

International Journal of Medical Science and Current Research (IJMSCR) Available online at: www.ijmscr.com Volume1, Issue 4, Page No: 149-158 November-December 2018



# Comparative Evaluation of Chlorhexidine and Probiotic Mouthrinses - A Randomized Controlled Clinical and Microbiological Study

Dr. Sonali Delmade\* , Dr.Rutuja Sindgi, Dr. Mona U. Shah, Dr. Yogesh Doshi, Dr. Cauvery Karbhari, Dr. Vidhi Kevadia

<sup>1</sup> Post Graduate student, <sup>2</sup> Post Graduate student, <sup>3</sup> Professor and HOD, <sup>4, 5</sup> Reader, <sup>6</sup> Senior Lecturer, <sup>7</sup>Post Graduate student Department of Periodontics and Oral Implantology, Pandit Deendayal Upadhyay Dental College, Solapur,Maharashtra, India

**Corresponding Author:** 

Dr. Sonali Shivaji Delmade, BDS Post Graduate student, Department of Periodontics and Oral Implantology, Pandit Deendayal Upadhyay Dental College, Solapur,Maharashtra, India

Type of Publication: Original Research Paper Conflicts of Interest: Nil

### ABSTRACT

**Background:** The aim of our clinical trial is to assess and compare anti-plaque and anti-inflammatory potential of Probiotic mouthrinse with 0.2 percent Chlorhexidine mouthrinse.

**Materials & Methods :** A randomized group study was being conducted for a period of 0 (baseline), 1month & 3 months on 50 systemically healthy individuals having chronic gingivitis. The study was divided into two groups. Group A consisting of 25 subjects were advised Probiotic mouthrinse and group B consisting of 25 subjects were advised Chlorhexidine mouthrinse. The oral prophylaxis was carried out for both groups at baseline. After proper oral hygiene instructions both groups were instructed to rinse their mouth with 10 ml of respective mouthrinses undiluted for 1 min, twice daily, 30 minutes after brushing.

**Results:** Clinical parameters such as Plaque Index, Gingival Index and Oral Hygiene Index were assessed at baseline, 1 month and 3 months. Bacterial culture was also assessed and Colony Forming Unit (CFU) was measured. At the end of 1 month and 3 months, the values of PI, GI, and OHI and also the bacterial count were reduced but were not statistically significant.

**Conclusion:** it was concluded that though Chlorhexidine is considered as a gold standard for plaque control, probiotics mouth rinses can be used as an alternative.

Keywords: Chlorhexidine, gingivitis, microorganisms, probiotics.

## **INTRODUCTION**

Primary cause of periodontal disease is bacterial irritation. Dental plaque accumulation is the prerequisite for the development of gingivitis. Current opinion favors the concept that plaque induced gingivitis always precedes periodontitis although not all gingivitis proceed to periodontitis. Long term success of periodontal treatment is dependent on satisfactory oral hygiene practiced individuals to maintain plaque by levels compatible with gingival health . Periodontal treatment is also directed towards eliminating subgingival plaque which itself is derived from supragingival plaque<sup>1</sup>.

Supragingival plaque control is thus fundamental to the prevention and management of periodontal disease and with appropriate advice and instructions from professionals, is primarily the responsibility of the individual, using tooth brushes and interdental cleaning<sup>2</sup>. Unfortunately,

### .....

it is a fact of life that a significant proportion of individuals fail to practice a high enough standard of plaque removal thus gingivitis is highly prevalent from an early age<sup>3,4</sup> Therefore, chemical agents have increasingly been used as adjuncts to mechanical plaque control. They are intended to augment, not to replace, mechanical plaque control<sup>5</sup>. It is now recognized that chemical antiplaque agents may be of value at inhibiting or plaque formation and thus gingival reducing inflammation<sup>4,6</sup>

The most tested and effective anti-microbial agent known today is Chlorhexidine (CHX) which has been used for more than two decades. However, due to certain limitations such as brown discoloration of teeth, oral mucosal erosion and bitter taste, search for an effective and safe alternative to CHX mouthwash has led to introduction of Probiotics. Probiotics, another potential tool of anti-plaque activity, have been reported to have beneficial effects on oral health.<sup>7</sup> Still, Probiotics are not widely used in clinical dental practice due to lack of awareness about them. This calls for actions to be taken in this direction because once the probiotics set a foothold in dentistry, they can be concomitantly beneficial for oral as well as systemic health of the human body and can apparently prove to be a panacea of health promotion.

Probiotics are defined as "live microorganisms administered in adequate amounts that when confer health benefits on the host". They repopulate the beneficial bacteria, which can help pathogenic bacteria and fight against kill infection. Probiotics administered orally may benefit oral health by preventing the growth of harmful microbiota or by modulating mucosal immunity in the oral cavity. Probiotics may be a area promising of research in periodontal therapy $^{8}$ .

However, only a few clinical studies have been so far conducted on the use of probiotics in the prevention of oral diseases. Thus taking into consideration, all these above facts, this study was carried out to test and compare the potential antiplaque and anti-inflammatory properties of Chlorhexidine and Probiotic in the form of a mouthwash.

### **Matrials and Methos**

After obtaining institutional ethical committee approval, A total of 50 systemically healthy subjects visiting Department the of Periodontology, at the Pandit Deendayal Upadhyay Dental College & Hospital, Solapur were recruited for the study.

The inclusion criteria for the study included:

- 1. Subjects of the age group 20 to 40 years and who agreed to comply with the study visits were included.
- 2. Subjects with gingivitis

The exclusion criteria of the study included:

1. History of systemic diseases

2. Pregnant, lactating females

3. History of oral prophylaxis 6 months prior to the study

4. Subjects with mouth breathing habit

5. Subjects with orthodontic and prosthodontic appliances

6. Subjects with deleterious habit such as smoking

7. History of nonsurgical and surgical periodontal therapy in the last 6 months.

# Study design and clinical measurements

A randomized clinical study was conducted. on 50 systemically healthy patients reporting to the outpatient department of Periodontics and Oral Implantology, at the Pandit Deendayal Upadhyay Dental College & Hospital, 19/1 Kegaon, Solapur with gingivitits.

The clinical parameters were recorded in a case history proforma.

The subjects were assessed for plaque and gingival inflammation by recording the Plaque Index (PI), (Silness and Loe 1964), Gingival Index (GI) (Loe and Silness in1963) and Oral Hygiene Index-Simplified (OHI-S) (Green and Vermillion in 1964) by a single investigator, experienced with index system recording, at baseline, 1 month and 3 months and colony forming unit( CFU) was assessed within the same intervals. Thorough scaling and polishing were performed and the

......................

patients were randomly divided into two groups consisting of 25 patients each as under:

Group B - Chlorhexidine mouthwash 0.02% (Rexidine® [ICPA])

Group A - Probiotic mouthrinse (Imubless [Mankind Pharma] + distilled water)



Fig. 1 0.2 % of Chlorhexidine antimicrobial mouthwash (HEXIDINE<sup>®</sup> [ICPA]) Mouthwash & Probiotic sachets (Imubless) with distilled water

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

The patients in Group A were given Imubless Satchets (probiotic formulation containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum and Bifidobacterium* lactis) and 10ml ampoules of distilled water which are commercially available as shown in Figure 1. The patients in group B were given Chlorhexdine mouthwash 0.02%.

The patients were demonstrated and instructed to prepare the experimental Probiotic mouthwash by mixing together the contents of the sachet and 10 ml of distilled 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 used immediately once prepared and could not be stored.

All the patients in both groups were advised to rinse their mouths with the respective mouthwashes prescribed to them for 3 months 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 PI, GI, and OHI-S recorded at baseline were repeated at 1 month and 3 months. Colony forming unit (CFU) was measured. Figure 2, 3, 4



Fig. 2 Sample collected at baseline



Fig. 3 sample collected after 3 months From Chlorhexidine group

## Statistical analysis

All the samples were subjected to statistical analysis. Comparison of the two groups was done using Independent T test and comparison within a group was done using ANOVA test for both the groups.

## Results

In our study, Gingival Index, Oral Hygiene Index and Plaque Index scores were assessed at 3 intervals –Baseline, 1 month and 3 months. The bacterial colony forming unit was also assessed at these intervals.

Mean value of, GI score at baseline was 2.29 for Chlorhexidine and 2.21 for Probiotics as mentioned in table 1 which decreased to 0.62 for Chlorhexidine and 0.76 for Probiotics which were not statistically significant (P=0.17). Mean value

# Fig. 4 Sample collected after 3 months from probiotic group

at baseline OHI score 4.16 of was for Chlorhexidine and for Probiotic it was 4.84 as mentioned in table 2. After 3 months there was no statistically significant difference observed (P= 0.25). Mean value of PI score at baseline was 2.19 and Probiotic 2.41 as mentioned in table 3. which decreased at the interval of 1 and 3 months but the difference was not statistically significant (P=0.51) Mean value of microbial count at baseline for Chlorhexdine was 1.71 and Probiotics it was 1.69 as mentioned in table 4 which decreased to 0.162 and 0.136 respectively which were not statistically significant (P=0.96) This inter-group comparison shows that though there was decrease in the GI. PI. OHI scores and the Microbial count also decreased after 3 months, no statistically significant difference was observed in the Chlorhexidine and Probiotic groups.

# Discussion

Since many years vigorous search has been made for chemical agents that could supplement patientdependent mechanical plaque control and thus reduce or prevent periodontal disease.

Tooth-brushing, when accomplished properly, results in effective plaque control. However, mechanical plaque control methods have certain inherent limitations <sup>9</sup>

Therefore, adjunctive chemical plaque control methods such as use of mouthwash have been suggested as additional therapeutic strategy to augment but definitely not to replace mechanical plaque control.<sup>10</sup> Mouthwash supplements routine mechanical oral hygiene procedures in controlling supragingival plaque formation.

Due to availability of a variety of mouthwashes with different active ingredients, there is always a patients and dilemma among practitioners regarding its choice. CHX, till date, is considered to be the most effective anti-plaque agent. But it certain side effects such has as brown discoloration of teeth, tongue, oral mucosal erosion, taste perturbation.

These side effects have led to the search for a better antiplaque agent without major side effects. Antibacterial mouthrinses act by nonspecifically reducing the levels of both friendly and harmful oral bacteria.

In contrast to this, Probiotics have been developed utilizing natural beneficial bacteria to promote a healthy balance of microorganisms in the oral cavity. These beneficial bacteria provide a natural defense mechanism against the harmful bacteria present in the mouth.

**Probiotics** mostly belong to the genera Bifidobacterium . Lactobacillus and For а microorganism to exert probiotics properties it has to resist oral environmental conditions and defense mechanism so that it is able to grow and colonize in the mouth and inhibit oral pathogens. The probiotics species should also be safe for the  $host^8$ .

A Probiotic mouthrinse contains *Nisin* which are bacteriocins produced by lactic acid bacteria cultured in a fermentor. These peptides are separated and purified from all other components including the lactic acid bacterial cells and then incorporated into the mouthrinse. Bacteriocins are synthesized ribosomes proteins by with a bacteriocidal mode of action. They usually act closely related species. against Thev are colorless, odorless and non - toxic, so they fit into the requirements of food preservatives<sup>1</sup>.

Bacteriocins differ from antibiotics: They have a relatively narrow killing spectrum and are only toxic to bacteria closely related to the producing strain. These toxins have been found in all major lineages of bacteria and more recently, have been described as universally produced by some members of the Archaea<sup>11</sup>.

Different mechanisms have been proposed for These include prevention their actions. of adhesion of pathogens to host tissues, stimulation and modulation of the mucosal immune system. reducing production This is done by of pro-inflammatory cytokines through actions on pathways, increasing production NFkB of anti-inflammatory cytokines such as interleukin-10 (IL-10) and host defense peptides such as beta-defensin 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 pathogenic bacteria has also been reported<sup>8</sup>.

Some experimental studies have explored the use of probiotics in periodontal diseases. In 2002 Grudianov et al. compared 3 groups consisting of gingivitis, periodontitis and control groups. It was found that probiotics were responsible for maintenance of normal microbiota in gingivitis and periodontitis patients as compared to control group<sup>12</sup>.

In 2006, Krasse et al. found reduced gingivitis on use of probiotics *Lactobacillus reuteri*. In Japan, two Randomized Controlled Trials (RCTs) were conducted in 2003 and 2006 which reported reduction of *P.gingivalis* in administration of *L.salivarius* as compared to placebo group.

### 

### Conclusion

Probiotic mouthrinse is found to be effective in reducing accumulation of plaque and gingival inflammation. Though Chlorhexidine is considered to be the gold standard for plaque control, Probiotic mouthrinse is also found to be an effective alternative. Further long term studies are required to determine their efficacy.

### Acknowledgements:

We are very grateful to Mrs. Seema Deshpande and Mrs. Bapat of for helping us in preparing bacterial culture and assessing the bacterial count required in our study.

## Reference

- 1. K. Noordin, S. Kamin. The effect of probiotic mouthrinse Original Article on plaque and gingival inflammation. *Annal Dent Univ Malaya* 2007; 14: 19–25.
- Axelsson P. Mechanical plaque control. In: Lang, N.P. & Karring, T., eds. Proceeding of the 1st European Workshop on Periodontology, London: Quintessence Publishing 1994; pp. 219-43.
- 3. Lavstedt S, Modeer T & Welander F . Plaque and gingivitis in a group of Swedish school children with particular reference to tooth brushing habits. *Acta Odontologica Scandinavia*1982; 40, 307-11.
- 4. Addy M. Chlorhexidine compared with locally delivered antimicrobials. A short review. *J Clin Periodontol* 1986; 13: 957-60.
- 5. Ciantar, M. (1995). Chemical agents in Periodontal Therapy: Use or Misuse? *Dental Update*; July / August; 32: 238-41.
- 13.

# Figure legends –

Figure 1: 0.2 % of Chlorhexidine antimicrobial mouthwash (HEXIDINE<sup>®</sup> [ICPA]) Mouthwash and probiotic sachets with distilled water

- Figure 2: Sample collected at baseline
- Figure 3: Sample collected after 3 months from chlorhexidine group
- Figure 4: Sample collected after 3 months in probiotic group

- Kornman KS. Anti-microbial agents. In: Löe, H.& Kleimann, D.V., eds. Dental plaque Control measures and Oral Hygiene Practices. *Oxford:IRL Press* 1986; pp. 121-42.
- 7. Stamatova I, Meurman JH. Probiotics: Health benefits in the mouth. *Am J Dent*. 2009;22(6):329-38.
- 8. Nadkerny PV, Ravishankar PL, Pramod V, Agarwal LA, Bhandari S. A comparative evaluation of the efficacy of probiotic and chlorhexidine mouthrinses on clinical inflammatory parameters of gingivitis: A randomized controlled clinical study. *J Indian Soc Periodontol* 2015;19:633-9.
- 9. Harris NO, Garcia-Godoy F, Nathe CN. Primary Preventive Dentistry. 8th ed. USA: *Pearson*; 2013.
- Aneja KR, Joshi R, Sharma C. The antimicrobial potential of ten often used mouthwashes against four dental caries pathogens. Jundishapur J Microbiol. 2010;3(1):15-27.
- 11. Torreblanca M, Meseguer I, Ventosa. Production of halocin is a practically universal feature of archael halophilic rods. *Lett Appl Microbiol*1994; 19: 201-5.
- 12. Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y,Yamaki K, *et al.* Improvement of periodontal condition by probiotics with *Lactobacillus salivarius* WB21: A randomized,double-blind, placebo-controlled study. *J Clin Periodonto* 2008;35:897-905.

#### .....

### Table Legends -

Table 1: Difference in Gingival Index scores between Chlorhexidine and Probiotics at different time intervals using Independent T-test.

Table 2: Difference in Oral Hygiene Index scores between Chlorhexidine and Probiotics at different time intervals using Independent T-test.

Table 3: Difference in Plaque index scores between Chlorhexidine and Probiotics at different time intervals using Independent T-test.

Table 4: Difference in microbial count between Chlorhexidine and Probiotics at different time intervals using Independent T-test.

Time interval	Groups	Mean	Standard Deviation	Mean Difference	95% Confidence Interval for difference		Т	р
					Lower	Upper		
Baseline	CHX	2.2960	.47124	.08400	15972	.32772	.693	.492
	Probio	2.2120	.38114					
1 month	CHX	1.6240	.45395	00800	26259	.24659	063	.950
	Probio	1.6320	.44132					
3 months	CHX	.6240	.38760	14000	34411	.06411	-1.379	.174
	Probio	.7640	.32772					

 Table No 1: Difference in Gingival Index scores between Chlorhexidine and Probiotics at different time intervals using Independent T-test

Time	Groups	Mean	Standard	Mean	95% Confidence		Т	Р
interval			Deviation	Difference	Interval for difference			
					Lower	Upper		
Baseline	CHX	4.6160	.97197	23200	69718	.23318	-1.003	.321
	Probio	4.8480	.62724					
1 month	CHX	2.1480	.49676	20400	46337	.05537	-1.581	.120
	Probio	2.3520	.41142					
3 months	CHX	.6160	.28384	08800	24031	.06431	-1.162	.251
	Probio	.7040	.25080					

<sub>se</sub>155

 Table No 2: Difference in Oral Hygiene Index scores between Chlorhexidine and Probiotics at different time intervals using Independent T-test

. . . . . . . . . .

Time interval	Groups	Mean	Standard Deviation	Mean Difference	95% Confidence Interval for difference		Т	Р
					Lower	Upper		
Baseline	СНХ	2.1960	.42375	22000	44091	.00091	-2.002	.051
	Probio	2.4160	.34962					
1 month	CHX	1.5120	.30183	06800	22517	.08917	870	.389
	Probio	1.5800	.24833					
3 months	CHX	.4880	.23331	04400	17840	.09040	658	.514
	Probio	.5320	.23930					

 Table No 3: Difference in Plaque index scores between Chlorhexidine and Probiotics at different time intervals using Independent T-test

Time	Groups	Mean	Standard	Mean	95% Confidence		Т	Р
interval			Deviation	Difference	Interval for difference			
					Lower	Upper		
Baseline	CHX	1.7120	.19218	.01600	09762	.12962	.283	.778
	Probio	1.6960	.20712					
1 month	CHX	1.3560	.19382	.06400	02887	.15687	1.386	.172
	Probio	1.2920	.12557					
3 months	CHX	.3840	.16248	.07200	01330	.15730	1.697	.096
	Probio	.3120	.13638					

 Table No 4: Difference in microbial count between Chlorhexidine and Probiotics at different time intervals using Independent T-test

 $\bar{P}_{age}156$ 



Graph 1: Difference in Gingival Index scores between Chlorhexidine and Probiotics at different time intervals



Graph 2: Difference in Oral Hygiene Index scores between Chlorhexidine and Probiotics at different time intervals



Graph 3: Difference in Plaque index scores between Chlorhexidine and Probiotics at different time intervals

 $\dot{P}_{age}15'$ 



Graph 4: Difference in microbial count between Chlorhexidine and Probiotics at different time intervals