COMPARATIVE EVALUATION OF CHANGE IN SALIVARY pH ON CONSUMPTION OF DRY READY TO EAT CEREALS, CEREALS WITH AND WITHOUT ADDED SUGAR IN MILK- AN IN -VIVO STUDY

Mahesh J[*], Sapna B, Veeresh DJ, Divya D
[*]Post graduate student, Department of Public Health Dentistry, Bapuji Dental Collage and Hospital, Davangere, India.
Reader, Department of Public Health Dentistry, Bapuji Dental Collage and Hospital, Davangere, India.
Professor, Department of Public Health Dentistry, Bapuji Dental Collage and Hospital, Davangere, India.
Intern, Bapuji Dental Collage and Hospital, Davangere, India.

Abstract

Background: Dry, Ready to Eat Cereals are a combination of refined sugar and starch, most commonly consumed breakfast in the modern human diet. The present study was done to investigate the effects of combination foods on salivary pH. **Objective:** To assess and compare the salivary pH changes after consumption of Dry Ready to Eat Cereals (REC), Cereals with plain and sugar added milk. **Method:** Thirty six adults of age 18 to 25 years were assessed for salivary pH at baseline followed by one minute, twenty minutes and one hour after consumption of dry ready to eat cereals, cereals with plain milk and sugar added milk as intervention groups. The differences in mean salivary pH within groups at different time interval was assessed using repeated measures ANOVA (Analysis of variance).

Results: There was significant increase in salivary pH at one minute from baseline and significant decrease at twenty minutes from one minute within the group in Dry REC and cereal with added sugar milk. There was no significant difference within the group of cereal with plain milk at different intervals of time. There is no statistical significant difference between the groups at different intervals of time (p value <0.05). **Conclusions:** Consumption of Dry REC, Cereals with plain and sugar added milk did not show much variation in salivary pH.

Introduction

Despite the advancements in oral disease science, dental caries continues to be a worldwide health concern, affecting humans of all the ages. It is an infectious bacterial biofilm and pH mediated disease expressed in a predominantly pathologic oral environment.[1] Although acid generating bacteria are the etiologic agents, dental caries has been considered as multifactorial disease since it is influenced by dietary habits as well host factors.[2] Saliva as a host factor plays an important role against dental caries. The caries process is dependent upon the interaction of protective and pathologic factors in saliva as well as the balance between the cariogenic and noncariogenic microbial populations that reside in saliva.[3]When the salivary pH decreases it leads to increased demineralization and caries initiation.[4]

A dynamic relation exists between sugars and oral health. Diet affects the quantity, pH and composition of the saliva. Sugars and other fermentable carbohydrates, after being hydrolysed by salivary amylase, provide substrate for the actions of oral bacteria, which in turn lower salivary pH. The resultant action is the beginning of tooth demineralization which contributes to dental caries.[5]

Dry, ready to eat sugar added cereals are a combination of refined sugar and starch, most commonly consumed breakfast in the modern human diet (processed food starches) which may possess significant cariogenic potential.[6] Beverages such as milk and other dietary products are considered tooth friendly, with a pH ranging from 6.4 to 6.7 is

considered to be anticariogenic. It is also a good source of calcium, phosphate, casein and lipids which are largely responsible for protective effect.[7]

Many investigations have assessed the acidogenicity and demineralizing potential of individual foods, however fewer attempts have been made to investigate the effects of combination foods and beverages on salivary pH.

Hence the aim of our research was to assess the change in salivary pH after consumption of dry ready to eat cereals, cereals with and without added sugar in milk? We hypothesized that there is a difference in salivary pH on consumption of dry ready to eat cereals, cereals with and without added sugar in milk.

Materials and methods

An in vivo, concurrent parallel, Experimental study was done among students aged 18-25 years of Bapuji Dental College and Hospital in Davangere city. The study was conducted at the clinical premises of Department of Public Health Dentistry, Bapuji Dental College and Hospital, Davangere. As the study is of its first kind sample size estimation was done according to article by Julious[8] a sample size of 12 subjects per group is recommended for a pilot study. So, total sample size of 12x3 (groups) = 36 were taken. A convenient sample of 36 students who fulfill the eligibility criteria were randomly selected. Eligibility criteria includes subjects who voluntarily consent to participate, Subjects aged between 18 – 25 years, Caries free individuals (DMFT=0), no incipient caries lesion, not allergic to any of the test products. Subjects who smoke and consume alcohol, who are on medications and subjects with systemic diseases that will affect their salivary flow rate and subjects undergoing orthodontic treatment were excluded from the study. Ethical approval was obtained from the Institutional Review Board of Bapuji Dental College and Hospital, Davangere. Informed consent was be taken from the study participants prior to the start of the study after explaining the purpose and the procedures involved in the study. Intervention Groups were GROUP A – Dry Ready to eat cereal (Kellogg's India private limited), GROUP B – Ready to eat cereal with plain milk, GROUP C– Ready to eat cereal with sugar added milk. Baseline salivary pH of all subjects was recorded on a given day in the morning. Subjects were randomly allocated to interventional groups and respective test products were administered. Random allocation was done by concealed randomization method. Random assignment of the participants to the three interventional groups was done by a separate person not involved in the study using lottery method. All subjects undergoing investigations were given prior instructions to refrain from eating for one hour before and after baseline collection of saliva. Respective groups was given 27 grams (single serving) of cereals, cereals with plain or sugar added milk chewed and swallowed for 5-10 minutes. Preparation of sugar added milk for 12 participants was done by mixing (12x 100ml) 1200ml of milk with 144 grams (36 tea spoon) of sugar.[9] Subjects were asked to pool their saliva (unstimulated) in the floor of the mouth for 60 seconds. The pooled saliva was spit into a sterile disposable cup. The procedure was repeated each time to collect the saliva sample. Approximately 2 ml of saliva was collected. Salivary pH was estimated using dental salivary pH indicator strips (GC company). The pH strip was dipped into the collected saliva and observed for 30 seconds for the colour change. The change in colour was compared with the coding reference given by the manufacturer and readings were entered. Two Examiners were involved in the study (Examiner 1 and Examiner 2) and were checked for interexaminer reliability. The kappa value was 0.876 indicating a strong measure of agreement. Salivary pH was recorded at baseline, then after intervention at different intervals of time was recorded at (1, 20 and 60 minutes) respectively.The Investigator and statistician were blinded**.** The data obtained were compiled systematically in Microsoft Excel sheet and subjected to statistical analysis (Statistical package for social sciences software 20). Data was normally distributed based on Kolmogrov Smirnov test. One way analysis of variance (ANOVA) was used to compare the mean salivary pH between the groups. Repeated measures ANOVA was employed to compare the means of salivary pH within the group followed by post hoc tukeys test.

Results

The mean and standard deviation of salivary pH in all groups at different intervals of time are been mentioned (table 1). There was significant increase in salivary pH at one minute from baseline and significant decrease at twenty minutes from one minute within the group in Dry REC and cereal with added sugar milk (Graph 1). There was statistically significant difference within the group with a p value of 0.013^* andthere was no significant difference between the groups at a p value of 0.576 (table 2).There was significant difference within Group A and Group C (p-value <0.05) i. e. Dry ready to eat cereals and cereals with sugar added milk with a p value of 0.028* and 0.001** respectively and no difference in Group B cereals with plain milk with p value of 0.167.

Schematic representation of random allocation:

Fig (1)
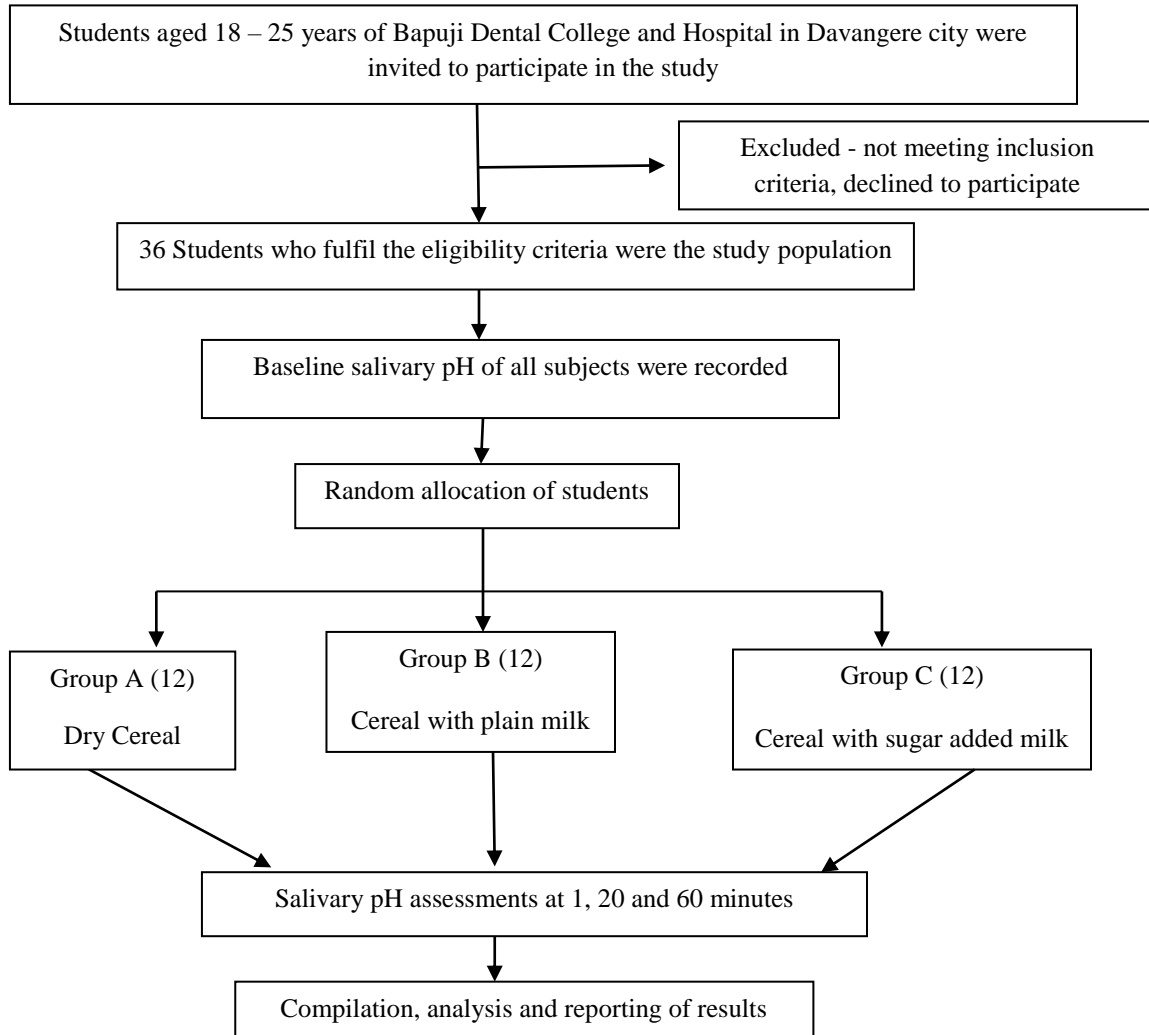
Pairwise comparison demonstrated significant difference in Dry ready to eat cereal at Baseline and One minute, One minute and twenty minutes, One minute and one hour with p value of 0.026*, 0.040*and 0.015* respectively.In cereals with plain milk at One minute and one hour with p value of 0.027*. In cereals with sugar added milk at Baseline and One minute, One minute and twenty minutes, One minute and one hour with p value of 0.000**, 0.001**and 0.000** respectively.There was no significant difference from twenty minutes to one hour within the same group. There was no significant difference within the group of cereals with plain milk at different intervals of time. There is no statistical significant difference between the groups at different intervals of time when compared between all the groups with p value of <0.05.

Tables:

Table 1: Mean and Standard deviation of salivary pH of all Group at different interval of time

Group	Baseline		One minute		Twenty minutes		One hour		n
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	
A	6.73	±0.215	7.03	±0.267	6.68	±0.356	6.77	±0.144	12
B	6.77	±0.223	6.88	±0.324	6.73	±0.355	6.65	±0.228	12
C	6.68	±0.180	6.95	±0.173	6.58	±0.395	6.68	±0.199	12

A- Dry Ready to Eat Cereal
B- Cereal with plain milk
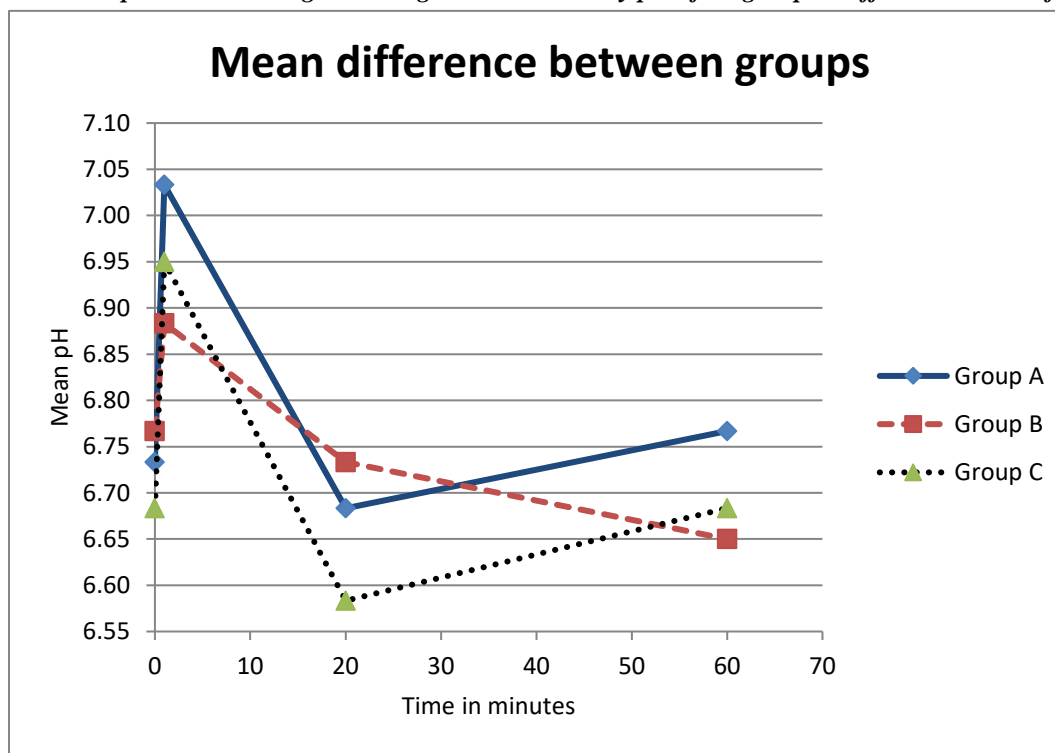C- Cereal with sugar added milk
n = number of samples

Table 2: Comparison of salivary pH within group and between groups

Comparison	F Value REPEATED MEASURES ANOVA	p value (probability)
Within Group A, B and C	6.917	.013*
Between Group A, B and C	.562	.576

* Statistically significant at $p < 0.05$ (2-tailed).
** Statistically highly significant at $p < 0.01$ (2-tailed).

Table 3: Difference in salivary pH within the (individual) group and Pairwise comparison within individual group A, B and C

Group	p-value within group	Pairwise comparison p-value					
		Baseline and One minute	Baseline and twenty minutes	Baseline and One hour	One minute and twenty minutes	One minute and one hour	Twenty minutes and one hour
A	.028*	.026*	.536	.658	.040*	.015*	.408
B	.167	.294	.812	.189	.069	.027*	.295
C	.001**	.000**	.293	1.000	.001**	.000**	.214

* Statistically significant at $p < 0.05$ (2-tailed).
** Statistically highly significant at $p < 0.01$ (2-tailed)

Graph 1: Line Graph demonstrating the change in mean salivary pH of all group at different interval of time.

Discussion

This study assessed and compared the change in salivary pH between and within the groups (i. e.) Dry Ready to eat cereal, cereal with plain milk and sugar added milk at different interval of time, by a randomized experiment concurrent parallel design. This design was used as there was no sufficient evidence to determine the wash out period. This study concentrated on combination of food, breakfast cereals as it is one of the routine diet which is followed in day to day life. It is one of top breakfast cereal marketed and consumed by the population. This study used a convenient sample of male and female undergraduate students of same college at age of 18- 25 who were residing in the hostel. Since civilized type of food is commonly used by this population as they are readily available, easy to prepare and its nutritive value. Therefore we used this population and also to minimize the effect of age on variations in salivary pH, all participants selected were of same age. Stephen's curve describes the changes in dental plaque pH in response to a carbohydrate challenge over a period of time. Characteristically the curve reveals a rapid drop in plaque pH that is attained after consumption of sugar. It normally takes at least 20 minutes for the plaque pH to reach its resting value. Based on the concept of Stephen's curve and few studies done to check the plaque and salivary pH changes after consumption of snacks and beverages, it was decided to check salivary pH changes at 1, 20 and 60 minutes time intervals.[10-11] Repeated measures ANOVA demonstrated that there was a significant difference within the group at different intervals of time this is due to the normal pattern of Stephen's curve. The raise in salivary pH at one minute (immediate) could be assumed due to the stimulation of the flow of saliva and due to less acidic nature of milk which is found in all the groups.[12] In group B (cereals with plain milk) there were no significant difference at different intervals except between one minute and one hour indicating plain milk is neutral and does not influence in much fall in pH. There was no statistical significant difference between the groups this might be attributed to the fact that the sugar used in dry ready to eat cereals were minimum and the amount of sugar mixed in sugar added milk is the standard amount being used for day to day diet. These finding cannot be compared exactly with other studies with the available literature search, since it is the first of its kind to compare the salivary pH among these products and Indian population, other studies have compared on different variables such as plaque pH and DMFT index. The results of this study shows similarity with other study that there was no convincing association to imply that sweetened cereal consumption increases the risk of caries.[13] The present study there was no

significant change within the cereals with plain milk, which synchronize with another study that milk is a protective factor and dilutes the salivary pH.[14] The limitations of this study could be a convenient small sample size been used (pilot study). The flow rate and the buffering capacity was not determined which also influence the salivary pH. The salivary pH may not be as important measure for buffering action as that of plaque pH. Further studies are required to improvise the external validity by selecting a larger population with consideration of plaque pH, salivary flow rate and buffering capacity. More emphasis should be given on population with high caries risk to know the actual imperil of combined cereal food and beverages.

Conclusion

Consumption of Dry REC, Cereals with plain and sugar added milk did not show much variation in salivary pH when compared with each other.

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