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Original Article

Clinical Efficacy between a Probiotic and 0.2% Chlorhexidine Mouth Rinse on Oral Health: A Randomized and Controlled Trial

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Abstract

Introduction: Chlorhexidine digluconate, a broad-spectrum antibacterial agent, is considered the gold standard in the dental field but its side effects limit its long-term use and acceptance as a mouthwash. Probiotics have an amazing potential for not only preventing the attack of oral pathogens and the ability to treat various oral diseases. The advantages of using probiotic strains are that the bacterial strains present in them are not harmful to the oral cavity and there is no occurrence of antibiotic resistance and no proven toxicities.

Aims and Objectives: The aim of the study was to clinically compare the efficacy of a Probiotic mouth rinse to chlorhexidine mouth rinse on the level of plaque accumulation and gingival inflammation.

Materials and Methods: The study was conducted among patients and their attendants visiting the Department of Public Health Dentistry at the Institute of Dental Sciences, Bareilly. Informed consent from each study participant was obtained. Ninety participants were randomly allocated into two groups – Group 1 – Chlorhexidine (0.2%) and Group 2 – Probiotic mouth rinse. Both the groups were instructed to rinse their mouth with 10 ml of their respective mouthrinse, for 1 min twice daily for 14 days. Clinical parameters such as plaque index, gingival index, and oral hygiene index (OHI)-simplified for each group were assessed at the baseline, 7th day, 14th day and 28th day, respectively. Intragroup and then intergroup comparison was done between both the groups.

Results: There was a decrease in mean scores of plaque index, gingival Index, and OHI. There was a significant improvement in all the scores from baseline to the 28th day within the groups but when intergroup comparisons were made there was no statistically significant difference.

Conclusion: In our study, there was an improvement in the oral hygiene status of both groups but the mean difference in the CHX group was more as compared to the probiotic over 28 days.

Key words: Chlorhexidine, Probiotic mouthwash, Plaque, Gingivitis, Oral hygiene

INTRODUCTION

Biofilms are increasingly being referred to as the cause of human infections and diseases. Plaque, respiratory infections, gastric ulcers, atherosclerosis, kidney stones, ear infections, prostatitis, and many other microbial-induced diseases are associated with biofilms.[1]

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These biofilms can be removed through proper oral hygiene maintenance and this includes measures to control plaque deposits. Plaque control can be achieved through chemical agents also, of which chlorhexidine digluconate is the most widely and effectively used anti-plaque and anti-gingivitis agent.[2]

Chlorhexidine gluconate is a cationic bisbiguanide with low toxicity and broad antimicrobial activity. When used as a mouthwash, it causes flushing, bacterial membrane destruction, concentration-dependent growth inhibition, and cell death and secondary interactions occur by inhibiting photolytic and glycoside enzymes. Chlorhexidine has increased substantivity in the oral cavity. However, some people experience bitter taste, light brown spots

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on the teeth, and loss of taste. These side effects limit its long-term use as a mouthwash.[2] Therefore, search for new rinse continues and the scientific focus has shifted toward biogenic agents.[3]

In addition to increasing bacterial resistance to antibiotics, reducing oral pathogens at this age requires new approaches such as complete bacterial replacement therapy.[4]

Probiotic therapy is a natural and alternative method that has been used to combat infectious disease by displacing pathogenic microorganisms with non-pathogenic endogenous or commensal bacteria.[5] According to the World Health Organization, probiotics are live microorganisms that when administered in sufficient amount to produce a health benefit for the host with minimal risk of side effects.[6] Consumption of foods that contain a live bacterial component such as milk, yogurt, and cheese has been advocated for centuries for their benefit on gastrointestinal health.[5]

Several studies have been conducted in the past one decade regarding the usefulness of various mouth rinses such as chlorhexidine, listerine, and fluoride, but only a few studies have been done on probiotic mouth rinse. There has been considerable clinical interest in the use of probiotic on day to day life because of developing antimicrobial drug resistance. Due to ill effects of staining teeth by chlorhexidine, additives of alcohol in listerine and fluoride toxicity and ingestion by children have led probiotic mouth rinse a clinical interest in dentistry. They recommended that probiotic mouthwash should be considered one of the effective oral hygiene regimens.[7] Thus, probiotic can also be used as a mouth rinse, due to its beneficial effect.

Very few studies have investigated the use of probiotic mouthwashes in India to reduce the clinical parameters of plaque, gingivitis, and oral hygiene so this study was undertaken to clinically compare the efficacy of a probiotic mouth rinse to a chlorhexidine mouth rinse (0.2%) on the level of plaque accumulation, gingival inflammation, and oral hygiene status.

MATERIALS AND METHODS

The present study was randomized and controlled trial with two parallel groups. The study was conducted in the Department of Public Health Dentistry, Institute of Dental Sciences Bareilly, Uttar Pradesh, India.

Ethical clearance was obtained from the Institutional Review Board (IRB) of the Institute of Dental Sciences, Bareilly, India. Informed consent, both written and verbal, was obtained from each study participant after explaining the nature of the study.

Sample Size

It was determined that 90 participants would be necessary to provide 80% power with an $\alpha$of 0.05 using G Power Software.

Inclusion Criteria

The following criteria were included in the study:

1. Subjects in the age range of 20–30 years
2. Subjects having a dentition with ≥20 evaluable teeth (minimum of five teeth per quadrant)
3. Patients willing to give informed voluntary written consent.

Exclusion Criteria

The following criteria were excluded from the study:

1. Subjects with any systemic diseases
2. Subjects on any other oral hygiene regimen other than routine tooth brushing
3. Subjects undergoing any specialized dental treatment, for example, orthodontic and prosthodontic treatment
4. History of oral prophylaxis within 6 months previous to the study
5. Tobacco consumers and smokers.
6. Subjects undergoing antibiotic therapy.

The kappa coefficient value for intraexaminer reliability for recording the plaque index, gingival index, and oral hygiene index (OHI) was found to be 0.84, 0.86, and 0.89, respectively. These values reflected high degree of conformity in observation.

Group Allocation

A complete clinical oral assessment of all participants was carried out based on inclusion and exclusion criteria from the outpatient Section Department of Public Health Dentistry, Institute of Dental Sciences, Bareilly, India.

Ninety study participants of age group between 20 and 30 years were selected based on inclusion and exclusion criteria.

In this randomized, controlled, and clinical trial, participants were enrolled and assigned to a computer generated table to assign the respective mouth rinse.

Study participants were randomly allocated into two groups with 45 participants in each group:

- Group 1 – Chlorhexidine mouth wash. (0.2% Chlorhexidine gluconate, Nitofresh, INTRA LIFE, Bangalore, India)
- Group 2 – Probiotic mouth rinse [Probilife-P (INTRA LIFE, Bangalore, India) + distilled water]

The participants in Group 2 were given Probilife-P Sachets (containing 1.25 billion freeze-dried bacterial cells of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Saccharomyces boulardii*) and 10 ml ampules of distilled water commercially available at the chemist.

The patients were demonstrated the preparation of the probiotic mouthwash by mixing the sachet contents with 10 mL of distilled water. Particular attention was paid to explain to the patient that the solution must be thoroughly mixed until all contents are completely dissolved in the water. The prepared mouth rinse cannot be stored, so it must be prepared and rinsed immediately.

Both the groups were instructed to rinse their mouth with 10 ml of their respective mouthrinse, undiluted for 1 min twice daily, 30 min after brushing for 14 days.

Clinical Examination

The clinical parameters were recorded in a case history proforma.

The following indices were recorded in the study participants:

1. Turesky-Gilmore-Glickman Modification of the Quigley-Hein Plaque Index (1970)
2. Loe H. and Silness J. Gingival Index (1963)
3. Greene J.C. and Vermillion J.R. OHI-Simplified (OHI-S) (1964).

The data obtained from each group were assessed individually at baseline, 7th day, 14th day, and 28th day, respectively, intragroup and then intergroup comparison was done between both the groups.

All of the examinations were conducted by one trained and calibrated examiner. The evaluation was carried out at baseline, 7th day, 14th day, and 28th day in the department.

Statistical Analysis

The statistical analysis was done using (Statistical Package for the Social Sciences) Version 22.0 statistical analysis software. Results were expressed as the mean and standard deviation. Repeated measures ANOVA was employed within the two groups and ANOVA was employed between the two groups.

RESULTS

The present study was conducted among 90 study participants with 45 participants in each group.

Table 1 shows the gender-wise distribution of the study participants in two groups. Out of 90 study participants, 41 were males and 49 were females. Twenty-two males and 23 females participated in the chlorhexidine group, 19 males and 26 females participated in the probiotic group.

The age group for the study was 20–30 years; however, the mean age was 25 years.

Tables 2 and 3 show a comparison of mean values of variables at baseline, 7th day, 14th day, and 28th day for chlorhexidine and probiotic mouth rinses.

OHI-S

For chlorhexidine group, the mean OHI-S score was 1.33 ± 0.21 at the baseline, which reduced to 0.83 ± 0.27 at 7th day, 0.33 ± 0.21 at 14th day, and 0.19 ± 0.09 at the 28th day.

Similarly, for the probiotic group, the mean OHI-S score at the baseline was 1.21 ± 0.21 which reduced to 0.80 ± 0.16 at 7th day, 0.30 ± 0.13 at 14th day, and 0.20 ± 0.12 at 28th day.

The difference in the mean values of OHI-S between baseline, 7th day, 14th, and 28th day in both groups was found to be statistically significant ($P < 0.001$).

Mean Plaque Score

For chlorhexidine group, the mean plaque score at the baseline was 0.30 ± 0.07 which reduced to 0.11 ± 0.05

Table 1: Gender wise distribution of study participants

Groups	Male	Female	Total
CHX	22	23	45
Probiotic	19	26	45

Table 2: Intragroup comparison of mean values of variables at baseline, day 7, day 14, and day 28 for Chlorhexidine

Variable		Mean value			p value
CHX	Day 0	Day 7	Day 14	Day 28	
OHI-S	1.33±0.21	0.83±0.27	0.33±0.21	0.19±0.09	0.001*
PI	0.30±0.07	0.11±0.05	0.06±0.03	0.04±0.02	0.001*
GI	0.78±0.08	0.19±0.06	0.06±0.03	0.04±0.02	0.001*

Repeated measure ANOVA; * Significance at p<0.05

Table 3: Intragroup comparison of mean values of variables at baseline, day 7, day 14, and day 28 for Probiotic

Variable		Mean value			p value
Probiotic	Day 0	Day 7	Day 14	Day 28	
OHI-S	1.21±0.21	0.80±0.16	0.30±0.13	0.20±0.12	0.001*
PI	0.30±0.06	0.12±0.05	0.07±0.02	0.04±0.02	0.001*
GI	0.77±0.12	0.18±0.11	0.07±0.02	0.04±0.01	0.001*

Repeated measure ANOVA; * Significance at p<0.05

Table 4: Intergroup comparison of mean values of variables between two groups.

Variable	Interval	Mean score		p value
		CHX	Probiotic	
OHI-S	Baseline	1.33±0.21	1.21±0.21	0.896 (NS)
	7th day	0.83±0.27	0.80±0.16	0.795 (NS)
	14th day	0.33±0.21	0.30±0.13	0.694 (NS)
	28th day	0.19±0.09	0.20±0.12	0.863 (NS)
PI	Baseline	0.30±0.07	0.30±0.06	1.286 (NS)
	7th day	0.11±0.05	0.12±0.05	1.277 (NS)
	14th day	0.06±0.03	0.07±0.02	0.177 (NS)
	28th day	0.04±0.02	0.04±0.02	0.156 (NS)
GI	Baseline	0.78±0.08	0.77±0.12	0.404 (NS)
	7th day	0.19±0.06	0.18±0.11	0.270 (NS)
	14th day	0.06±0.03	0.07±0.02	0.092 (NS)
	28th day	0.04±0.02	0.04±0.01	0.081 (NS)

ANOVA test; NS – Not significant; * significance at *P*<0.05

at day 7, 0.06 ± 0.03 at day 14, and 0.04 ± 0.02 at day 28.

Similarly, for the probiotic group, the mean plaque score at the baseline was 0.30 ± 0.06 which reduced to 0.12 ± 0.05 at 7th day, 0.07 ± 0.02 at 14th day, and 0.04 ± 0.02 at 28th day.

The difference in the mean values of Plaque Index between day 0, day 7, day 14, and day 28 in both groups was found to be statistically significant ($P < 0.001$).

Mean Gingival Score

For chlorhexidine group, the mean gingival score at the baseline was 0.78 ± 0.08 which reduced to 0.19 ± 0.06 at day 7, 0.06 ± 0.03 at day 14, and 0.04 ± 0.02 at day 28.

Similarly, for the probiotic group, the mean gingival score at baseline was 0.77 ± 0.12 which reduced to 0.18 ± 0.11 at 7th day, 0.07 ± 0.02 at 14th day, and 0.04 ± 0.01 at 28th day.

The difference in the mean values of the Gingival Index between day 0, day 7, day 14, and day 28 in both groups was found to be statistically significant ($P < 0.001$). On the 28th day, plaque scores, gingival scores, and OHI-S scores were significantly lower for both the chlorhexidine and probiotic groups; however, the reduction in all the mean scores was found to be greater for chlorhexidine group than the probiotic group.

Intergroup comparison in all time periods between the two groups was not found to be statistically significant [Table 4].

When an intergroup comparison was done between the probiotic and chlorhexidine mouth rinse with respect to the oral hygiene status, there was a reduction in mean score; however, it was not found to be statistically significant.

When probiotic and chlorhexidine mouth rinse were compared regarding the plaque index scores, the reduction was seen in both the groups but it was not found to be statistically significant and when the two mouth rinses were compared regarding the gingival index, reduction in the gingival scores was observed; however, it was also not found to be statistically significant.

DISCUSSION

Chlorhexidine has been regarded as the "gold" standard anti-plaque and anti-gingivitis agent in dentistry. Antibacterial mouth rinses work by reducing the levels of both healthy and harmful oral bacteria non-specifically.

Probiotic technology represents an innovative approach to maintain oral health by utilizing healthy oral microflora to provide a natural defense against harmful bacteria. *Lactobacillus and Bifidobacterium* genera constitute the most probiotic species.[8]

The first probiotic species included in the study are *L. acidophilus* and *Bifidobacterium bifidum*. In dentistry, the previous studies of *Lactobacillus* strains such as *L. rhamnosus*, *Lactobacillus casei*, *Lactobacillus reuteri*, or *Lactobacillus* mixtures have shown mixed results for oral microflora.[9]

Several experimental studies are investigating the use of probiotics in periodontal disease. Krasse *et al.* conducted a study in patients with moderate-to-severe gingivitis who received either $2 \times 10^{[8]}$ CFU/day or an appropriate placebo of either type of *L. reuteri* (LR-1 or LR-2). *L. reuteri* effectively reduced gingivitis and plaque formation in patients with moderate-to-severe gingivitis.[10]

Harini and Anegundi evaluated the clinical efficacy of a probiotic and chlorhexidine mouth rinses in children on plaque accumulation and gingival inflammation for 14 days and concluded that the probiotic mouthwash effectively reduced the plaque accumulation and gingival inflammation.[11]

The present study was designed to evaluate and compare the efficacy of probiotic and chlorhexidine mouthwashes on plaque accumulation and gingival inflammation.

The indices used were Plaque Index (Turesky-Gilmore-Glickman Modification of The Quigley-Hein Plaque Index), Gingival Index (Loe H and Silness J), and OHI-S at baseline, 7th day, 14th day, and 28th day. The age group selected to carry out this study was 20–30 years.

The results obtained showed that there was a reduction in the plaque accumulation, gingival bleeding, and improvement in oral hygiene status after 28 days in both groups. These results add to the body of data supporting

the efficacy of these two products for anti-plaque and anti-gingivitis.

The advantage of using a probiotic mouthwash is that it contains friendly commensals, there are no concerns about antibiotic resistance, and there are no known toxic effects associated with the use.[12]

In the present study, probiotics (Probilife-P) contained 1.25 billion freeze-dried bacterial cells from *L. acidophilus*, *L. rhamnosus*, *B. longum*, and *S. boulardii*.

Lactobacilli produces a low molecular weight bacteriocin that has an inhibitory effect against a wide range of bacterial species associated with oral diseases.[13]

L. rhamnosus shows high antimicrobial activity and high resistance to environmental stress.[14]

Bifidobacterium species metabolize lactose and generates lactic ions from lactic acid and also produces beneficial short-chain fatty acids with vitamin synthesis.[15] *S. boulardii* has antibacterial properties.[16] Since the synthesis of compounds such as bacteriocin or biosurfactant, inhibition of cell association, colonization, and invasion of pathogenic bacteria may explain the anti-plaque effect of probiotics. Hence, in the present study, probiotics improved gingival health due to the above-mentioned facts.[17]

The results of our study showed a significant reduction in plaque levels and gingival inflammation at the end of 28th day in chlorhexidine group which was in accordance with studies done by Singh *et al*., where chlorhexidine showed a statistically significant difference in the plaque levels when compared with HiOra Regular Mouthwash[17] and Biswas *et al*., who found that CHX was better in improving plaque and gingival index scores than the herbal mouth rinse.[18] and Mishra *et al*., where chlorhexidine showed maximum reduction in Plaque Index, followed by herbal and probiotic mouthwash at the end of 1 week.[19] This was in contrast with the study conducted by Purunaik *et al*., where probiotic mouth rinse was significantly more effective in the reduction of plaque level and gingivitis than chlorhexidine at 14th day,[20] and Parwani *et al*., where both chlorhexidine and herbal mouthwash showed no significant difference in the plaque scores.[21]

In our study, there was an improvement in the oral hygiene status of both groups but the mean difference in the CHX group was more as compared to the probiotic over 28 days, this was found to be in contrast with the study conducted by Nadkerny *et al*., who showed equal efficacy of probiotic and chlorhexidine mouthwashes in the reduction of OHI-S, PI, and GI at the end of 28th day[22] and Shetty *et al*., where there were no statistically significant differences between CHX and HiOra groups with regards to OHI, PI, and GI.[23]

However, we would like to point out that the most important limitation of probiotic preparations is that they must be used immediately after preparation and cannot be stored. Therefore, we recommend an appropriate probiotic dispensing agent to help improve patient compliance.[24]

No adverse effects on the oral mucosa such as ulcerations were noted with probiotic mouth rinse. Likewise, it would be interesting to evaluate the additional gastrointestinal effects of probiotics in studies instructing patients to swish and swallow probiotic mouthwash rather than expectorate.

CONCLUSION

Probiotics are a newer approach to the maintenance of human health and also implied on the maintenance of oral health. Probiotics used to treat oral diseases can reduce the cost of traditional treatment and prevention programs. The idea of replacing harmful microbes with innocent, inactive, or genetically modified bacteria is fascinating.

Most of the studies conducted with probiotic strains originally suggest for gut health. The interest in oral probiotics has been growing since the last few decades.

The literature shows that the improvement in oral health is mainly due to reduction in caries causative microorganisms. With an increasing global problem of antibiotic resistance and ill effects of certain mouthwash, probiotics contribute to the effective treatment of microbial diseases.

The possibilities of using probiotics with a focus on disease prevention and optimal health for people of all ages are enormous. Efforts should be made to raise awareness of general dentists about these aspects of oral healthcare.

The presence of probiotics in the local microbiota of the human oral cavity should be investigated, as these bacteria have the advantage of being ideally adaptable to the oral ecosystem and much more scientific research is needed to better understand these tiny creatures and expand their applications.

Hence, our study concludes that:

1. Probiotic mouth rinse was found to be a potent plaque inhibitor
2. Probiotic mouth rinses can be used as an adjunct in maintaining oral health and preventing healthy oral status as they are safe with no side effects having good anti-plaque and anti-gingivitis action.

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