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Comparative Evaluation of the Cariogenicity of Three Breakfast Cereals amongst Corporate Employees of an Industry – A Single Blinded Randomized Control Study

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Abstract

Introduction – Diet is one of the essential components of the multi-factorial etiology of the dental caries. Various dietary constituents attribute to different levels of cariogenicity. So, this study was conducted with the aim to compare the cariogenicity of three breakfast cereals amongst corporate employees.

Methods- A total of thirty caries-free employees (30-50years) fulfilling the eligibility criteria were included in the study. They were randomly allocated to three different breakfast cereal groups (one unflavored – Plain cornflakes and two flavored – Fruit and Nut and Chocos). They were asked to consume 30mg of cereals in 60ml plain milk. Their salivary samples were taken at baseline and 30 minutes after cereal consumption and salivary pH and microbial flora was determined. Statistical analysis was done using Paired t-test and ANOVA at 95% confidence interval (p value <0.05).

Results: All the three cereal groups showed statistically significant difference (p value <0.05) in the salivary pH levels at baseline and after 30 minutes of cereal consumption, with the highest decline in the salivary pH among flavored cereal group as compared to unflavored cereal group. The salivary microbial flora showed a significant increase in the colony forming units of streptococcus species and pseudomonas species after 30 minutes of consumption of all three cereals. However, the inter-group comparison between flavored and unflavored cereal groups did not show statistically significant results.

Conclusion: All three cereals are equally conducive in creating an acidogenic oral environment, after their consumption. Moreover, all the cereals have a similar cariogenic potential, regardless of their flavors.

Keywords: Cariogenicity, diet, microorganisms, salivary pH.

Introduction

Diet has a local effect on the oral health, primarily on the integrity of the teeth, pH and composition of the saliva and plaque. Dental research studies on food type, texture, composition, retentiveness, consistency and their effect on dental hard tissues have been growing since ancient times[1,2].

Simple sugars are considered cariogenic as against complex sugars or starch.1 There has been a growing concern within the dental profession about the cariogenic potential of various foods. Ideally, assessment of food cariogenicity involves several factors which encompass the host, the diet, and those microorganisms contained in plaque and saliva which ferment carbohydrates and produce acids that ultimately cause dental decay through the demineralization process[3]. The amount of saliva concentration and presence of cariogenic bacteria will favor the development of caries.

The production of acids by microorganisms within the dental plaque continues until the carbohydrate substrate is metabolized. It also is known that the plaque's pH goes from acidic to normal (or the resting level) within a few minutes and depends on the presence of saliva. This is primarily due to the carbonate and phosphate pH buffering agents in saliva. In essence, an equilibrium exists within the dental plaque whereby the pH of the plaque decreases each time the host ingests a snack or meal that contains fermentable carbohydrates; afterwards, the pH returns to the resting level because of saliva.[4]

Stephan and Englander and colleagues [5] reported plaque pH responses after plaque exposure to foods and beverages that contain sucrose or other fermentable carbohydrates. Within three to five minutes after such exposures, the pH of the plaque decreases below the so-called critical pH values of 5.5 and 6.0 for enamel and dentin, respectively, and demineralization of the underlying enamel or dentin is initiated [4].

Sucrose and starches are the predominant dietary carbohydrates in modern societies. The causal relationship between sucrose and dental caries development is indisputable. An in-depth evaluation of the sucrose-caries relationship requires the consideration of several critical cariogenic determinants like the intensity (i.e., the amount and frequency) of exposure of tooth surfaces to both sugars and starches, the bioavailability of the sugars, the nature of the microbial flora of dental plaque, the pH-lowering capacity of dental plaque, and the flow rate of saliva[6]. This is a difficult issue to study in humans because of the variability of the human diet, so views are based principally on extrapolations from animal studies and laboratory research [7]. Based on this knowledge, some studies have attempted to compare the caries-producing potentials of foods by measuring the amounts of acid or enamel demineralization they produced when incubated with saliva or oral bacteria [8].

There is an increased use of processed snack and convenience foods by consumers, especially amongst the corporate industrial employees so investigations of the cariogenic potential of these foods are necessary to identify products that may be potentially detrimental to teeth. This will enable the clinician and patient to recognize and select foods which are less caries conducive [2].

Studies of caries in animals, human plaque pH response, and enamel/dentin demineralization leave no doubt that processed food starches in modern human diets possess a significant cariogenic potential. However, the available studies with humans do not provide unequivocal data on their actual cariogenicity [6]. Therefore, it was appropriate to investigate the change in salivary pH levels and microbiota to assess the relative cariogenicity. Hence, this

study was conducted with the aim to compare the cariogenicity of flavored (chocos and fruit and nut) and unflavored (plain cornflakes) breakfast cereals amongst corporate employees. The cereals in this study were chosen because they were commonly consumed for breakfast as a complementary meal in their industry.

Materials and Methods

This is a randomized controlled study conducted after obtaining relevant permissions from the Scientific Advisory Committee and Institutional ethics committee (IEC no. SDCH/IEC/OUT/2017-18/58).

A. Sample Size Determination

Sample size for the study was determined by using the means of two groups, from the previous study[1], considering the following formula,

$$\frac{2\,(Z_{\alpha} + Z_{1-\beta})^2\,\sigma^2}{(m_1 - m_2)}$$

where $Z_{\alpha} = 1.96$, $Z_{1-\beta} = 0.84(80\%)$,

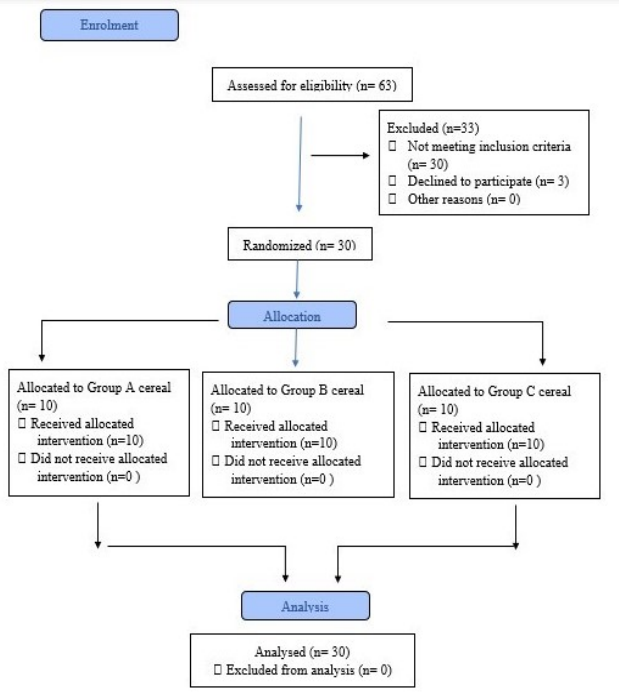
σ = difference between SD, m_1 = mean of first group[1], m_2 = mean of second group[1]

$$\frac{2(1.96 + 0.84)^2\,(0.03)^2}{(5.88 - 5.43)} = 6$$

Sample size = 6, for each group. Hence, the total sample size determined was 18.

B. Eligibility Criteria

Caries-free employees (30-50years) of genders, willing to participate and giving written informed consent were included in the study. Only those who had refrained from eating or drinking at least 2 -3 hours before the study (as mentioned in the participant information sheet) were included in the study. Those employees giving history of previous allergy to cereals or having history of acute infections or systemic conditions were excluded from the study. Moreover, the employees with adverse tissue abuse habits like smoking, tobacco chewing, etc. were also excluded from the study. The total number of participants fulfilling the eligibility criteria was 30. A Consort flow diagram 2010 (Flow diagram) was followed for the selection of the study participants as follows: -



C. Sampling Technique and Procedure

A single blinded randomized control study was conducted among 30 employees of an industry in Pune. The Employees were randomly allocated into three cereal groups – Group A - Fruit and Nut flavor, Group B - Chocolate flavor and Group C - Plain Cornflakes (control group).

D. Blinding and Randomization

The allocation concealment was done by using Sequentially Numbered Opaque Sealed Envelopes (SNOSE), wherein the principal investigator was blinded to the groups allocated to the participants. The principal investigator collected the baseline salivary samples and randomly gave an envelope to each participant. These opaque sealed envelopes had a chit with letter either A, B or C written in the chit which were carried by the employees to the co-investigator. The type of cereals given to the employees were based upon the alphabet corresponding to the particular cereal which was pre-decided by the co-investigator and remained the same for

the entire study. This was blinded for the principal investigator and was revealed only after the statistical analysis of the study was complete.

E. Study Procedure

The employees were refrained from eating or drinking for at least 2 hours before the study. They were asked to consume 30 gms of cereals with 60 mL of plain milk (for each group). The saliva samples were collected by draining method, where saliva was allowed to drool passively from the corner of the mouth. (Unstimulated saliva). The saliva samples were collected of all three groups before and 30 minutes after the consumption of cereals into the sterilized plastic cups and then transferred using a funnel into the test-tube. Approximately 5ml of saliva was collected.

F. Laboratory Analysis

The salivary pH levels of samples collected at Baseline and after (30 minutes) consumption of cereals were determined by using standard pH meter, previously calibrated with pH buffers from 4,7 and 9. The salivary microbial flora of the same samples was determined and enlisted by using Blood Agar and MacConkey's agar culture media plates. (Figure 1)

Figure 1: - Colony forming Units (CFU's) seen on Blood Agar, No growth MacConkey's agar

G. Statistical Analysis

The data recorded was entered in Microsoft Excel 2013. Frequency analysis was done of demographic status by using Statistical Package for Social Sciences (IBM SPSS Statistics V21.0) statistical software. Mean and standard deviation of the Salivary pH and Colony forming units was derived. Comparison at baseline and 30 minutes after the cereal consumption within the groups was done by using Paired t-test and One-way ANOVA was used to determine the difference between the groups followed by bonferroni post hoc test at 95% confidence intervals (p value <0.05)

Results

A single blinded randomized control study was conducted with the aim to compare the cariogenicity of three breakfast cereals amongst corporate employees in an Industry. A total of 30 employees fulfilled the eligibility criteria and were included in our study. The employees were within the age groups of 30-50 years.

Amongst them, there were 9 (47.3%) males and 10 (52.7%) females in the age group of 30-40 years and 7 (63.6%) males and 4 (36.4%) females in the age group of 41-50 years.

In our study, the relative cariogenicity of three different breakfast cereals was assessed using the Salivary pH levels and the type of oral microbiota and their colony forming units (CFU's). The oral microbiota was determined on two types of culture media plates – MacConkey's agar and Blood Agar. Pseudomonas species was identified on MacConkey's Agar and Streptococcus species and Coagulase Negative Streptococcus species (CONS) were identified on Blood Agar.

The participants in the present study were allocated to three cereal groups namely Group A (Unflavored – Plain cornflakes), Group B (Flavored – Chocos) and Group C (Flavored – Fruit and Nut). The mean salivary pH level at baseline for Group A was 6.90 + 0.46, Group B was 6.71 + 0.56 and Group C was 6.82 + 0.63 and after 30 minutes of cereal consumption, a marked decline in the pH was

observed, it was 6.01 + 0.54, 5.52 + 0.89 and 5.97 + 0.67 in the cereal groups A, B and C respectively. This intra-group comparison of the salivary pH levels at baseline and after 30 minutes of cereal consumption was statistically significant in all the three cereal groups (p value <0.05). This shows that there was a drop in the level of salivary pH (acidic) in all the three groups after consumption of cereals. (Table 1)

Table 1: Intra-group comparison of Mean Salivary pH at Baseline and after 30 minutes of cereal consumption between three cereal groups

Type of Cereal group	Mean (±SD) Salivary pH		Intra-group comparison	
	At Baseline	After 30 minutes of cereal consumption	t value	p value
Group A (Unflavoured cereal – Plain cornflakes)	6.9 ±0.46	6 ± 0.54	5.63	0.01*
Group B (Flavoured cereal – Chocos)	6.7 ± 0.56	5.5 ± 0.89	3.01	0.02*
Group C (Flavoured cereal – Fruit and Nut)	6.8 ± 0.63	5.9 ± 0.67	5.32	0.01*

*p value <0.05 significant

Our study results showed that the mean CFU's of Streptococcus species at baseline were 102.5±101, 50±46.5 and 50±46.2 for Group A, Group B and Group C respectively and after 30 minutes of cereal consumption were 168±103.2, 225±103.5 and 175±89.1 for Group A, Group B and Group C respectively. This suggests that the CFU's increased from baseline as compared to post-consumption of cereals and this difference was highly statistically significant. (p value < 0.01) (Table 2). In our study, the mean CFU's of Pseudomonas species at baseline were 0.63 ± 1.18, 0.63 ±1.76, 1.38 ± 2.66 for Group A, Group B and Group C respectively and after 30 minutes of cereal consumption were 4.88 ± 4.39, 2.88 ± 4.45 and 4 ± 5.75 for Group A, Group B and Group C respectively. This suggests that the CFU's increased from baseline as compared to post-consumption of cereals and this difference was highly statistically significant. (p value < 0.01) (Table 2). In our study, the mean CFU's of Pseudomonas species at baseline were 0.63 + 1.18, 0.63 +1.76, 1.38 + 2.66 for Group A, Group B and Group C respectively and after 30 minutes of cereal consumption were 4.88 + 4.39, 2.88 + 4.45 and 4 + 5.75 for Group A, Group B and Group C respectively. This difference in the number of CFU's at baseline and after 30 minutes of cereal consumption was highly statistically significant. (p value < 0.01) (Table 3).

Table 2: Intra-group comparison of Mean Colony forming units (CFU's) of Streptococcus at Baseline and after 30 minutes of cereal Consumption between three cereal groups

Type of Cereal group	Colony forming units (CFU's) of Streptococcus (Mean ± SD)		Intra-group comparison	
	At Baseline	After 30 minutes of cereal consumption	t value	p value
Group A (Unflavoured cereal – Plain cornflakes)	102.5±101	168±103.2	2.79	0.02*
Group B (Flavoured cereal – Chocos)	50±46.5	225±103.5	7.56	0.000***
Group C (Flavoured cereal – Fruit and Nut)	50±46.2	175±89.1	5.94	0.001**

*p value <0.05 significant, **p value <0.01 highly significant, ***p value <0.001 very highly significant

Table 3: Intra-group comparison of Mean Colony forming units (CFU's) of Pseudomonas at Baseline and after 30

minutes of cereal consumption between three cereal groups

Type of Cereal group	Colony forming units (CFU's) of Pseudomonas (Mean ± SD)		Intra-group comparison	
	At Baseline	After 30 minutes of cereal consumption	t value	p value
Group A (Unflavoured cereal – Plain cornflakes)	0.63 ± 1.18	4.88 ± 4.39	3.28	0.049*
Group B (Flavored cereal – Chocos)	0.63 ±1.76	2.88 ± 4.45	2.02	0.01*
Group C (Flavored cereal – Fruit and Nut)	1.38 ± 2.66	4 ± 5.75	2.02	0.005**

*p value <0.05 significant, **p value <0.01 highly significant

Inter-group comparison was done to determine the specific cereal groups that are causing significant cariogenicity and this comparison did not show any statistically significant results suggesting that both the Unflavored and flavored cereals have almost similar cariogenicity. (Table 4). Thus, all the three cereal groups showed significant differences between pre and post consumption of cereals However, there was no significant difference in the relative cariogenicity between the unflavored and flavored cereals.

Table 4 – Inter-group comparison of Salivary pH levels and Colony forming units (CFU'S) of Streptococcus and Pseudomonas species at baseline and after 30 minutes of cereal consumption between three cereal groups

Parameter			Comparison between three groups
Salivary pH	At Baseline	F value	0.23
		p value	0.796
	After 30 minutes	F value	1.13
		p value	0.340
Colony forming units (CFU's) of Streptococcus	At Baseline	F value	1.25
		p value	0.305
	After 30 minutes	F value	0.77
		p value	0.472
Colony Forming Units (CFU's) of Pseudomonas species	At Baseline	F value	0.38
		p value	0.685
	After 30 minutes	F value	0.33
		p value	0.720

(*p value <0.05 statistically significant)

Discussion

Dental caries is a multifactorial and chronic bacterial disease that involves the destruction of tooth hard tissue structure. It is directly caused by the acid produced by oral bacteria fermentation of dietary carbohydrates in dental plaque. Several studies [9, 10] have assessed oral clearance, pH changes in the oral environment and carbohydrate content of food. The inherent properties of food that make it cariogenic have also been researched. Most of these studies have been conducted on dietary foods consumed by the western or European population. Information on the physical nature of foods that are available and consumed in India is lacking. Studies on cariogenicity of diet give importance to their sugar content. Retentive, sticky foods may be potentially more cariogenic than foods that are cleared rapidly from the oral cavity. Retention of food in the oral cavity for prolonged periods increases the potential of starch to break down into sugars. This may accentuate demineralization of teeth further increasing the risk of dental caries. Caldwell's comprehensive summary of physical, chemical, physiological, and anatomic factors involved in the retention and clearance of food from the mouth showed that the physical and chemical factors of food caused intraoral food retention and delayed clearance[9].

In our study, the relative cariogenicity of breakfast cereals was determined by using the salivary pH levels and the

colony forming units (CFU's) of streptococcus and pseudomonas species. The salivary pH levels showed a significant fall (acidic pH) before and after the consumption of the cereals. There was a marked difference in pH levels of all the three cereal groups, which was in consensus with the study conducted by Abdul *et al* (2014)[1], wherein the salivary pH levels had a maximum drop within 10-15 minutes after the consumption of different flavored cereals. Such similar results may be attributed to the fact that commercially available breakfast cereals were used in both the studies and a similar methodology was being followed.

Drummond BK *et al* (2002)[11], Pollard MA (1995)[12], Fosdick *et al* (1957)[13], Sonmez A and Aras S (2007)[14] and Papa M *et al* (2010)[15] reported similar results, wherein the salivary pH levels dropped down to the critical pH after consumption of different types of processed formulations like cheese, yoghurt and different canned fruit juices. A study conducted by Hegde *et al* (2009)[16] also reported similar reduced salivary pH levels after the consumption of filled and unfilled chocolates at different time intervals. Moreover, Chaudhary S *et al* (2011)[17] reported a lower salivary Our study showed that the CFU's of streptococcus species increased after the consumption of cereals. This was in consensus with the study conducted by Minton K and Berry C (1985)[3], wherein the streptococcus mutans level increased, when tested in-vitro in twelve different cereal groups. Moreover, the cariogenicity was found to be related to sugar content and most cereals exhibited an increased salivary retention time. These similar results may be attributed to the fact that there was homogeneous selection of pre-sweetened cereals in both the studies, with similar laboratory conditions.

As reported by Chaudhary S *et al* (2011)[17], there was a highly significant increase in the levels of colony-forming units of Streptococcus mutans after ingestion of different commercially available infant milk formulae. This was identical to the findings of our study. Moreover, similar results were reported by Thaweboon S *et al* (2007)[18], Osawa K *et al* (2001)[19], Petti S *et al* (2008)[20] and Marshall TA *et al* (2005)[21] indicating an increase in the number of salivary and plaque microorganisms including different strains of streptococci. This may be due to fact that the streptococcus mutans are acidogenic and aciduric in nature, and when present in the environment of fermentable carbohydrates exhibit their cariogenic ability, as is seen in the above studies conducted.

Dissimilar results were reported by Paula *et al* (2015)[22] wherein the colony count of S. mutans and Lactobacillus sp. was not statistically significant, when compared within the workers from the confectionaries and sugar industries. This may be due to the fact that there was no direct intervention in this study as compared to our study. The salivary pH levels and microbial counts checked in their study were analysed passively without giving any food formulations.

Our study inferred that the physical properties like texture, consistency, size etc., of the food formulations play a significant role in determining their cariogenic potential. Similarly, Gupta P *et al* (2013)[7], Neeraja G *et al* (2018)[10], Curzon ME and Hefferren JJ (2001)[23], Duggal MS and Loveren LC (2001)[24] and Frostell G (1970)[25] reviewed in their research that the role of sugar or other fermentable carbohydrates is majorly influenced by the granular structure of the food items consumed. Moreover, the overall physical properties of food items play a role in evaluating the relationship between food retention and initiation and progression of dental caries, hence selection of the appropriate diet in day-to-day life is important.

The present study concluded that the salivary buffer capacity was lowered and differed at various time intervals after the consumption of breakfast cereals. This pH value after the consumption of infant milk formulae, when observed at three different time intervals. This similarity may be due to the fact that the processed commercially available food formulations may be using simple sugars in their preparations, which may be rendering the pH acidic and making these food items more cariogenic was consistent with the findings reported by Chifor I *et al* (2014)[26] in an interventional study, wherein a negative correlation was found between the cariogenic food items and salivary buffer capacity. This may be attributed to the fact that the acidic environment in the oral cavity reduces the buffering activity of the saliva and increases the microbial count.

Conclusion

The results of our study can be extrapolated to other population within the country. As there are many misconceptions regarding the caries-prone or anti-caries potential of different food items, the study indicates the need of spreading awareness regarding the selection of appropriate food items in the diet and identifying the cariogenic potential of different food formulations available commercially in the market.

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