FRUCTOOLIGOSACCHARIDE INTAKE AFFECTS THE COLONIZATION OF *S*. *Mitis* ESTABLISHMENT IN ORAL CAVITY ALONG WITH GUT BACTERIA IN YOUNG SCHOOL CHILDREN OF URBAN VADODARA

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ABSTRACT

**Background:** Streptococcus mitis are commensal bacteria that colonize hard surfaces in the oral cavity such as dental hard tissues as well as mucous membranes and are part of the oral flora. *S. mitis* is resistant to some antibiotics such as penicillin and cefotaxime and thus represent a potential clinical problem in the prevention of oral diseases. Due to the emergence of antibiotic resistance and frequent recolonization of treated sites with pathogenic bacteria, there was need for a new treatment paradigm to be introduced to periodontal disease.

**Objective:** To determine the impact of Fructooligosaccharide supplementation on the establishment of *S. mitis* in mouth and *Bifidobacteria, Lactobacilli* and *E.coli* in the gut of children having fair oral hygiene status of urban Vadodara.

**Methods:** 255 children (8-12 years) were enrolled in the study from the Primary school of urban Vadodara and were assessed for their oral hygiene status, using Oral hygiene index-simplified. Sixty children having fair oral hygiene were purposively selected and grouped as experimental and control group. They were supplemented with, Fructooligosaccharide (FOS) incorporated buttermilk and plain buttermilk, for a period of 4 weeks. Pre data was collected on their basic oral hygiene practices using a structured questionnaire. Their saliva samples were analysed for the...
presence of *S.mitis* and stool samples in subsamples (n=30) were analysed for *Bifidobacteria*, *Lactobacilli* and *E.coli*, both at the baseline and after the intervention period. **Results:** The baseline data showed a strong association between oral hygiene status of children with age and gender as well as with establishment of *S.mitis*. Female children had poorer oral hygiene as compared to their male counterparts. Intervention brought about improvement in oral hygiene status in both the groups with the greater reduction in *S.mitis* counts (16.03%) in subjects of experimental group. Gut *E.coli* also significantly reduced (17.92%) upon supplementation with buttermilk+FOS along with a significant rise in Gut *Bifidobacteria* (11.58%) and *Lactobacilli* (11.58%). The oral hygiene status of the subjects significantly improved from fair to good (70.24%). **Conclusion:** Daily intake of 7g FOS with buttermilk and plain buttermilk helped to improve the oral hygiene status along with the improved beneficial gut microflora in children initially having fair oral hygiene status. The establishment of *S.mitis* was significantly reduced in both experimental and control groups.

**KEYWORDS:** *S.mitis, Bifidobacteria, Lactobacilli* and *E.coli.*

**INTRODUCTION**

Oral diseases and dental caries are infectious diseases resulting from the interaction of oral bacteria residing dental plaque and the host. The indigenous bacteria residing dental plaque are thought to be a relatively stable community of high species diversity, which may vary from site to site throughout the mouth. *Streptococcus mitis* have been identified as the first and most dominant oral microbe to colonize the oral cavities of newborn infants and young children. With the eruption of primary teeth, the number and complexity of the microflora in the oral environment increase.[1,2,3] The strong resistance of *S.mitis* to antibiotics including penicillin, further represent a potential clinical problem in the prevention of oral diseases.

With the threat of widespread antibiotic resistance rendering many antibiotics useless against important diseases, there is an increased necessity not only to minimise antibiotic use and develop novel non-antibiotic-based treatments, but raise the profile of disease prevention. There is a public appetite for new therapies that are perceived to be natural, for example, manipulation of the resident microbiota by the ingestion of probiotic or prebiotics. These changing attitudes are also relevant to the prevention of dental diseases and there is an increased interest in the use of strategies that do not involve conventional antimicrobial agents for oral care.[4,5]
Treatment of periodontal disease in recent years has moved toward an antibiotic/antimicrobial model of disease management. With increase in the incidence of resistance to antibiotics, probiotics and prebiotics may be a promising area of research in periodontal therapy. Also, prebiotics have been proved to be an aid to complement probiotics in the treatment of oral diseases. There are limited food based strategies currently available to improve oral health. Besides, there are limited studies on the impact of FOS on the reduction of S. mitis colonisation in oral health among children. Food based approach can help in improving the oral health of school going children and reduce the prevalence of poor oral health.

Thus, keeping this in mind the present study was planned with the objective to study “the impact of FOS supplementation on oral health (S.mitis establishment) and on gut health of young school children of urban Vadodara”.

MATERIALS AND METHODS
Enrolment of subjects
Two hundred and fifty five (255) subjects were enrolled in the study in the age group 8-12 years from the Maharana Pratap School of urban Vadodara based on the permission given by Vadodara Municipal Seva Sadan (VMSS). Children between 8 to 12 years are most suitable for the study related to dental problems as below 8 years they do not have all the teeth protruded and do not have hormonal induced dental problems.

The study was approved by the departmental ethical committee with Clearance number: (IECHR/2013/9).

Study design
Pre post intervention trial was used as a study design. Clinical signs of oral hygiene were examined by certified dentist using OHI-S for oral hygiene status in children. With purposive selection method sixty subjects (n=60) with fair oral hygiene were divided into two equal groups as control group (n=30) and experimental group (n=30). Pre intervention data was collected for both groups, stool and saliva samples were collected for microbial analysis. For intervention, the experimental group was given FOS (7g) and buttermilk (150 ml) and the control group was intervened with only buttermilk (150 ml) for period of 1 month. Buttermilk (Goras) was procured daily from Baroda dairy, Vadodara, through a local vender and FOS was procured from S.A. Pharmachem Pvt. Ltd. All the parents were informed about the
intervention and a written consent was signed for the same. Also information sheet for the
details of Fructooligosaccharide was provided to the parents, principle as well as municipal
board in charge of the school.

Collection of basic information of the subjects
Pre intervention data was collected using survey method for the prevalence of oral hygiene,
nutritional status of children, and basic oral hygiene practices using structured questionnaire.
The questionnaires were made in user-friendly language (Gujarati) so that the information
could be easily filled. All the forms were pre tested before handing it over to the parents for
filling it at home.

Visible Dental check up
All the children in the studying in same school from 8-12 years (3rd – 6th standard) of both the
genders were given non-invasive dental check up by certified dentist. There were two
hundred and fifty five (n=255) children who were diagnosed on the basis of oral hygiene
index-simplified.\[6\]

The OHI-S has two components, the Debris Index and the Calculus Index. Each of these
indexes, in turn, is based on numerical determinations representing the amount of debris or
calculus found on the preselected tooth surfaces. The six surfaces examined for the OHI-S are
selected from four posterior and two anterior teeth.

\[
\text{Debris index} = \frac{(\text{The buccal score}) + (\text{The lingual score})}{(\text{Total number of examined buccal and lingual surfaces})}
\]

\[
\text{Calculus index} = \frac{(\text{The buccal score}) + (\text{The lingual score})}{(\text{Total number of examined buccal and lingual surfaces})}
\]

The average individual or group debris and calculus scores are combined to obtain simplified
Oral Hygiene Index, as follows.

**Oral Hygiene Index= Debris Index + Calculus Index**

Oral Hygiene Index-Simplified was recorded for each patient and the values were interpreted
as:

<table>
<thead>
<tr>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1.2</td>
<td>1.3 to 3</td>
<td>3.1 to 6</td>
</tr>
</tbody>
</table>
Determination of the gut microflora

Gut micro flora with respect to Bifidobacteria, Lactobacillus and E.coli. were analysed. Sterile airtight containers were given to subjects to bring stool sample for the microbial analysis. The containers were immediately transferred to -18°C within half an hour of the sample collection. For enumeration of all gut bacteria1 gram of fresh fecal sample was accurately weighed and added to 99ml of 0.1% peptone water for homogenization. This provided 1% (wt. /vol.) fecal slurry, from which 1ml of dilution was diluted serially in peptone water as required. As the serial dilutions were ready, 0.1ml of dilution was pipette from each dilution bottle to the petriplate and then the respective media were added. For uniform distribution of sample in the petriplate, proper clockwise and anticlockwise rotations were provided. The whole procedure was undertaken taken in laminar flow so that the chances of contamination were reduced and a sterile environment was maintained.Further the plates were placed in an incubator at 37°C for the period of 48 hours for Bifidobacteria and Lactobacilli and E.coli was incubated at 37°C for 24 hours. After the incubation, the colonies were counted in a colony counter and were recorded in a unit of log_{10} CFU/gm.

Determination of the oral microflora

Saliva sample (2ml) was collected from the subject with passive drool method in sterile air tight containers. The containers were immediately transferred to -18°C within half an hour of the sample collection. For enumeration oral microflora, 1ml of fresh saliva sample is added to 99ml of 0.1% peptone water and serial dilutions were prepared as required. From these dilutions 0.1ml were added in to the petriplates, and thanselective media namely Rogosa SL agar (Hi media) media was addedfor the growth of S. mitis. For uniform distribution of the sample, petriplates were rotated in clockwise and anticlockwise directions. This procedure was carried out inside laminar flow to maintain sterile environment and avoid contamination. The petriplates were sealed in a desiccator and kept in an incubator at 37°C for 96 hours.After the incubation period, the colonies were counted in a colony counter and were recorded.The microorganisms were counted using colony counter and the number of colonies were reported as log values of these colonies per gram of sample (log_{10} CFU/g).

Intervention with Fructooligosaccharide and buttermilk

Children in the control group were provided with plain buttermilk (150ml) and experimental group was provided with FOS (7g) added into the buttermilk (150ml) for 30 working days.
Plain buttermilk and FOS added buttermilk were provided daily to the subjects and recorded for its intake by the subjects on daily basis to monitor the compliance.

**Post-intervention data**

Dental check-up was done on the subjects post intervention by certified dentists. Stool sample and saliva sample were also collected immediately for microbial analysis.

**Statistical analysis**

The data was analysed using the Statistical Package for the Social Sciences (SPSS 16.0 version) and WHO Anthro Plus. Paired 't' test was used to assess the difference between the means of the same group before and after the study period. Chi-square was used to test the differences between the frequency distribution.

**RESULTS**

**Oral hygiene status**

Among the 255 children, 58% children were having good oral hygiene whereas 41.96% were having fair and poor oral hygiene. By using Oral Hygiene Index Simplified the children were categorised as good, fair and poor.

The oral hygiene status among the gender revealed that among females 26.27% had fair oral hygiene and among male children 15.68% had fair oral hygiene status. The data showed that oral hygiene status of males were much better than females.

Data also depicted a significant association between the age, brushing routine and nutritional status with the oral hygiene status of the subjects, where females have higher percentage of fair oral hygiene (26.27%). Younger children (8-10 years) had significantly poorer oral hygiene as compared to older children (11-12 years). Frequency of brushing showed no significant effect on oral hygiene status where, children brushing once a day had high rate of fair oral hygiene. There was a significant association between oral hygiene status and nutritional status.

**Effect of supplementation on oral hygiene index scores**

It was observed that there was a shift in the oral hygiene status of the subjects from fair to good by 70.24(↓) after supplementation (Table 1).
Table 1. Impact of supplementation on oral hygiene scores of the subjects.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental group (n=30)</th>
<th>Control group (n=30)</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHI-scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.05 ± 0.30</td>
<td>1.93 ± 0.24</td>
<td>0.87 NS</td>
</tr>
<tr>
<td>Post</td>
<td>0.61 ± 0.11</td>
<td>1.51 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Paired t</td>
<td>12.04**</td>
<td>3.23*</td>
<td></td>
</tr>
<tr>
<td>% difference</td>
<td>70.24</td>
<td>21.76</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p<0.05, **significant at p<0.01, ***significant at p<0.001.

NS=non significant (0-1.5=Good, 1.6-3= Fair, 3.1-6=Poor).

Gender difference in oral hygiene status of children after intervention

Table 2 reveals that both in males and females the improvement in the OHI scores were observed and this improvement was statistically significant (p<0.001).

Table 2. Improvement in scores of males and females after supplementation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Experimental</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>1.97±0.54</td>
<td>1.95±0.590</td>
<td>0.846 NS</td>
</tr>
<tr>
<td>Post</td>
<td>1.60±0.685</td>
<td>0.58±0.13</td>
<td>5.60136***</td>
</tr>
<tr>
<td>Paired t</td>
<td>1.95NS</td>
<td>8.65**</td>
<td></td>
</tr>
<tr>
<td>% Difference</td>
<td>18.78</td>
<td>70.25</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>1.84±0.34</td>
<td>2.14±0.59</td>
<td>0.670NS</td>
</tr>
<tr>
<td>Post</td>
<td>1.30±0.36</td>
<td>0.63±0.304</td>
<td>4.817*</td>
</tr>
<tr>
<td>Paired t</td>
<td>3.13*</td>
<td>8.66**</td>
<td></td>
</tr>
<tr>
<td>% Difference</td>
<td>29.34</td>
<td>70.56</td>
<td></td>
</tr>
</tbody>
</table>

NS=non significant, **significant at p<0.01, ***significant at p<0.001.

Impact of supplementation on gut and salivary microorganisms

Table 3 and 4 reveal that there was significant rise in mean values of *Bifidobacteria* and *Lactobacilli* among the children of the experimental group which was 8.72 CFU/gm to 9.73 CFU/gm and 6.61 CFU/fm to 8.34 CFU/gm respectively. There was a significant reduction seen in the *E.coli* mean counts from 5.30 CFU/gm to 4.35 CFU/gm and in *S.mitis* mean count from 5.76 CFU/ml to 4.87 CFU/ml of the same group. Control group which was supplemented with plain buttermilk also showed significant results with regards to mean counts of microorganisms.

Table 3. Impact of supplementation on gut microorganisms.

<table>
<thead>
<tr>
<th>Parameters (Log_{10} CFU/gm)</th>
<th>Experimental N=15</th>
<th>Control N=15</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacteria Pre</td>
<td>8.72 ± 1.10</td>
<td>9.33 ± 0.83</td>
<td>1.48 NS</td>
</tr>
<tr>
<td>Post</td>
<td>9.73 ± 0.15</td>
<td>9.99 ± 0.59</td>
<td>1.72 NS</td>
</tr>
<tr>
<td>Paired t value</td>
<td>3.31**</td>
<td>2.52*</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Impact of supplementation on salivary microorganism - S.mitis.

<table>
<thead>
<tr>
<th>Parameters (Log_{10}CFU/gm)</th>
<th>Experimental N=30</th>
<th>Control N=30</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>5.76 ± 0.44</td>
<td>5.80 ± 0.47</td>
<td>0.27 NS</td>
</tr>
<tr>
<td>Post</td>
<td>4.87 ± 0.86</td>
<td>5.36 ± 0.75</td>
<td>2.15 *</td>
</tr>
<tr>
<td>Paired t value</td>
<td>5.30**</td>
<td>2.45**</td>
<td></td>
</tr>
<tr>
<td>% difference</td>
<td>16.03↓</td>
<td>6.94</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p≤0.05, **significant at p≤0.01, ***significant at p≤0.001, NS=non significant.

DISCUSSION

The mouth represents the beginning of the gastrointestinal tract. Since probiotics have been used to successfully treat gastrointestinal diseases\cite{7}, an attempt was be made to use the same treatment with regard to oral diseases. Several mechanisms have been proposed to explain how probiotics work including formation of a protective biofilm on the oral tissues, modification of the surrounding environment, secretion of antimicrobial substances, production of short chain fatty acids, etc.\cite{8} There are a handful of studies that have been conducted on the effects of probiotics in oral health and prevention of periodontal diseases. In a parallel, double-blind, randomized, placebo-controlled study with 59 patientshaving moderate-to-severe gingivitis, Lactobacillus reuteri strains were administered via chewing gum twice a day for 2 weeks at a concentration of 1×10^8 CFU (colony forming unit). Results showed an improvement in clinical parameters along with reduction in the pathogenic bacteria in the group consuming probiotics chewing gum.

L. rhamnosus is one of the most extensively studied probiotic and of particular interest in oral biology. Many controlled studies have also shown the effectiveness of L. rhamnosus in reducing caries.\cite{5} In a seven-month study on kindergarten children who received probiotic L.
*rhamnosus* showed less dental caries and lower levels of *S. mutans* in the probiotic milk-consum ing group.\[^9\] Another study aimed at benefit of cheese containing *Lactobacillus rhamnosus* showed that probiotic intervention helped in reducing the highest level of *Streptococcus mutans* in the oral cavity.\[^10\]

In a one-way crossover, open-label placebo-controlled study 72 subjects were given a mouth rinsing solution containing probiotics strain and were asked to rinse twice, three times a day, with 15 ml. of the solution. There was a significant, 20% reduction in plaque scores when the *Weissellacibaria* CMS1-containing rinse was used. The results indicated *Weissellacibaria* isolates possess the ability to inhibit biofilm formation on the teeth.\[^11\]

To study the effect of *Bifidobacteria* a doubleblind, randomized crossover study was performed. A statistically significant reduction of salivary *mutans streptococci* was recorded after the probiotic yoghurt consumption containing *Bifidobacterium*, which was in contrast to the controls. Concluding that probiotic *Bifidobacteria* in yoghurt may reduce the levels of selected caries-associated microorganisms in saliva.\[^12\] Another study using *Bifidobacterium lactis* a statistically significant reduction (p<0.05) of salivary *mutans streptococci* was recorded after consumption of the probiotic ice-cream in adults.\[^13\] A recent study revealed positive relationship between the gut *Bifidobacteria* and oral *Lactobacilli* upon feeding the school going children with buttermilk as well as FOS containing buttermilk. (Sheth *et al* 2016).

Most studies on the effects of probiotics on caries prevention are aimed at decreasing the number of *mutans streptococci*. The present study showed significant shift in oral hygiene status from fair to good after FOS added buttermilk supplementation leading to a significant improvement in the oral hygiene status. The oral health with regards to *S.mitis* showed significant change with the reduction (16.03%) in their growth over a period of one month. Along with this the gut flora of subjects in relation to *Bifidobacteria, Lactobacilli* and *E.coli* also showed significant changes. Indicating a plausible role of gut micro flora on oral health. The reduction in the count of *S. mitis* might be because of the *Lactobacilli* species which are likely to be present in the buttermilk, along with prebiotic FOS which may have helped in the multiplication of these probiotic organisms.

A study conducted ON FOS, concluded that doses between 4 and 15g/day give rise to significant increases in numbers of *Bifidobacteria* within the gut microflora, and that the
degree of bifidogenesis is related to the initial numbers of faecal Bifidobacteria.\textsuperscript{[15]} Another study confirmed that Bifidobacterium infantis not only grew well on FOS but also inhibited Escherichia coli and Clostridium perfringens.\textsuperscript{[16]} However, the present study is one of the pioneering study reporting a concomitant improvement in both gut beneficial bacteria and oral bacteria.

**CONCLUSION**

Daily consumption of buttermilk which is likely to have probiotic bacteria/ lactic cultures along with 7gm FOS as a prebiotic significantly reduces S. mitis colonization in the oral cavity along with improvement in the Oral Hygiene Index scores and increased colonization of beneficial bacteria. The research is still in the initial stage. Further studies need to be carried out in terms of establishing prebiotic profile of buttermilk so as to authenticate its role in oral health.

**REFERENCES**


