Original article

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Relationship between Caries status (caries & caries free group), Salivary flow rate, Buffering capacity, Salivary Mutans Streptococcus count and Sugar intake among 3-5 year old preschool children

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Abstract

Objective: The predictive value of a single diagnostic test on an individual can be misleading in disease of a multifactorial nature such as dental caries. Hence this study was undertaken to seek the relationship of salivary microbiologic parameters. (Mutans streptococcus count) and salivary physiochemical parameters (flow rate, buffering capacity) and sucrose intake with the occurrence of dental decay on the primary dentition among 3-5 years old of preschool children.

Materials and Methods: The present study comprised of 100 healthy children in the age group of 3-5 years, divided into two categories on the basis of their caries experience

Group 1:50 children with no detectable caries & Group 2:50 children with caries.

Children in caries group are categorized into Grade 1: 1 – 3 decayed teeth, Grade 2: 3 – 5,

Grade 3: > 5. A questionnaire was specifically designed which sought to discover the frequency of sugar consumption rather than the total amount of sugar eaten. The Stimulated saliva was collected to determine Flow rate, buffering capacity and Mutans streptococci count.

Results: There was a highly significant relation between frequency of sugar consumption, microbial parameter and the caries experience ($P < 0.001$) and an inverse relation between salivary parameters (salivary flow rate, buffering capacity) and the caries experience. ($P < 0.001$).

Conclusion: In conclusion, it is observed that there was a significant relation between caries experience and salivary flow rate, buffering capacity, salivary streptococcus mutans count, and sugar intake. This multifactorial approach of identifying high caries risk group children and implementation of preventive strategies represents an improvement over an approach that relied solely on single parameter.

Key words: Salivary flow; Buffering capacity; Dental caries; Streptococci mutans.

Introduction

In a period marked by brilliant achievements in the prevention and treatment of disease, dental caries still remains one of the most widespread afflictions in modern man. Dental caries is a common, complex, chronic disease that results from an imbalance between multiple potential etiological [risk] factors and multiple protective factors overtime. Ultimately this disease process can cause loss of tooth structure through demineralization or frank cavitations [cavities]. The disease progression can be stopped if the factors responsible are nullified in the initial stage. It is thus important to identify high caries risk markers as well as individuals to implement preventive and interceptive procedures. With regard to its multifactorial nature, in evaluating the caries risk of a patient a number of factors must be taken into consideration.

In the year 1912 *Miller* [1] recorded that the flow of saliva plays an important role in the pathogenesis of dental caries.He proved the fact that the individuals with the diminished flow of saliva developed severe, rapid

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spreading caries lesion. The relationship of flow rate to caries is further compounded where one considers the matter of buffering capacity of saliva. The salivary buffer capacity is an important host factor to maintain a suitable pH with its bicarbonate content in saliva. The micro flora involved in the dental caries activity is streptococcus mutans. It has a unique feature of producing extra cellular polysaccharides eg.dextran or glucan which increases the tenacity of plaque as well as limits the diffusion of acids which increases the acidity to bring about demineralization of tooth enamel. It has been accepted for many years, and almost without question since the Vipeholm study, that sugar consumption is a major risk factor for dental caries. [2]In developing countries an increase in the consumption of sugary foods or changes in eating habits have been associated with the dental caries.

Based on the above facts, it clearly indicates that, the predictive value of a single diagnostic test on an individual can be misleading in disease of a multifactorial nature such as dental caries. Hence this study was undertaken to seek the relationship of salivary microbiologic parameters. (Mutans streptococcus count) and salivary physiochemical parameters (flow rate, buffering capacity) and sucrose intake with the occurrence of dental decay on the primary dentition among 3-5 years old of preschool children. These parameters were taken as assays that could be used as risk indicators of dental caries.The 3-5 yrs age groups presented with their primary dentition could be of interest because controlling the level of s.mutans in the early age could be beneficial, so that the upcoming dentition would not be challenged by cariogenic ability of these organisms.

Materials and methods

The present study comprised of 100 healthy children in the age group of 3-5 years from local nursery schools in Chennai. All children were in the primary dentition stage. A proforma was prepared to record the data. The children with the history of antibiotic consumption within last 3 months or were receiving any other antimicrobial agents concurrent with this study period, fluoride use or living in fluoridated area, any prior dental treatment were not included in the study. This was possible to study the natural occurrence of dental decay that was unclouded by the effects of treatment and preventive measures.

Dental examination was carried out on a chair, under natural lighting conditions. The teeth were wiped with a 2x2 gauze piece. The diagnosis of dental caries was predominantly by the visual methods and this was augmented by the gentle use of explorer or Ash's number 54 probe to remove dental plaque and confirm the softness of caries lesions and the caries experienced was assessed in the entire mouth with the dft index. (Grubbel 1944). This value gave the caries status of the individual. Children were divided into two categories on the basis of their caries experience.

Group 1:50 children with no detectable caries.

Group 2:50 children with caries.

Children in caries group are categorized in to three grades depending on the number of decayed tooth. Grade 1: 1 – 3 decayed teeth, Grade 2: 3 – 5, Grade 3: > 5

Dietary Questionnaire

Parents were asked to attend with their child in the school and at that time they were questioned closely about the child's dietary habits. A questionnaire was specifically designed which sought to discover the frequency of sugar consumption rather than the total amount of sugar eaten.

Parents were asked what the child ate at meal times and between meals on weekdays and at weekends. From the completed questionnaire, it was possible to calculate the number of occasions that sugar was consumed with meals, snacks, sweets and drinks, To this figure, calculated for 5 week days, was added the number of occasions sugar was similarly consumed at week ends and the total for 1 week termed as "sugar index" for the child was determined. [3]

Collection of Salivary sample

The salivary samples were collected from the subjects on second visits. The tests were carried out using mid-morning (9 am-11 am) saliva samples. The investigations of *Weinberger et al (1990)* [4] showed that any food ingestion can alter the salivary physiochemical properties as well as microbial load. Therefore the salivary samples were collected at least 1.5 hrs-2hrs after the breakfast.

Salivary flow rate

The secretion of whole mixed saliva was measured after stimulation. Prior to stimulation the subjects were asked to eliminate any existing saliva in the mouth. Each child was given a sugarless chewing gum and was instructed to chew on both sides. Stimulated secretion was collected in sterile saliva collecting cups (Fig 1). Collection was tried for a period of 5 minutes. Since the composition of saliva depends on the duration of stimulation, the saliva collected during the first minute has a different composition from saliva collected after 5 minutes of constant stimulation.Then salivary flow rate was measured and was calculated per minute.[5]

Stimulated salivary flow rate expressed in ml/min			
	Hypo salivation	Low	Normal
Stimulated saliva	Grade 1 = < 0.7	Grade 2 = 0.7-1	Grade 3 = >1

Salivary buffering capacity (pH)

The estimation was carried out using a pH indicator paper that had been impregnated with acid. A small volume of saliva was taken with the pipette from the salivary samples and added to the strips. After 5 minutes the colour of the strips were compared with the colour coded chart which had numerical value. The colours had been chosen to indicate low, medium or good buffering capacity. [6]

pH	Inference
Grade 1 = =4	Low
Grade 2 = 4.5-5.5	Medium
Grade 3 = =6	High

Salivary microbiological tests

The collected samples were inoculated on Mitis salivarius bacitracin agar medium (MSB agar), which is a highly selective medium for S.mutans, for counting colony-forming units (C.F.U). The isolated plates of Mitis salivarius bacitracin agar was incubated at 37 °C in a candle jar environment for 48 hours. After 2 days the number of s.mutans were counted and recorded semi quantitatively as Grade 0 = '0' (no detectable cfu), Grade 1 = 1-9 cfu (Low), Grade 2 = 10-100 cfu (Medium) and Grade 3 = > 100 cfu (High) and the results were tabulated.The confirmation of s.mutans was done by smear examination (Fig 2) and biochemical tests.

Results

The analysis was done using chi-square test and the results from Table 1 indicated that 73.5% of the children who were caries free had < 32 frequency of sugar consumption. 62.5% of children with Grade 3 dft had > 32 frequency of sugar consumption. The children with increased frequency of sugar intake (>32) showed a rise in dft and children with decreased frequency of sugar intake (<32) were caries free (Graph-1) . There was a statistically significant relationship between frequency of sugar consumption and caries experience in the children corresponding to the p-value of 0.001 .The results from Table 2 showed 100% of children in Grade 3 dft had hyposalivation, 63% of children in Grade 3 dft had low flow rate, 70% of children who were caries free had normal flow rate. 63% of children in Grade 3 dft had medium buffering capacity and 68.5% of children who were caries free had high buffering capacity. The children with decreased flow rate and buffering capacity showed a rise in dft and the children who were caries free had normal flow rate and high buffering capacity (Graph-

2A,2B). Thus the relationship between salivary parameters and caries experience were statistically highly significant (p < 0.001). The results (Table 3) showed 100% of children who were caries free had no detectable mutans count, 69.6% of children in Grade 3 dft had highest mutans count (Grade 3). This suggested a direct relationship between mutans streptococci level in saliva and dft, with increase levels of mutans streptococci associated with increased dft and no mutans in caries free group (p < 0.001) (Graph-3).

Table 1: Relationship between Caries status and Sugar Index

Sugar Index in Grades	dft in Grades								Chi-Square Value	P-Value
	0		1		2		3			
	No.	%	No.	%	No.	%	No.	%		
1	50	73.5	15	22.1	3	4.4	0	0	88.97	<0.001***
2	0	0	0	0	12	37.5	20	62.5		

NS = Not significant
* = P < 0.05 significant at 5%
** = P < 0.01 significant at 1%
*** = P < 0.001 significant at 0.01%

Graph 1: Relationship between Caries status (Grade 0, 1, 2, 3) and Sugar Index (Grade 1,2)

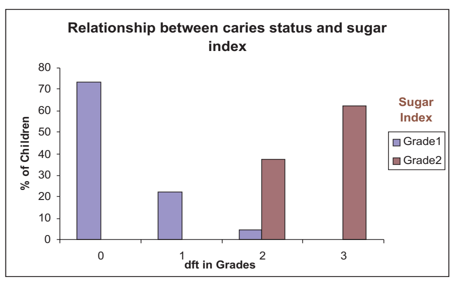
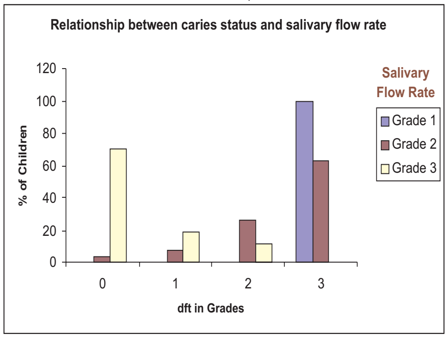


Table 2: Relationship between Caries status and Salivary Parameters

Variables in Grade		dft in Grades								Chi-Square Value	P-Value
		0		1		2		3			
		No.	%	No.	%	No.	%	No.	%		
Salivary Flow Rate	1	0	0	0	0	0	0	3	100.0	72.47	<0.001***
	2	1	3.7	2	7.4	7	25.9	17	63.0		
	3	49	70.0	13	18.6	8	11.4	0	0		
Salivary Buffering Capacity	1	0	0	0	0	0	0	0	0	59.33	<0.001***
	2	0	0	2	7.4	8	29.6	17	63.0		
	3	50	68.5	13	17.8	7	9.6	3	4.1		

NS = Not significant
* = P < 0.05 significant at 5%
** = P < 0.01 significant at 1%
*** = P < 0.001 significant at 0.01%

Graph 2A: Relationship between Caries status (Grade 0,1,2,3) and Salivary Flow Rate (Grade 1,2,3)



Graph 2B: Relationship between Caries status (Grade 0,1,2,3) and Salivary Buffering Capacity (Grade 1,2,3)

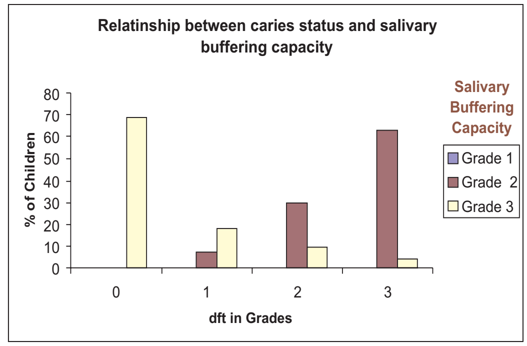


Table 3: Relationship between Caries status and Salivary Mutans Streptococcus Count

Salivary mutans Streptococcus counts in Grade	dft in Grades								Chi-Square Value	P-Value
	0		1		2		3			
	No.	%	No.	%	No.	%	No.	%		
0	16	100.0	0	0	0	0	0	0	112.88	<0.001***
1	34	82.9	7	17.1	0	0	0	0		
2	0	0	8	40.0	8	40.0	4	20.0		
3	0	0	0	0	7	30.4	16	69.6		

NS = Not significant

* = P < 0.05 significant at 5%

** = P < 0.01 significant at 1%

*** = P < 0.001 significant at 0.01%

Graph 3: Relationship between Caries status (Grade 0,1,2,3) and Salivary Mutans Streptococcus Count (Grade 0,1,2,3)

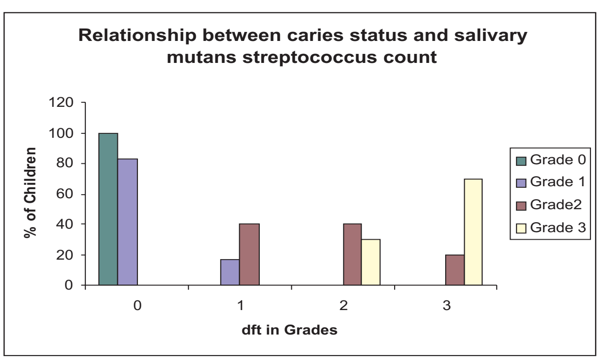
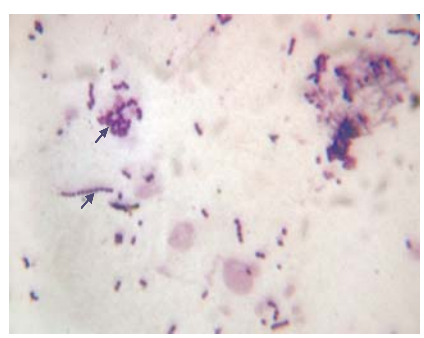


Fig 1: Determination of Salivary flow rate & buffering capacity

Fig 2: Microscopic view of mutans streptococcus



Discussion

The earlier model to represent the key factors involved in dental caries was put forth by *Fitzgerald and Keyes*. Recently, the concept of the caries balance was first published by Featherstone in 1999 [7] in an attempt to simplify the key factors involved in dental caries progression or reversal and make them readily applicable in clinical practice and easily understandable for the patient. The three pathological factors are cariogenic bacteria, fermentable carbohydrates and saliva dysfunction and the protective factors are saliva components and salivary flow, fluoride and antibacterial therapy. As per the concept of Featherstone, the caries experience tend to be related to microbial, non-microbial salivary parameters [5] and frequency of sugar intake .The current study focused on the relation of these variables with the caries experience among 3 – 5 year old preschool children. As this age group presented with their primary dentition could be of interest, so as to control the level of cariogenic organisms at this early age, so that the upcoming dentition would not be challenged by the cariogenic ability of these organisms.

Culturing plaque samples from discrete sites, such as occlusal fissures or proximal area, is an ideal method for the purpose of detecting and quantitating the mutans streptococcus that have colonized on teeth. However, it is often not practical to sample a large number of dental sites, hence the use of salivary sample to provide a workable alternative to assaying mutans streptococcus. The stimulated salivary samples were collected to determine the flow rate, buffering capacity and mutans count. The caries process occurs during and immediately following ingestion of cariogenic foods. It is precisely during this period, that the saliva is stimulated. This would suggest that stimulated rather than resting saliva should be studied. [8]

The children with increased frequency of sugar intake (>32) showed a rise in dft and children with decreased frequency of sugar intake (<32) were caries free. As mentioned in the other studies by Holbrook et al 1989 [3], Akapta et al

1992 [9], Mazengo et al 1996 [10], there was a statistically significant relationship between frequency of sugar consumption and caries experience ($p < 0.001$). The etiologic role of diet, particularly that of fermentable carbohydrates, in dental caries is well accepted, Perhaps the most conclusive proof was provided by the now classical Vipeholm Study (1946 – 1950). The frequencies with which cariogenic foods are eaten are more important. The more frequently, the more cariogenic they become.

The children with decreased flow rate and buffering capacity showed a rise in dft and the children who were caries free had normal flow rate and high buffering capacity. The results of the present study correlated with the other studies that have examined the relationship of salivary parameters on caries experience and the inverse relation between salivary parameters and caries experience. (Dan Ericsson et al 198[11], Purohit et al 1996 [12]). The saliva has a number of various functions; obviously the most important one is the clearance of oral micro-organisms and food components from the mouth to the gut. This balance can be disturbed either by extensive growth of bacteria as a consequence of for example, poor oral hygiene, abundant use of fermentable carbohydrates or some systemic diseases or by reduced salivary flow rate which in turn results in increase caries attack. The flow of the whole saliva is of clinical relevance for the susceptibility and activity of dental caries. [5]

The relationship between mutans streptococci and caries experience had been reported for several population of preschool children. Typically, the mean dft and dfs have been shown to increase with the increasing levels of mutans streptococci. (Chosack et al 1988 [13], Holbrook et al 1989 [3], Alalussua et al 1989 [14], Weinberger et al 1989 [4]). The amount of mutans streptococci in saliva is related to number of colonized surfaces on the teeth. The highest mutans streptococci levels may predict high-risk children at an early age. Alaluusua and Renken 1982 [14], Kohler and colleagues 1988 [15], Fujiwara et al 1991 [16], Roeters et al 1995 [17], clearly demonstrated that early infection with mutans streptococci is a significant risk factor for future development of dental caries.

So from this study it is observed that there was a significant relation between caries experience and salivary flow rate, buffering capacity, salivary streptococcus mutans count, and sugar intake. This multifactorial approach of identifying high caries risk group children and implementation of preventive strategies represents an improvement over an approach that relied solely on single parameter.

Conclusion

The following conclusions were drawn from the study:

1. There was a highly significant relation between frequency of sugar consumption and caries experience. ($P < 0.001$)
2. There was an inverse relation between salivary parameters (salivary flow rate, buffering capacity) and the caries experience. ($P < 0.001$)
3. There was a direct relation between microbial parameter (salivary mutans streptococcus count) and the caries experience. ($P < 0.001$)

The present study demonstrated the importance of considering sugar intake, microbial and non microbial salivary parameters when assessing the caries activity in children. Although strong evidence exists that these parameters affect the caries process, on an individual or population basis they offer only little predictive value. Therefore future research should focus on exploring combinations, or clusters of various parameters which are typical for caries active and inactive individuals.Hence future research should focus on the other reliable parameters that can be incorporated to predict the caries status of the individual.

References

1. Miller W.D.A study of certain questions relating to pathology of teeth. *D Cosmos* 1904; 46: 981-1001.
2. Szpunar S.M, Eklund S.A, Burt B.A. Sugar consumption and caries risk in schoolchildren with low caries experience. *Community Dent Oral Epidemiol* 1995; 23: 142-146.
3. Holbrook W.P, Kristinsson M.J, Steinunn Gunnarsdottir.Caries prevalence, Streptococcus mutans and sugar intake among 4-year-old urban children in Iceland. *Community Dent Oral Epidemiol* 1989; 17: 292-295.
4. Weinberger S.J. Wright G.Z. Variables influencing Streptococcus mutans testing. *Pediatric Dentistry* 1990; 12(5): 312-315.
5. Tenovuo J. Salivary parameters of relevance for assessing caries activity in individuals and populations. *Community Dent Oral Epidemiol* 1997; 25: 82-86.
6. Helena Tukia,Kilmala,Tarma Tenorono.Intra and interindividual variation in salivary flow rate, buffer effect, lactobacilli and mutans streptococci among 11-12 yr old school children.*Acta Odontol Scand* 1993; 51: 31-37.
7. Featherstone J.D.B.Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiol* 1999; 27: 31-40.
8. Guggenheim.*Cariology today in Guggenheim* ed: Denmark; Aio Print as, Odense, 1994; 333-350.
9. Akpata E.S, Shammery A.R., Saeed H.I. Dental caries, sugar consumption and restorative dental care in 12-13-year-old children in Riyadh, Saudi Arabia. *Community Dent Oral Epidemiol* 1992; 20: 343-346.
10. Mazengo C.M, Tenovuo J, Hausen H. Dental caries in relation to diet, saliva and cariogenic microorganisms in Tanzanians of selected age groups. *Community Dent Oral Epidemiol* 1996; 24: 169-174.
11. Dan Ericson, Bratthall.D.Simplified method to estimate salivary buffer capacity. *Scand J Dent Res* 1989; 97: 405-407.
12. Purohit V.D, Damle S.G. Salivary counts of mutans streptococci, lactobacilli, flow rate and buffering capacity in caries free and caries active children. *J Indian Soc Pedo Prev Dent* 1996; 14 (4): 97-106.
13. Chosack A, Cleaton-Jones P, Woods A.Caries prevalence and serevity in the primary dentition

and St.Mutans in the saliva of preschool children in South Africa. *Community Dent Oral Epidemiol* 1988; 16: 289-291.

14. Alaluusua S, Myllarniemi S, Kallio. M. Streptococcus mutans Infection Level and Caries in a Group of 5-Year-Old Children. *Caries Research* 1989; 23: 190-194.
15. Kobler B, Bratthall D.Practical method to facilitate estimation of streptococcus mutans level in the saliva. *J Clin Microbial* 1979; 9: 584-588.
16. Fujiwara T, Sasada E, Mima N, Ooshima T. Caries prevalence and salivary mutans streptococci in 0-2-year-old children of Japan. *Community Dent Oral Epidemiol* 1991; 19: 151-154.
17. Roeters R.J.M.Lactobacilli, mutans streptococci, and dental caries: a longitudinal study in 2 – years – old children up to the age 5 years. *Caries Res* 1995; 29: 272-279.