**
Probiotic	20	1.8960	0.2455	0.53612	0.13640	<0.001**
Chlorhexidine	20	1.7895	0.6320	0.38880	0.13927	<0.001**
Warm saline rinse	20	1.9455	1.0985	0.47710	0.20623	<0.001**
Total	80	1.8981	0.5571	0.47847	0.38593	

(p<0.05 - Significant*, p< 0.001 - Highly significant**, SD: Standard Deviation)

Table 3B: : Comparison of the **Papilla Bleeding Index (PBI)** values in terms of {Mean(SD)} at different time intervals among all the groups using **Tukey's post hoc analysis**

	Synbiotic	Probiotic	Chlorhexidine	Normal saline
Synbiotic	-	0.999	0.416	<0.001**
Probiotic	0.999	-	0.414	<0.001**
Chlorhexidine	0.416	0.414	-	<0.001**
Warm saline rinse	<0.001**	<0.001**	<0.001**	-

(p<0.05 - Significant*, p< 0.001 - Highly significant** ,SD: Standard Deviation)