A RANDOMISED CONTROLLED TRIAL OF CLINICAL EFFICACY BETWEEN A PROBIOTIC AND A CHLORHEXIDINE MOUTH RINSE.

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Background: With the increasing antibiotic resistance of bacteria, new methods such as whole bacteria replacement therapy for decreasing oral cavity pathogens are needed.

Aim: To clinically compare the efficacy of a Probiotic mouth rinse to a Chlorhexidine mouth rinse on the level of plaque accumulation and gingival inflammation.

Methods: Sixty children, between 6 and 8 years of age, with a similar baseline scoring of Plaque index and Sulcus bleeding index were given a thorough oral prophylaxis and oral hygiene instructions. They were then divided into two groups: Group 1: Probiotic mouth rinse group and Group 2: Chlorhexidine mouth rinse group. On the 14th day all the participants were recalled for re-examination of the level of plaque accumulation and gingival inflammation.

Results: Probiotic mouth rinse group had a statistically lower plaque and gingival bleeding index on the 14th day as compared to the baseline.

Conclusion: Probiotic mouth rinses can also be considered as one of the effective regimens in maintaining oral hygiene.
increasing antibiotic resistance of bacteria, new methods such as whole bacteria replacement therapy for decreasing oral cavity pathogens is the need of this era.  

The World Health Organization in 2001 defined probiotics as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host". Probiotics have proved to inhibit plaque formation by lowering the salivary pH and producing antioxidants which utilize the free electrons required for mineralization of plaque. Plaque associated bacteria is unable to form plaque in this new condition, therefore, indirectly helping to prevent periodontal diseases.

Probiotics have an amazing potential for not only preventing the attack of oral pathogens but also the ability to treat various oral diseases, thereby, assuring healthy living and increased longevity.

The aim of this study was to clinically compare the efficacy of a Probiotic mouth rinse to that of a Chlorhexidine mouth rinse on the level of plaque accumulation and gingival inflammation.

**Materials and Methods:-**

Ethical clearance was obtained from the Institutional Review Board of Meenakshi Ammal Dental College & Hospital, Maduravoyal, Chennai – 600095, India.

The pilot study consisted of 60 children who visited the Department of Pediatric and Preventive Dentistry at Meenakshi Ammal Dental College and Hospital, Chennai.

**Inclusion criteria:-**
1. Children between 6 and 8 years of age.
2. Healthy children without any known systemic illness.
3. No recent history of use of antimicrobial agents/ any other drugs.
4. Children and parents willing to comply with the oral hygiene instructions.
5. Children having a plaque index of 2/ above 2.

**Exclusion criteria:-**
1. Children who have unmanageable behaviour.
2. Children using any other oral hygiene aids other than routine tooth brushing.
3. Children and parents who cannot comply with the oral hygiene instructions.
4. Children who cannot come for the recall visit.

A written informed consent was obtained from the parents/ guardians of children who participated in the study.

Oral examination was performed to assess the plaque (Silness & Loe) and the gingival bleeding index (Muhlemann & Son) on day one. Plaque index was recorded by visual examination of the designated teeth under room lighting. Sulcus bleeding index was measured by running a straight probe along the free gingival margin and waiting for about 30 seconds to record bleeding. The initial assessment was followed by a thorough oral prophylaxis and oral hygiene instructions. The children were then randomized by simple randomization into two groups:

- **Group 1:** Probiotic mouth rinse (Darolac, Aristo pharmaceuticals)
- **Group 2:** Chlorhexidine mouth rinse 0.02% (Hexidine® [ICPA])

The designated mouth rinses were dispensed to the respective groups through other staff of the department due to the double-blind design of the investigation.

The patients in Group 1 were given Darolac Sachets (a probiotic formulation containing Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus sporogenes, Bifidobacterium longum, and Saccharomyces boulardii) and 10 ml ampules of distilled water. The parents/ Guardians were demonstrated and instructed to prepare the probiotic mouth rinse by mixing together the contents of the sachet and 10 ml of distilled water. Emphasis was made to clearly explain that the solution had to be stirred thoroughly until all the contents were completely dissolved in the distilled water. The preparation had to be made freshly each time and rinsed immediately once prepared and could not be stored.
The children were instructed to rinse their mouth with 10ml of their respective mouth rinses (1:1 dilution for chlorhexidine) after tooth brushing at night by swishing it for 60 seconds and then expectorating it. They were advised not to eat or drink after using their mouth rinse. The parents/Guardians were asked to supervise the children during the use of mouth rinse.

All the participants were recalled on the 14th day for re-examination of the level of plaque accumulation and gingival inflammation.

Statistical Analysis:-
An Independent t test and Mann-Whitney test were performed in order to study the intra and inter group variability respectively. The confidence interval was kept at 95%.

Results:-
The plaque index had considerably reduced from 2.25 to 0.35 and 2.45 to 0.50 in the chlorhexidine and probiotic groups respectively. In terms of bleeding index the 14th day value was 0.00 for both the chlorhexidine and the probiotic groups as compared to the baseline which was 0.70 for chlorhexidine group and 0.85 for the probiotic group. A p value of 0.000 indicates a high statistical significance.

A Wilcoxon signed rank test also showed high significance for both the chlorhexidine and the probiotic groups in terms of both plaque index and gingival bleeding index.

Table 1:- Baseline characteristics

|                      | Chlorhexidine group | Probiotic group |
|----------------------|---------------------|-----------------|
| Males                | 55%                 | 50%             |
| Females              | 45%                 | 50%             |
| Average age          | 7.3 years           | 6.8 years       |
| Plaque Index         | 2.5                 | 2.3             |
| Bleeding Index       | 0.6                 | 0.7             |

Table 2:- Group statistics of the plaque index and gingival bleeding index on baseline and 14th day for both the chlorhexidine and the probiotic groups.

|                     | PLAQUE INDEX | BLEEDING INDEX |
|---------------------|--------------|----------------|
|                     | MEAN         | STD. DEV. | P VALUE | MEAN | STD. DEV. | P VALUE |
| CHLORHEXIDINE GROUP |              |           |         |      |           |         |
| BASELINE            | 2.25         | 0.444    | 0.000   | 0.70 | 0.571     | 0.000   |
| 14TH DAY            | 0.35         | 0.489    |         | 0.000|           |         |
| PROBIOTIC GROUP     |              |           |         |      |           |         |
| BASELINE            | 2.45         | 0.686    | 0.000   | 0.85 | 0.587     | 0.000   |
| 14TH DAY            | 0.50         | 0.513    |         | 0.000|           |         |

Table 3:- Wilcoxon signed rank test for the chx group.

|                     | PLAQUE INDEX - DAY 14 - PLAQUE INDEX - BASELINE | BLEEDING INDEX - DAY 14 - BLEEDING INDEX - BASELINE |
|---------------------|-----------------------------------------------|-----------------------------------------------|
| Z                   | -4.089a                                        | -3.500a                                       |
| Asymp. Sig. (2-tailed) | .000                                           | .000                                           |

a. Based on positive ranks.
b. Wilcoxon Signed Ranks Test
Table 4: Wilcoxon signed rank test for the probiotic group

|                      | PLAQUE INDEX - DAY 14 - PLAQUE INDEX - BASELINE | BLEEDING INDEX - DAY 14 - BLEEDING INDEX - BASELINE |
|----------------------|-----------------------------------------------|-----------------------------------------------------|
| Z                    | -4.056<sup>a</sup>                           | -3.690<sup>a</sup>                                  |
| Asy mp. Sig. (2-tailed) | .000                                          | .000                                               |

<sup>a</sup> Based on positive ranks.
<sup>b</sup> Wilcoxon Signed Ranks Test

**Discussion:**

Oral hygiene habits among majority of the population include regular tooth brushing, but, a positive synergistic effect is seen in the oral cavity, when mouth rinse is used as an adjunct.

Mouth rinses are intended to augment and not to replace mechanical plaque control. Chlorhexidine has been reported to have a number of local side effects. These side effects are brown discoloration of the teeth and tongue, taste perturbation and oral mucosal erosion. These side effects associated with its use have stimulated the search for an alternative antiplaque agent. Antibacterial mouth rinses act by nonspecifically reducing the levels of both friendly and harmful oral bacteria whereas, probiotics has been developed using natural beneficial bacteria to promote a healthy balance of microorganisms in the mouth.

Milward and Wilson (1989)<sup>5</sup> studied the effect of chlorhexidine on *Streptococcus sanguis* biofilm and found that 72-h biofilms were more resistant to chlorhexidine than 24-h plaque biofilms. But till date, *in vivo* resistance of *S. mutans* to chlorhexidine has not been documented in the literature.

Probiotic bacteria guard the oral health by competing with the oral pathogens for nutrients, site of adhesion and growth factors. Once adhered to the oral cavity, probiotic bacteria aggregate and inhibit the adhesion of the harmful microorganisms by producing bacteriocins or other antimicrobial compounds such as acids or peroxides. Thus, Probiotics help to prevent the inflammation of oral cavity and the oral tissue destruction by oral pathogens.<sup>6, 7</sup>

Probiotic first needs to adhere successfully to the surfaces of oral cavity in order to avoid or reduce its rapid exclusion from the oral cavity. Studies have shown that the pretreatment with lysozymes can increase adhesion properties of the lactobacilli without affecting its viability.<sup>8</sup> The lactobacilli adhesion mechanism involves hydrophobicity and surface charge in addition to specific carbohydrate and/or proteinaceous components. Another factor that needs to be considered while evaluating establishment of probiotics in the oral cavity is saliva-mediated aggregation. Those microorganisms that have the ability to co-aggregate may have greater advantage over non co-aggregating organisms which are easily removed from the mouth.<sup>9-12</sup>

Unlike adults who have an already established oral micro flora, in children the microbial ecosystem is still in the process of being established. An early installation and colonization of probiotics in the oral environment would be the first step for an anticipated long-term effect, but there are limited data available to support this event. Therefore, it seems especially important to carry out studies on infants because it is very likely that the chance of a permanent colonization of probiotics increases with a regular exposure from early childhood.<sup>13</sup> Several studies suggest that consumption of products containing probiotic lactobacilli or bifidobacteria could reduce the number of mutans streptococci in saliva.

The advantages of using probiotic strains are, bacterial strains present in them are not harmful to the oral cavity, There is no antibiotic resistance occurring and there is no proven toxicities related to their use.

According to Hatakka *et al*<sup>14</sup> Milk containing probiotic bacterium LGG has beneficial effect on children’s dental health. Zahradnik *et al.*<sup>15</sup> said that probiotics were safe for daily use as an aid in maintaining dental and periodontal
health. Keller and Twetman found that despite their strong acidogenic abilities, there was no evidence of an increase in plaque acidity after exposure to probiotic bacteria.

Jothika et al. found a sustained decrease in the bacterial count of S. mutans in dental plaque after 30th day of usage of a probiotic mouth rinse. There was an increase in S. mutans count in dental plaque in a four week period after exposure to CHX, this increased plaque accumulation or S. mutans could have been due to development of drug resistance toward chlorhexidine.

The results of our study showed the anti-plaque and the anti-gingivitis effects of a probiotic mouth rinse to be similar to that of a chlorhexidine mouth rinse.

Conclusion:
Probiotic mouth rinses have a statistically similar and equivalent effect as compared to chlorhexidine mouth rinses. Probiotic mouth rinses can be considered as one of the effective regimens in maintaining oral hygiene.

Future research should be aimed at identifying the long-term effects of oral probiotics and to know whether the oral probiotics should come from the oral cavity or general lactobacilli products and whether it should be used as an adjunctive therapy or mono-therapy. This will aid in blossoming this mode of treatment.

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