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AN INTERVENTIONAL TRIAL WITH FRUCTOOLIGOSACCHARIDE AS A STRATEGY TO IMPROVE ORAL HEALTH (*LACTOBACILLI* ESTABLISHMENT) AND GUT HEALTH OF YOUNG SCHOOL CHILDREN OF URBAN VADODARA

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ABSTRACT

Background: Oral health is an essential component of health throughout life. Millions of children suffer from oral problems. In India 60 to 65% population suffer from dental caries with 50 to 90% from periodontal diseases. Thus there is an urgent need to develop a strategy to prevent the increasing prevalence of dental diseases as indicated by OHI-S, by manipulating the resident microflora of oral cavity. **Objective:** To evaluate the impact of fructooligosaccharide supplementation to school going children and its effect on oral hygiene along with changes in *oral Lactobacilli* in mouth and *in Bifidobacteria, Lactobacilli*and *E.coli* in gut of children. **Materials and methods:** 255 children were enrolled (8-12years) 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. Pre data was collected on their basic oral hygiene practices using a structured questionnaire. Oral *Lactobacilli* in saliva and gut *Bifidobacterium* and *Lactobacilli*were determined using standard methods. Further, the experimental group was intervened with FOS (7g) in buttermilk (150 ml) and the control group was intervened with only buttermilk (150 ml) for period of 1 month. **Results:** The result of study revealed that at the baseline, most children (41.9%) suffered from fair and poor oral hygiene. The mean score for OHI-S at baseline was 2.003. Buttermilk and FOS added buttermilk intervention for 1 month resulted in improvement in mean score OHI by 70%. The intervention brought about an increase in gut*Bifidobacteria* and *Lactobacilli* by 11.5% and 25% respectively, whereas the *E.coli* decreased by 17%.Oral *Lactobacilli* increased by 16.03%. **Conclusion:** The study concludes that daily dose of 7g of FOS along with buttermilk help improving clinical signs of oral hygiene in children and improves colonization of beneficial bacteria such as oral *Lactobacilli*,gut *Bifidobacteria* and gut *Lactobacilli*along with a reduction in colonization of *E.coli* in young school going childrenof urban Vadodara.

KEYWORDS: Oral health; *Bifidobacteria*; *Lactobacilli*; Fructooligosaccharide; Buttermilk.

INTRODUCTION

The World Health Organization (2003) defines oral health as a "state of being free from chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft lip and palate, periodontal (gum) disease, tooth decay and tooth loss, another diseases and disorders that affect the oral cavity".

According to US department of health and services (2005), Oral health in children encompasses a broad range of dental and oral disorders. In addition to caries and gingival disease, children can also suffer from malocclusion (poor bite) and from birth defects such as cleft lip/palate. More than 65% of all cases of children suffer from poor oral health symptoms worldwide. The consequences of widespread poor oral health can be seen on the personal, population, and health systems level, as caries and periodontal disease deteriorate individual health and wellbeing, decrease economic productivity, and act as a significant risk factors for other systemic health ailments.[1]

The concept of microbial ecological change as a mechanism for preventing dental disease is important one, while altered microbial ecology may lead to dental disease. New methods such as probiotic and prebiotic approaches (i.e. whole bacteria replacement therapy) to eliminate pathogenic members of the microbiota is investigated. Bacteriotherapy is an alternative and promising way to combat infections by using harmless

bacteria to displace pathogenic microorganisms. Probiotics and prebiotics are one of these new agents which are widely used for their therapeutic action. Although, limited research is available that shows probiotics and prebiotics may help dental improvement.[2]

Prebiotics have been proved to be an aid to complement probiotics in the treatment of oral diseases. Oral hygiene is a burden for the society like other non communicable diseases. There are limited food based strategies currently available to improve oral health. Besides, there are limited studies on the impact of FOS on oral health and gut 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.
Hence the study was undertaken to investigate the role of above mentioned factors on oral hygiene as well as effect of buttermilk supplementation and FOS added buttermilk supplementation on oral and gut health of children.

MATERIALS AND METHODS

Selection of the School

Two hundred and fifty five children (8-12 years) were selected from Maharana Pratap School of urban Vadodara. The school was selected based on the permission given by Vadodara Municiple Seva Sadan (VMSS) to undertake the study. The study was approved by the departmental ethical committee with Clearance number: (IECHR/2013/9).

Selection of the subjects

Two hundred and fifty five (255) subjects were enrolled in the study in theage group 8-12 years. Children below 8years of age do not have all the teeth protruded and the age of children from 13years are considered to have hormonal induced dental problems. Hence, this age was considered most appropriate to carry out study related to dental problems.

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 out of which one hundred and seven (n=107) subjects were found to have fair oral hygiene. With purposive selection method sixty subjects (n=60) with fair oral hygiene (OHI=1.3-3.0) were divided into two equal groups as control group (n=30) and experimental group (n=30). For both groups, stool and saliva samples were collected for microbial analysis. Further, the experimental group was intervened with FOS (7g) and buttermilk (150 ml) and the control group was intervened with only buttermilk (150 ml) for period of 1 month (30 days). All the parents were informed about the intervention and a written consent was signed for the same (Appendix-1). 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 general information of the subjects

A survey method was used to obtain the data pertaining to prevalence of oral hygiene, nutritional status of children, basic oral hygiene practices, parent's education and socio economic status using structured questionnaire. The questionnaire was pretested and necessary changes were made before handing it over to the parents for filling it at home. The forms were made in user-friendly language (Gujarati) so that the information could be easily filled.

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 (255) children who were diagnosed on the basis of oral hygiene index-simplified.[3] 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.

Debris

	Right molar		Anterior		Left molar		Total	
	B	L	La	La	B	L	B	L
Upper								
Lower								

B-Buccal, L-Lingual, La- Labial

$$\text{Debris Index} = \frac{(\text{The buccal scores}) + (\text{The lingual scores})}{(\text{Total number of examined buccal and lingual surfaces})}$$

Calculus

	Right molar		Anterior		Left molar		Total	
	B	L	La	La	B	L	B	L
Upper								
Lower								

B-Buccal, L-Lingual, La- Labial

$$\text{Calculus Index} = \frac{(\text{The buccal scores}) + (\text{The lingual scores})}{(\text{Total number of examined buccal and lingual surfaces})}$$

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:

Good	Fair	Poor
0 to 1.2	1.3 to 3	3.1 to 6

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^{0}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 was reduced and a sterile environment was maintained.Further the plates were placed in an incubator at 37^{0}C for the period of 48 hours for *Bifidobacteria and Lactobacilli*and *E.coli* was incubated at 37^{0}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 *Lactobacilli*

2ml of saliva sample was collected from the subject with passive drool method [4] in sterile air tight containers. The containers were immediately transferred to -18^{0}C within half an hour of the sample collection. For enumeration *oral Lactobacillus*, 1ml of fresh saliva sample is added to 99ml of 0.1% peptone water and from this further serial dilutions were prepared as required. From these dilutions 0.1ml were added in to the petriplates, and then Rogosa SL agar (Hi media) media for the culture growth was added. 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^{0}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)

Fructooligosaccharide and buttermilk intervention to the subjects

The children with fair oral hygiene were divided into two groups, experimental group and control group. Control group was provided with plain buttermilk (150ml) and Experimental group was provided with FOS (7g) added into the buttermilk (150ml) for 30 working days. Buttermilk (*Goras)* was procured daily from Baroda dairy, Vadodara, through a local vender and FOS was procured from S.A. Pharmachem Pvt. ltd. Plain buttermilk and FOS added buttermilk were provided daily to the subjects and recorded for its intake by the subjects on daily basis so that compliance could be studied.

Post-intervention data

The stool sample and saliva sample were collected immediately after the intervention period of 30 days and were analysed for microbial load. Visible dental check-up was also done on the subjects.

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 AND DISCUSSION

Oral hygiene status of the children

Out of the Two fifty five (255) children most children (58%) were found to have a good oral hygiene, but also a large proportion of children (41.9%) suffered from fair oral hygiene.

Figure 1 reveals that there existed a gender difference and age group difference with oral hygiene status of children. Male children had a better oral hygiene status than female children. Also, younger children (8-10 years) had fairer oral hygiene than older group of children (11-12 years). 60 children with fair oral hygiene were chosen for the intervention.

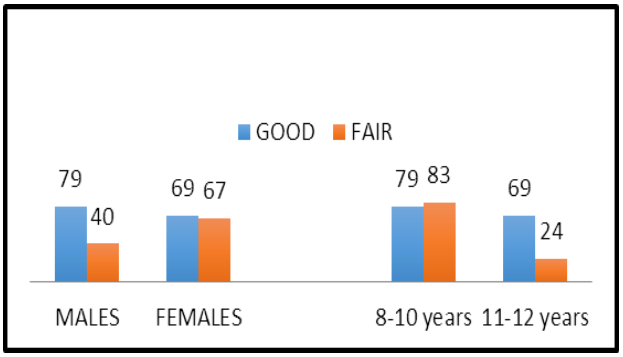

Figure 1: Number of male and female children and age group of children having good and fair oral hygiene.

Changes in the oral hygiene index scores after intervention to the subjects

Table 1 reveals that oral hygiene index significantly ($p<0.05$) improved from fair (2.003) to good (0.61) oral hygiene by 70%.

Table 1. Mean oral hygiene scores of subjects before and after intervention.

Parameters		Control (N=30)	Experimental (N=30)	*t* value
OHI-scores	Pre	1.93±0.24	2.05±0.30	
	Post	1.51±0.38	0.61±0.11	0.857^{NS}
	Paired t test	3.23*	12.04**	7.43***
	%difference	21.76 ↓	70.24 ↓	

(NS= non significant, ***significant at p<0.001)

Gender difference and age group difference in oral hygiene status of children after intervention.

Table 2 reveals that both in males and females the improvement in the score of oral hygiene status and this improvement was statically significant (p<0.001)

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

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

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

Changes in the mean counts of oral and gut micro flora after supplementation

A significant increase in counts of *Lactobacilli, Bifidobacteria* and oral *Lactobacilli* and reduction in *E.coli* was observed after intervention in experimental group. Post intervention, percent difference showed significant improvement in counts of beneficial bacteria in both the groups. However in experimental group percent improvement was higher indicating an additional effect of FOS added to buttermilk on the oral health and gut health. Table 3 reveals that there was a significant rise in both *Bifidobacteria*(11.58%) and *Lactobacilli* (25.03%) of gut and oral *Lactobacilli* (13.17%) of saliva along with, a significant decrease in counts of *E.coli* (17.9%) in the gut.

Table 3. Mean counts of oral and gut micro flora before and after intervention.

Parameters (log_{10}cfu/gm)		Control	Experimental	*t* value
Gut *Bifidobacteria* (N=30)	Pre	9.33±0.69	8.72±1.21	1.48^{NS}
	Post	9.99±0.35	9.73±0.023	1.72^{NS}
	Paired t test	2.52*	3.31**	
	%difference	7.41 ↑	11.58 ↑	
Gut *Lactobacilli* (N=30)	Pre	6.70±1.31	6.671+1.70	0.18^{NS}
	Post	7.36±0.566	8.34±0.63	3.53**
	Paired t Test	2.07*	4.72***	
	%difference	9.85 ↑	25.03 ↑	
***Gut E.coli* (N=30)**	Pre	5.30±0.78	5.30±0.78	1.67^{NS}
	Post	5.35±0.05	4.35±0.05	14.06***
	Paired t test	0.25^{NS}	4.77**	
	%difference	0.9 ↑	17.92 ↓	
Oral *Lactobacilli* (N=60) (log_{10} CFU/ml)	Pre	5.41±0.45	5.39±0.92	0.11^{NS}
	Post	5.8±0.17	6.10±0.24	1.87^{NS}
	Paired t test	3.61***	3.53***	
	%difference	7.2 ↑	13.17 ↑	

(*significant from the baseline value at P<0.05,** significant from the baseline value at P<0.01, *** significant from the baseline value at P<0.001, NS= Non significant)

DISCUSSION

Oral health problems are ranking on fifth position when it comes to non communicable diseases worldwide.[5] Oral health is an important aspect of health for all children, and is more important for children with special health needs. Because oral hygiene affects one's aesthetics and communication, it has strong biological, psychological, and social projections.[6]

The baseline data of the present study shows that 41.9% of total screened subjects suffered from fair oral hygiene and the female children (26.2%) had poorer scores of oral hygiene index than male children (15.68%). In the present study, results in context to age of children were also obtained as 8-10 years (32.4%) had poorer oral hygiene scores than 11-12 years (9.4%).Also, female children (26.2%) had poorer scores of oral hygiene index than male children (15.68%). Results of oral hygiene scores, with regards to sex of children are similar to one study and contradicting to another.

In the present study after FOS intervention the counts of oral *Lactobacilli*increased by 16.03% significantly. Similar studies where FOS added lemon juice, curd, and other base materials fed to younger children and elderly resulted in significant increase in establishment of *Lactobacilli* and *bifidobacteria* in the gut of children with a significant reduction in establishment of *Enteric pathogens*.[7,8,9] Significant increase in the number of colonies of beneficial bacteria with regards *bifidobacteria* (11.58%) and *Lactobacilli* (25.03%) in the gut with significant decrease in establishment of *E.coli* (17.02%) was also observed in the present study. However, no studies have been reported for change in micro flora of oral cavity with prebiotic intervention

CONCLUSION

The study concludes that daily dose of 7g of FOS along with buttermilk help improving clinical signs of oral hygiene in children and improves colonization of beneficial bacteria such as oral *Lactobacilli* (16.03%); gut *Bifidobacteria* (11.5%) and gut *Lactobacilli* (25%). A strong negative correlation was found between oral *Lactobacilli* and gut E.coli. A reduction in colonization of *E.coli* in gut of young school going children by 17 % was observed.

FUTURE SCOPE OF INVESTIGATION

Effect of FOS on other oral diseases and dental problems can be studied. Also, studies need to be undertaken to develop FOS added ready to consume drinks that can be up scaled for its market potential and commercialization. And such drinks, be subjected to various groups of population for studying its potential health benefits in health and disease.

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