To Evaluate the Effect of Probiotic Mouthrinse on Plaque and Gingivitis among 15-16 Year Old School Children of Mysore City, India-Randomized Controlled Trial

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Abstract

Introduction: Probiotic concept of using beneficial bacteria has recently gained popularity in medical research. New methods such as probiotics has given a new dimension for both general and oral health.Objectives: This study aimed to investigate the efficacy of a Probiotic mouthrinse in reducing plaque and gingivitis among schoolchildren aged 15-16 years.Methods: This was a randomized, controlled, double blind clinical trial. 90 subjects granting their parental informed consent and willing to participate completed the trial. The sample was randomized by computer generated table into Group A -0.2

Index terms—

1. Gingivitis has been largely distributed among children and adults. Hence, it becomes important to consider alternatives for better oral health care. 2. A new possibility; to control plaque and gingivitis levels by means of a natural product that seems to overcome adverse effects of chlorhexidine mouthrinse such as altered taste and tooth staining is provided. 3. The product investigated was proven to be efficient and safe in a 14-day treatment. Also, it was well accepted by study participants.

1 I. Introduction

ore than 1000 bacterial species have been identified from the human mouth. These microorganisms are easily grown and produce dental plaque in the mouth environment, due to the considered as a microbiota consisting on average of Author : e-mail: dentisttips@gmail.com more than 400 species in each gram of plaque removed from the teeth. These live together within a biofilm community through the exploitation of very specific ecological niches **??**. Dental disease such as dental caries and periodontal disease remains a "silent epidemic" in the world that threatens children and adults. The oral streptococci especially mutans streptococci are related with the development of caries. The adhesion of oral streptococci such as Streptococcus mutansto tooth surfaces has the major role in their pathogenicity. Going along with the increasing antibiotic resistance of bacteria, new methods such as whole bacteria replacement therapy for decreasing of oral cavity pathogens must be investigated. 2 The mere spell of the word microorganism often gives a threat of health hazard. But, friendly microorganisms called Probiotics have changed this concept and have given a new dimension for both general and oral health 3 The definition of "probiotics" has been adopted by the International Scientific Association and the World Health Organization: "Live microorganisms, if administered in adequate amounts, confer a health benefit on the host" 4 The basic rationale behind the tautology of probiotics was that the human body lives in a heavily contaminated environment associated with millions of bacteria and probiotics can be utilised by replacing pathogenic microorganisms with healthy ones. This concept of using beneficial bacteria has gained much popularity in the field of medical research in recent years where antibiotic resistance is an increasing global problem 5 The first species introduced into research were Lactobacillus acidophilus and Bifidobacteriumbifidum , and among a number of potential benefits that have been proposed are reduced susceptibility to infections

, reductions to allergies and lactose intolerance, as well as lowered blood pressure and serum cholesterol values.Within dentistry, previous studies with lactobacilli strains such as L.rhamnosus , L. acidophilus and L.

To our knowledge in India, none of these formulations are readily available for oral health, so there exists a need to explore easily available alternative approach to bacterial mediated oral disease such as gingivitis.

Hence this study was undertaken to test the hypothesis that Short term administration of probioticmouth rinse is effective in reducing plaque and gingivitis II.

2 Materials and Methods

3 a) Sample size calculation

This study is a double blind Randomized controlled trial and powered to evaluate the effect of probiotic mouth rinse on plaque and gingivitis.From a review of key papers the ideal sample size toassure adequate power for that Randomized Controlled Trial was calculated considering potential mean difference of 1.5 between control and test groups for the difference between subject values on Quigley Hein Plaque index. It was determined that 30 subjects pergroup would be necessary to provide80% power with an ? of 0.05. i.

4 Subject population, inclusion and exclusion criteria

Subjects were selected from the populationby simple random sampling. In brief, the 90 eligible subjects were thoroughlyinformed of the nature, potentialrisks and benefits of their participationin the study and signed a termof Informed Consent.

b) The inclusion criteria were as follows ii.

5 Experimental design, allocation concealment

The medical and dental records of all subjects were recorded by a questionnaire.

In this double blinded randomized placebo controlled clinical trial , subjects were enrolled and assigned to a computer generated table by the examiner who assigned the coded mouthrinses according to treatment groups after Baseline examination into :

Group A -0.2% of CHX mouthrinse Group B -Probiotic mouthrinse Group C -Placebo mouthrinse The subjects and the examiner were blinded regarding the product allocation.Knowledge of the randomization list obtained by computer generated table was limited to the study coordinator.

6 d) Preparation of Mouthrinses

JSS University pharmacy prepared the mouthrinses in undistinguishable packets and sent them to the study coordinator, who marked the code number of each subject on the packets, according to the therapy assigned and gave them to the examiner. The random allocation sequence was generated by the clinical investigator.To maintain full blinding of the results , the randomization table was held by the study coordinator remotely from all the assessment and was not broken until the data was collected and analysed. The randomization was concealed by using sequentially numbered ; identical appearing containers to subject assigned treatment. The mouthrinses were decoded after the data was analysed.

Probiotic product: probiotic mouthrinse was prepared by using commertially available probiotic product Darolac (Aristo pharmaceuticals, india) containing 1 gm powder of 1.25 billion freeze dried combination , it comprised of a mixture of , Lactobacillus acidophilus, lactobacillus rhamnosus, bifidobacteriumlongum, and Saccharomyces boulardii .Each sachet powder was dissolved in 20ml of water in a measuring cup and used as a mouth rinse 5 .The placebo mouthrinse was prepared using distilled water.

Chlorhexidinegluconate mouthwash (Proprietary name: Clohex, concentration 0.2%) was procured from the market and given to the pharmacy manufacturing center. It was then diluted and the final concentration of Chlorhexidinegluconate was 0.2% such that 20 ml was dispensed at one time. Both solutions were made of identical colors to eliminate bias Investigators calibration: The examiner participated in calibration exercise that was performed by taking measurement in duplicate at randomly chosen teeth in subjects who were not included in the study. Calibration was accepted when the results were identical on >85% of occasions Treatment protocol : when the subjects volunteered for the study and before they received a packet containing mouthrinses and instruction for use, baselineplaque index Tureskey modification of Quigley & Hein Plaque index(QHI) 7 and gingival index 8 (Loe H. and Silness P., Volume XIV Issue IV Version I Year ( ) J which thegingivae are scored on a four-pointscale from 0 (absence of inflammation) to 3 (severe-inflammation). Supragingival plaque was scored onthe buccal and lingual surfaces of allscorable teeth using the Tureskymodification of the Quigley-HeinPlaqueIndex (Turesky et al. 1970).Following disclosing with an erythrosine solution, plaque was scored on a six-point scale from 0 (no plaque) to 5 (plaque covers two thirds or more of the tooth surface).

Each subject was given one of the test products with a given code according to the assigned group. 20 ml of mouth rinse was dispensed for each individual using a measuring cup & subjects were instructed to swish the mouth rinse for 60 seconds & then expectorate. The procedure was performed once daily morning, after breakfast & was supervised by the examiner by visiting the school every day in the morning. The subjects were given with the assigned treatment group the mouthrinses in a packet and instructed to repeat the procedure

before retiring to bed at night for the next 14 days. During the intervention period,no influence on personal oral hygiene procedures was exerted the subjects were encouraged to maintain routine oral hygiene & also instructed to maintain strict compliance.

A day after the 14 days of intervention, gingival & plaque indices were recorded using same indices by same examiner .This study protocol was approved by the JSS University Research Ethics Committee.

Clinical monitoring: clinical monitoring was performed by single examiner at baseline and 14 day.

Monitoring of compliance and adverseevents: The monitoring of compliance was assessed by instructing the subjects to return the old packets containing mouthrinses and received a new packets of mouthrinses. The single examiner was responsible for conducting the enquiry on adverse events and also monitoring of compliance During the study period no dropouts and withdrawals were encountered.

7 Primary outcome variables: All clinical measurements

were obtained in all subjects at baseline and 14 day. It was defined that the primary outcome variable to determine the superiority of one treatment over the other would be differences between groups in the reduction of plaque and gingival index compared from baseline to follow up.

Statistical analysis: The significance of difference within each group (over the course of study) was sought using paired student t test . Data was analysed with statistical SPSS software package.The level of significance was set at 5%.

8 III.

Results 90 subjects were included in entire study with 30 subjects allocated in each group . On comparison of plaque scores from Baseline to 14 th day there was astatisticallysignificant reduction with mean differences of 1.05 and 0.87 for chlorhexidine and probiotic group($p<0.05$).The reduction in mean plaque score was found to be greater for chlorhexidine group than the probiotic group.But their was no statistically significant reduction in placebo group for plaque scores.

On comparison of gingival scores from baseline to 14 th day there was a statistically significant reduction with mean differences of 0.30and 0.31 for chlorhexidine and probiotic($p<0.05$). Although the probioticmouthrinse was significantly more effective than chlorhexidine at 14 day ($p<0.01$). But their was no statistically significant difference in placebo group. interdentalpapillae of all scorable teeth wasscored using the Loe-Silness Gingival Index(Loe&Silness 1963) in

9 Table-2 Comparision of plaque scores

10 IV. Discussion

This controlled comparative clinical trial demonstrated that the probioticmouthrinse and the chlorhexidinemouthrinse produced significant reductions in supragingival plaque and gingivitis when used as adjuncts tosubjects' usual mechanical oral hygiene procedures. These findings add to the body of data supporting the effectivenessof these two antiplaque/ antigingivitis products.The finding that the respective 14 day plaque and gingivitisreduction indicates that the two active mouthrinseshad omparable clinical effectiveness. The data in the study compares favourably with those from the study performed by Krasse et al 9 , A 14-day intake of L. reuteriled to the establishment of the strain in the oral cavity and significant reduction of gingivitis and plaque in patients with moderate to severe gingivitis.

A gingival infection is caused by a mix of Gram positive and Gram negative species and characterized by pronounced leucocyte infiltration and inflammatory exudation in the marginal area. The mechanism of probiotic action in the oral cavity is not fully understood, but is commonly explained by the combination of Local and systemic immunomodulation as well as non immunologic defense mechanisms. The study reported by SvanteTwetman et al 10 , that have examined Shortterm effect of chewing gums containing probiotic Lactobacillus reuteri on the levels of inflammatory mediators in gingival crevicularfluid.The authors reported significant reduction in Cytokines TNF-? and IL-1? , which are considered central mediators of proinflammatory cascade causing damage. This result, to some extent explained the mechanism of probiotic action in the oral cavity.

In the light of present findings, our study results also showed a significant reduction in gingival status on short -term administration of probiotic mouthrinse. The results are also in consistent with study doneby to Kanget al 11 , studies on three strains ofL. Reuteridemonstrated a centrifuged supernatant inhibitory effect on periodontopathic and cariogenic bacteria, all three inhibited the growth ofthe periodontopathic bacteria and S. mutansby more than 90%. This novel observation was also revealed in a study done by Margarita et al 12 , it was concluded that L. Reuteri containing probiotic tablets are able to colonize the saliva and the subgingival habitat of some gingivitis patients. The use of the probiotic was associated with a reduction of total bacterial counts in saliva and reductions in the numbers of selected periodontal pathogens.

It is probably the production ofsome compounds such as bacteriocin or biosurfactant, which is the most likely reason for the antimicrobial effect of the probiotic powder 13,14 . Another crucial realm of probiotic bacterial clinical impact is mechanism by which they act, thus improving the intestine and over all health .Several reports have documented the ability ofprobiotic bacteria to inhibit; cell association, colonization and invasion by

pathogenic bacteria. 13,15,16 In a study done by khanfari 17 , the research aimed to investigate the induction or reduction of S. mutansgrowth as it is a dominant bacterium producing dental plaque. In conclusion, the results showed that probiotic strains and probiotic chocolate can inhibit the growth of oral isolates of S. mutans, but their capacity differed significantly between the various strains.

In our study we used probioticmouthrinse combination of lactobacillus strains and strain of bifidobacterium and Sacchromyces that contains 1.25 billion freeze dried bacterial combination.It is possible, in the complex environment of the human mouth , that probiotic "cocktails" of multiple strains would be more effective than any single probiotic agent. This combination of probiotic strain was similar to those used by Haukoja et al 16 .The author reported the clinical treatment of periodontitis and gingivitis seems to be a potential target for probiotic lactic acid bacteria or bifidobacteria.A basic prerequisite to be an oral probiotics is the ability to bond and inhabitant over the oral mucosal surfaces. Action of the probiotic strains on the oral cavity is dubious as oral mucosa is not their

11 Gingival scores

12 Table-3 Comparision of gingival scores

13 Baseline on 14th day

Volume XIV Issue IV Version I Year ( ) J strains maintain oro microbiological balance. But the there is negligible proof that these lactobacilli strains are momentary or stable oral colonizers.

In the present study only the effect of short term administration of probiotics was assessed. As this also resulted in significant reduction of plaque and gingival status it seems plausible that prolonged administration of probiotic preparations may have a preventive role against development of plaque and gingivitis.

The subjects selected in this study were 15-16 years age group, which was important for the present study, for the assessment of periodontal disease indicators in adolescents 18 .This age group is considered to markperiodontal manifestations related to endogenous sex hormones 19 . Puberty marks initiation of changes from maturation into adulthood 20 . Several cross-sectional and longitudinal studies 21,22,23 have demonstrated an increase in gingival inflammation without accompanying an increase in plaque levels during puberty. Both estradiol and progesterone have been shown toselectively accumulate by P.intermedia as a substitute for vitamin K, and thus postulated to be acting as a growth factor for this microorganism. 24 Another reason for selecting this age group was the intellectual ability of the child. In accordance with Jean Piaget at the age of seven years a child largely corresponds to an increase in cognitive development where by the child develops a sense of semi-logical reasoning to infer physical cause-effect relationnships.Thus in this age group a positive compliance could be expected from a child. To our knowledge only one study reported use of oral probiotic in the age group between 7-14 years 5 .

14 V. Conclusion

Probiotic therapies, once discussed primarily in the context of "complementary" or "integrative" medicine, are entering the therapeutic mainstream in maintaining the oral health. This concept prompts a new Horizon on use of probiotic mouthrinse in reduction of plaque and gingivitis.

15 VI. Limitations

It is pertinent to highlight some limitations of this study in order to subsidize future clinical trials in this field as follows.

(i)Probiotic effects are strain-specific, thus each individual bacterial strain needs to be tested separately, and the effects described for one strain cannot be directly applied to others. Unfortunately, mislabelling of strains in probiotic products seems to be a common problem . On the other hand, multispecies or multistrain probiotic products can be even more effective than products with only one bacterial strain, making the scientific evaluation of the mechanisms of the probiotic activity even a more complicated task.

16 VII. Recommendations

Enlarging the duration of treatment may be an alternative to assess the effects of prolonged use on oral mucosa and teeth. In addition, since our findings have indicated a good safety pattern of the product in a 14-day regimen, long-term trials are now encouraged to check [1]

Figure 1:

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