Original Article

Dental caries experience and salivary *Streptococcus mutans*, lactobacilli scores, salivary flow rate, and salivary buffering capacity among 6-year-old Indian school children

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Abstract

Context: Dental caries is a disease of multifactorial etiology. A variety of potential predictors have been examined for the association with caries increments in longitudinal and cross-sectional studies. **Aims:** The aim of this study was to assess the possible relationship among salivary cariogenic microflora, buffer capacity, secretion rate, and caries experience among 6-year-old school-going children in Davangere city, India. **Settings and Design:** A total of 196 6-year-old school children were selected by a two-stage random sampling method. **Materials and Methods:** Parents were interrogated regarding sociodemographic details. Clinical examination of children was conducted to assess dental caries experience, and stimulated saliva was collected to assess *S. mutans* levels, lactobacilli, salivary flow, and buffering capacity of saliva. **Statistical Analysis:** The difference in proportions was tested using Kruskal–Wallis analysis of variance (ANOVA) followed by the Mann–Whitney *U*-test for intragroup comparison, and the difference in mean was tested using ANOVA and independent sample *t*-test as necessary. Caries experience was correlated with salivary factors using Spearman's correlation coefficient. **Results:** Out of 196 children, 96 were boys and 100 were girls. Overall, 97 (49.49%) children were caries free (dmft, DMFT = 0) and 99 (50.51%) children presented with caries (dmft, DMFT>0). The mean dmft and dmfs score for the overall group was 3.20 and 5.43, respectively. The mean DMFT and DMFS score was 0.23 and 0.25, respectively. A highly significant correlation was seen between mean the caries score and salivary variables. **Conclusions:** High levels of salivary microbiological counts in correlation with the caries data stress the importance of these factors and urge the necessity of elective preventive programs in this region.

Key words: *Colony-forming unit, dental caries experience, lactobacillus, salivary buffering capacity, salivary flow, Streptococcus mutans*

INTRODUCTION

Dental caries is a localized and transmissible pathological infectious process that results in the destruction of hard dental tissues.[1] It is one of the main oral health problems in both industrialized and increasing in developing countries, and it affects 60–90% of school-going children, and adults.[2] In India, children comprise 40% of a rapidly growing population. The prevalence of dental caries varies from 33.7% to 90% in the child population and is increasing at an alarming rate.[3,4] A variety of potential predictors have been examined for an association with caries increments in longitudinal and cross-sectional studies, including initial or baseline caries prevalence scores for permanent and primary teeth, characteristics of occlusal morphology, levels of *Streptococcus mutans* and

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	PMID: ***
	DOI: 10.4103/2231-0762.97697

lactobacilli, salivary buffering capacity, dietary factors, sex, race, and socioeconomic status.[3-12]

The importance of *S. mutans*, lactobacilli, and saliva in the development of dental caries has been reviewed extensively through many cross-sectional studies.[3-8] The studies have showed that the subjects with active caries tend to harbor a higher number of mutans streptococci and lactobacilli in their saliva and plaque than those who are caries free. Moreover, longitudinal studies have demonstrated that there is an increase overtime in the number of *S. mutans* and lactobacilli associated with the onset and progression of caries.[9-13]

Many attempts have been made recently to identify those children who are more susceptible to caries development so as to implement specific prevention programs for them.

This study is part of an ongoing longitudinal study where we have tried to evaluate the possible relationship among salivary cariogenic microflora, buffer capacity, secretion rate, and caries experience among 6-year-old school-going children in Davangere city, Karnataka, India. More specifically, we have tried to find an answer to the following questions:

(1) Is there an association between caries experience and different levels of *S. mutans* in saliva?
(2) Is there an association between caries experience and different levels of lactobacilli in saliva?
(3) Is there an association between caries experience and buffering capacity of saliva?
(4) Is there an association between caries experience and salivary flow rates?

MATERIALS AND METHODS

Davangere is the district headquarter of Davangere district, divided into North and South zones. The total number of public schools was 61 and private schools were 105 in number (2007–2008 data). Approximately, 10% of schools (6 public and 10 private schools) were selected by a lottery method to meet the sample size of 196. A two-stage random sampling method was followed with a school as the primary sampling unit and individual child the unit of enquiry. Children were selected proportionate to the number of children in each school by systematic random sampling. All required and relevant information about the number and location of schools in Davangere was obtained from the office of DDPI, Davangere.

Ethical clearance was taken from the ethical committee of Government Dental College, Bangalore, Karnataka, India. Necessary permission was taken from school authorities and the written informed consent was taken from the parents before the start of the study.

Inclusion criteria

- Children who were permanent residents of Davangere
- Children with informed consent from the parent/guardian.

Exclusion criteria

- Children who were severely ill
- Children having difficulty in opening the mouth
- Children who had taken antibiotics in the last month
- Children with orthodontic appliances.

The study was carried out using a specific pretested pro forma, which consisted of two parts.

First part consisted of general information of school children regarding age and sex and the second part consisted of clinical oral examination. The oral examination of school children was carried out under natural light using a plane mouth mirror, and WHO probe. The sterilization of instruments was done by autoclaving. No radiographs were used. The same examiner examined all children.

The intra-examiner calibration was performed with respect to the diagnostic criteria of caries. A significant correlation was found with a kappa value of 0.96, $P < 0.05$, for dental caries.

Caries status was determined by DMFS, DMFT, dmft, and dmfs (WHO Dentition Status and Treatment Need – Basic Oral Health Survey 1997 Pro Forma).[14]

LABORATORY PROCEDURES FOR ASSESSING SALIVARY SAMPLES FOR *S. MUTANS*, LACTOBACILLI, SALIVARY BUFFERING CAPACITY, AND SALIVARY FLOW

Stimulated saliva was collected from each subject and microbiological assay and salivary tests commenced within 24 h of saliva collection.

Method of saliva collection

Saliva collection was scheduled after the clinical

examination. Two students at a time were made to sit comfortably on the chair. Children were made to swallow the preexisting saliva, in order to clear the mouth of any residual unstimulated saliva. After this, each student was asked to chew a standard piece of paraffin wax, for 5 min and the stimulated saliva was collected. The saliva samples of all the participants were identified by a code number during the period of sample collection and processing.

Salivary flow rate

Once the participant could chew comfortably on the wax, he or she was asked to expectorate all saliva, formed over a 5-min period, into the graduated sterile cylinder. After the disappearance of the salivary froth, the secretion rate was estimated in milliliters per minute:
Salivary secretion: 0, >0.7 ml/min; 1, 0.3–0.7 ml/min; 2, <0.3 ml/min.

Buffering capacity of saliva

To estimate the buffering capacity, 3 ml of the 0.005 mol/l HCl solution was added to 1 ml of saliva. The sample was shaken and the stopper was then removed to eliminate carbon dioxide. The sample was allowed to stand for 10 min and the final pH was measured with the commercially purchased Hindicorm pH paper, which had a predetermined pH range, and categorized accordingly (pH range 3.5–6, 6.5–9): Salivary buffering capacity: 0, pH > 6.0; 1, pH 4.5–5.5; 2, pH < 4.0.

MICROBIOLOGICAL PROCEDURE

The sample was transported to the laboratory immediately after collection using the thioglycollate broth and processed on the same day. The sample was vortexed (15 s) and diluted 1:1000 in an isotonic saline solution prior to inoculation. One loop (1/1000th ml of sample) was inoculated on the Mitis Salivarius agar with the potassium tellurite medium, bacitracin, and 20% sucrose. The plates were incubated at 37°C anaerobically. For lactobacilli, one loop of the diluted sample was inoculated on Rogosa SL agar plates and incubated aerobically for 72 h. After 72 h, colony characteristics were studied and the number of colony-forming units of SM (CFU/ml) and LBS (CFU/ml) of saliva was determined using a colony counter.

Statistical analysis

The Statistical Package for Social Science (SPSS), version 17, was used for analysis. The difference in proportions was tested using Kruskal–Wallis analysis of variance (ANOVA) followed by the Mann–Whitney *U*-test for intragroup comparison, and the difference in means was tested using ANOVA followed by Tukey's post hoc test and independent sample *t*-test as necessary. Dental caries experience was correlated with salivary factors using Spearman's correlation coefficient. The level of statistical significance was assumed at $P < 0.05$.

RESULTS

Caries prevalence

The total number of study participants was 196 out of which 96 (48.98%) were boys and 100 (51.02%) were girls. Overall, 97 (49.49%) children were caries free (dft, DMFT = 0) and 99 (50.51%) children presented with caries (dft, DMFT>0), out of whom 42 (42.42%) were boys and 57 (57.58%) were girls, and there was no significant diffrence between the two groups ($P = 0.086$, two-sided). The mean dmft and dmfs score for the overall group was 3.20 and 5.43, respectively. The mean DMFT and DMFS score was 0.23 and 0.25, respectively. Table 1 shows mean dmft and dmfs scores according to sex; a statistically significant difference (independent sample *t*-test; $P < 0.05$) was found between mean ft and mean fs scores between sexes.

Table 1: Distribution of study participants with mean dt, ft, mt, dmft, ds, fs, ms, and dmfs according to sex

Sex		dmft	dt	ft	mt	ds	fs	ms	dmfs
Boys	Mean	3.11	3.03	0.08	0.00	5.25	0.23	0.00	5.48
	N	96	96	96	96	96	96	96	96
	Std. deviation	4.477	4.404	0.427	0.000	8.822	1.326	0.000	9.023
Girls	Mean	3.28	3.32	0.02	0.00	5.35	0.04	0.00	5.39
	N	100	100	100	100	100	100	100	100
	Std. deviation	4.048	4.027	0.141	0.000	8.349	0.281	0.000	8.441
Total	Mean	3.20	3.18	0.05	0.00	5.30	0.13	0.00	5.43
	N	196	196	196	196	196	196	196	196
	Std. deviation	4.253	4.208	0.316	0.000	8.562	0.952	0.000	8.708

Table 2: Distribution of study participants according to salivary variables and sex

Sex	Salivary flow rate (ml/min)		Salivary buffering capacity			Salivary *S. mutans* level (CFU/ml)				Salivary lactobacilli level (CFU/ml)			
	0 (>0.7)	1 (0.3–0.7)	0 (>6)	1 (4.5–5.5)	2 (<4)	0 (negligible)	1 <104	2 104–105	3 >105	0 <103	1 104	2 105	3 >106
Boys (n)	86	10	74	21	1	1	24	47	24	15	35	34	12
Percentage	89.58	10.42	77.08	21.87	1.04	1.04	25	48.96	25	15.62	36.46	31.42	12.5
Girls (n)	89	11	73	25	2	2	19	45	34	10	32	40	18
Percentage	89	11	73	25	2	2	19	45	34	10	32	40	18
Total (n)	175	21	147	46	3	3	43	92	58	25	67	74	30
Percentage	89.29	10.71	75	23.47	1.53	1.53	21.94	46.94	29.59	12.75	34.18	37.75	15.31

Table 2 shows the distribution of study participants according to salivary factors.

Salivary secretion rates

The secretion rate was between 0.4 and 1.5 ml/min in the examined sample with a mean flow rate of 0.96 ± 0.23 ml/min. Majority (89.29%) of study participants presented with a salivary flow rate score of 0 (>0.7 ml/min; Table 2).

Salivary buffering capacity

The analysis of the buffer capacity of saliva showed that the majority (75%) of participants presented with a buffer capacity of >6 pH, followed by 23.47% with a buffer score of 1 (pH 4.5–5.5) and 1.53% with a buffer score of 2 (pH < 4.5; Table 2).

Salivary *S. mutans*

A total of 98.47% participants carried detectable salivary levels of *S. mutans*; 46.94% of these had a *S. mutans* score of 2 (10^4-10^5 CFU/ml) and 29.59% a score of 3 (>10^5 CFU/ml; Table 2).

Salivary lactobacilli

A total of 87.24% participants carried detectable salivary levels of lactobacilli; 37.75% of these had a score of 2 (10^5 CFU/ml) and 34.18% a score of 1 (10^4 CFU/ml; Table 2).

Caries status according to salivary variables

A total of 78 (78.79%) children with caries had a normal salivary flow rate (≥0.7 ml/min). A total of 51 (51.51%) children with caries had a salivary buffering capacity of pH > 6 and the difference was statistically significant. Majority (49.49%) of children with caries presented with a lactobacillus score of 2 and the difference was statistically significant. Majority (48.48%) of children with caries had a *S. mutans* level score of 3 and the difference was statistically significant [Table 3].

Mean caries score and salivary parameters

A statistically significant difference was seen between *S. mutans* scores and mean DMFT, dmft and dmfs scores. A statistically significant difference was seen between mean dmft and dmfs scores and the lactobacillus level. A statistically significant difference was also seen between mean DMFT, DMFS, dmft, and dmfs scores and salivary buffering capacity and salivary flow rate [Table 4].

Correlations among caries prevalence and salivary factors

Table 5 shows the correlation coefficients of mean dmft, dmfs, DMFT, and DMFS values with salivary findings in the total sample. All the variables showed a highly significant ($P<0.01$) correlation with both dmft and DMFT values.

DISCUSSION

This study is part of an ongoing longitudinal study, with an aim to assess the possible relationship among salivary cariogenic microflora, buffer capacity, secretion rate, and caries experience among 6-year-old school-going children. The results showed that out of 196 study participants, 99 (50.51%) children presented with caries (dft, DMFT>0), out of whom 42 (42.42%) were boys and 57 (57.58%) were girls and there was no significant difference between the two groups. The mean dmft and dmfs score was 3.23 and 5.43, respectively, and the mean DMFT and DMFS score was 0.23 and 0.25, respectively. The study conducted by Gamboa *et al*. showed an overall caries prevalence of 66% (35/53) among 3- to 5-year-old children in Columbia, which was slightly higher compared to present study results.[15] The study conducted by Alaluusua among 5-year-old children in Helsinki showed that 38.25% of children presented with caries with a mean dmfs score

Table 3: Distribution of study participants according to salivary variables and caries status

Caries status	Salivary flow rate (ml/min)		Salivary buffering capacity			Salivary *S. mutans* level (CFU/ml)				Salivary lactobacilli level (CFU/ml)			
	0 (>0.7)	1 (0.3–0.7)	0 (>6)	1 (4.5–5.5)	2 (<4)	0 (negligible)	1 <104	2 104–105	3 > 105	0 < 103	1 104	2 105	3 > 106
na	97	0	96	1	0	2	37	48	10	20	49	25	3
Percentage	100		98.97	1.03		2.06	38.14	49.48	10.31	20.62	50.51	25.77	3.09
nb	78	21	51	45	3	1	6	44	48	5	18	49	27
Percentage	78.79	21.21	51.51	45.45	3.03	1.01	6.06	44.44	48.48	5.05	18.18	49.49	27.27
Kruskal–Wallis H	$P < 0.001$		$\chi^2 = 58.548$, $P < 0.001$			$\chi^2 = 47.494$, $P < 0.001$				$\chi^2 = 50.055$, $P < 0.001$			
Mann–Whitney U	819.000		1, 2>0			3>2>1				2>0, 1 and 3>0, 1			

[a]caries free, number of individuals divided by the total number, 97 (dft, DFT, DFS = 0), [b]With caries, number of individuals divided by the total number, 99 (dft, DFT, DFS>0).

Table 4: Distribution of study participants with mean dmft, dmfs, DMFT, and DMFS scores according to salivary variables

Salivary variables		dmft	dmfs	DMFT	DMFS
Salivary flow rate (ml/min)	0 (>0.7) n = 175	2.34 ± 3.425	3.51 ± 6.017	0.19 ± 0.632	0.21 ± 0.724
	1 (0.3–0.7) n = 21	10.33 ± 3.799	21.43 ± 11.116	0.57 ± 1.076	0.57 ± 1.076
Independent sample t-test		$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.05$
Salivary buffer capacity in pH	0 (pH > 6) n = 147	1.60 ± 2.79	2.12 ± 3.97	0.15 ± 0.58	0.17 ± 0.70
	1 (pH 4.5–5.5) n = 46	7.72 ± 4.26	14.20 ± 10.16	0.43 ± 0.91	0.43 ± 0.91
ANOVA	2 (pH < 4) n = 3	3.20 ± 4.25	5.43 ± 8.70	0.23 ± 0.69	0.25 ± 0.77
	F	77.046	98.728	7.101	5.256
	P	<0.001	<0.001	0.001	<0.05
	Tukey's post hoc test	2>1>0	2>1>0	2>0, 1>0	2>0
Salivary *S. mutans* (CFU/ml) of saliva	0 (negligible) n = 3	0.67 ± 1.15	0.67 ± 1.15	00	00
ANOVA	1 (<104) n = 43	0.74 ± 2.07	1.14 ± 3.24	0.05 ± 0.30	0.05 ± 0.30
	2 (104–105] n = 92	2.24 ± 3.05	3.14 ± 4.71	0.18 ± 0.61	0.21 ± 0.73
	3 (>105) n = 58	6.67 ± 5.01	12.50 ± 11.90	0.47 ± 0.95	0.48 ± 1.01
	F	27.603	25.731	3.553	3.034
	P	<0.001	<0.001	<0.05	<0.05
	Tukey's post hoc test	3>0, 1, 2	3>0, 1, 2	3>1	3>1
Salivary lactobacillus (CFU/ml) of saliva	0 (<103) n = 25	0.88 ± 2.02	1.16 ± 2.64	0.16 ± 0.62	0.16 ± 0.62
	1 (104) n = 67	1.36 ± 2.56	1.94 ± 4.07	0.09 ± 0.45	0.12 ± 0.66
	2 (105) n = 74	3.95 ± 4.05	5.92 ± 6.63	0.28 ± 0.76	0.30 ± 0.82
	3 (>106) n = 30	7.40 ± 5.44	15.60 ± 14.28	0.50 ± 0.93	0.50 ± 0.93
ANOVA	F	22.991	27.043	2.691	1.911
	P	<0.001	<0.001	<0.05	NS
	Tukey's post hoc test	3>2>0, 3>2>1	3>2>0, 3>2>1	3>1	NS

Table 5: Correlations of dmft, dmfs, DMFT, and DMFS with salivary findings

Variable	Correlation coefficients			
	dmft	dmfs	DMFT	DMFS
Salivary flow rate	0. 489[a]	0.513[a]	0.167[b]	0.166[b]
Salivary buffering capacity	0.645[a]	0.670[a]	0.286[a]	0.285[a]
Salivary *S. mutans* level	0.536[a]	0.539[a]	0.243[a]	0.243[a]
Salivary lactobacilli	0.515[a]	0.525[a]	0.211[a]	0.211[a]

[a]Correlation is significant at the 0.01 level, [b]Correlation is significant at the 0.05 level

of 2.2 for the overall group, which was lower compared to present study results.[16] In the present study, a total of 98.47% participants carried detectable salivary levels of *S. mutans*; 46.94% of these had a *S. mutans* score of 2 (10^4–10^5 CFU/ml) and 29.59% had a score of 3 (>10^5 CFU/ml). A total of 87.24% participants carried detectable salivary levels of lactobacilli and 37.75% of these had a score of 2 (10^5 CFU/ml; Table 2). A statistically significant difference was seen between the mean caries score and the salivary *S. mutans* count and lactobacilli [Table 4]. In contrast to this, the study conducted by Gamboa *et al*. showed that only 62% of study participants presented with positive salivary *S. mutans* and there was no statistically significant difference between the *S. mutans* count and dental caries experience.[15] The study conducted by Alaluusua *et al*. showed that 54% of the saliva samples were not detected with *S. mutans*, and only 6% of children showed a high salivary level of *S. mutans* and a statistically significant difference was seen between salivary *S. mutans* levels and mean caries scores.[16] The study conducted by Bretz *et al*. among 3- to 6-year-old children from Brazil showed that 70% and 46% of the children had moderate-to-high salivary levels of mutans streptococci and lactobacilli, respectively, and a high correlation was seen between salivary levels of *S. mutans* and lactobacilli and dental caries prevalence.[17] This results tallies with the present study result, which showed a highly significant correlation between salivary microflora and the mean caries score [Table 5].

A lot of research has been done to find out and prove the influence of salivary *S. mutans* and lactobacilli on caries experience on the child populations.[3-8,18-20] Our results also showed a statistically significant relation between different levels of *S. mutans* and lactobacilli and caries experience [Tables 3 and 4]. The study conducted by Campus *et al*. showed similar results.[8] Although high levels of *S. mutans* and lactobacilli indicate a cariogenic environment, many authors[21,22] believe that this may be partly due to different microbiological sampling methods (stimulated or nonstimulated saliva, plaque, or total saliva). In addition, the type of research study may also influence the correlation between streptococcus levels and disease. However, the study in 2002 by Dasanayake *et al*., using the "Dentocult MS" set, among 2–17 year old Sri Lankan children and the study conducted by Gudkina and Brinkmane among 6 and 12 year olds in Riga found no positive correlation between caries and the salivary *S. mutans* level.[23,24]

The present study showed that the salivary secretion rate for the overall sample was 0.4–1.5 ml/min with a mean flow rate of 0.96 ± 0.23 ml/min. The analysis of the buffer capacity of saliva showed that the majority (75%) of participants presented with a buffer capacity of >6 pH, followed by 23.47% with a buffer score of 1 (pH 4.5–5.5; Table 2). A highly significant (P<0.01) correlation was seen between the mean caries score and the salivary buffer capacity and flow rate [Table 5]. This result is in contrast to the study conducted previously[25,26], and which showed no significant difference between the salivary pH and salivary flow rate and the mean caries score.

Comparison of all results with other studies was not possible, as disparity between results exists. This could be attributed to the difference in dmft values, dietary patterns, oral hygiene practices, genetic factors, and several other factors peculiar to the study population. Also, distinctness in the technique of sampling saliva and cultivation of bacteria can contribute to the variation.

To conclude, high levels of salivary microbiological counts in correlation with the caries data stress the importance of these factors and urge the necessity of elective preventive programs in this region, and this study can serve as a basis to develop a caries prediction model using other variables such as oral hygiene status, dietary factors, sociodemographic details, etc., in the future.

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How to cite this article: Sakeenabi B, Hiremath SS. Dental caries experience and salivary Streptococcus mutans, lactobacilli scores, salivary flow rate, and salivary buffering capacity among 6-year-old Indian school children. J Int Soc Prevent Communit Dent 2011;1:45-51.

Source of Support: Nil, **Conflict of Interest:** None declared.