ORIGINAL RESEARCH ARTICLE

'INDIVIDUAL VARIATION OF SALIVARY FACTORS AND ITS VARIATION IN RELATION TO DENTAL CARIES EXPERIENCE AMONG TWELVE YEAR OLD RESIDENTIAL SCHOOL CHILDREN IN DAVANGERE CITY'- A SIX MONTHS FOLLOW UP STUDY

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ABSTRACT

Background: Salivary counts of Mutans Streptococci and Lactobacilli, combined with the measurement of salivary flow rate and buffer effect are frequently used for diagnostic and predictive purposes in cariology. The intra and inter individual variation is particularly important at the time of tooth emergence, which allows new tooth surfaces to become colonized. Thus there is a need of combination of diagnostic tests to target high risk caries groups. The purpose of the present study was to evaluate the variations in salivary factors (salivary flow rate, buffering capacity, Mutans streptococci counts and Lactobacilli counts) among children with different caries experience, to evaluate individual variations of salivary factors at different time intervals.

Methodology: A six month follow up study was conducted among ninety school children aged between 12 and 13 years, from a residential school in Davangere city. The study subjects were grouped according to their caries experience. Resting saliva was collected from the study subjects. Salivary flow rate was expressed in ml / min. Salivary buffering capacity was assessed using Ericsson's method modified for smaller volumes. Mutans Streptococci was quantified in the laboratory by culturing on Mitis Salivarius Bacitracin agar and Lactobacilli by culturing on Rogosa SL agar. Follow up samples were taken at 6, 12 and 24 weeks. Statistical analysis was done using Kruskal Wallis and Mann Whitney U tests.

Results: All the salivary factors studied showed significant relationship with caries experience. No significant variation was observed over a period of 24 weeks.

Conclusion: The study indicated that the salivary variables studied are a valuable diagnostic tool to assess caries experience and predict caries risk.

Key words: Caries; caries experience; flow rate; buffering capacity; Mutans streptococci; Lactobacilli.

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INTRODUCTION

Dental caries is a microbial disease of the calcified tissues of the teeth, characterized by demineralization of inorganic portion and destruction of organic substances of the tooth leading to cavity formation. Dental caries is a disease with a multifactorial aetiology, as it is due to the interaction of various factors: diet, the host's susceptibility and the presence of microorganisms over a certain length of time.

Among the oral diseases, dental caries is the most common chronic disease of mankind. It affects persons of both sexes in all races, all socio-economic strata and every age group. As children reach school age, they will have an increasing incidence of carious lesions because of change in dietary habits which includes refined carbohydrates and sweeteners. It is also profoundly affected by other factors like oral hygiene and saliva.[1]

The factors related to the development of dental caries are extremely relevant in the disease process. The microorganisms with cariogenic capacity do not determine the presence of dental caries. It is necessary to have suitable substrates and physiological conditions in the host to allow implantation and survival of these microorganisms in order to facilitate the development of caries. That is why caries is considered to be a multifactorial disease.[2]

Due to the multifactorial and complex etiology, unfortunately there is no single test available that can fully explain or predict the disease. [3] Salivary counts of Mutans streptococci and Lactobacilli, combined with the measurement of salivary flow rate and buffer effect are frequently used for diagnostic and predictive purposes in cariology. The variability of these tests on an individual level is not well documented. For the purpose of predicting and diagnosing dental caries, the intra and inter individual variation is particularly important at the time of tooth emergence, which allows new tooth surfaces to become colonized.[4]

Thus there is a need of combination of diagnostic tests to target high risk caries groups. The purpose of the present study was to evaluate the variations in salivary factors (salivary flow rate, buffering capacity, Mutans streptococci counts and Lactobacilli counts) among children with different caries experience, to evaluate individual variations of salivary factors at different time intervals.

METHODOLOGY

The present study is a six month follow up study conducted among ninety school children aged between 12 and 13 years, from a residential school in Davangere city, to evaluate the intra and inter individual variation in salivary factors in relation to their caries experience. Prior to the start of study, the study proposal was submitted for approval and clearance from the Ethical review board of College of Dental Sciences, Davangere. Permission to conduct the study was obtained from the school authorities of the residential school. A specially prepared and pretested format in English language, exclusively designed for recording all the relevant data pertaining to general information, salivary variables, and microbiological findings were used. Pilot study was conducted before the main study to check the feasibility and validity of the study. By standardizing all the materials and methods, the study was conducted by considering a total of 9 children, 3 in each group (caries free, medium caries, high caries). Pilot study assessments were utilized for proper planning and execution of the main study and also to finalize the procedure, method and analysis of the saliva samples. These 9 children who participated in the pilot study were not included in the main study. All the examinations and laboratory analysis were carried out by a single examiner (i.e., investigator himself) and recording was done by another person, who was familiar with the proforma. Sample size determination was based on the prevalence of Lactobacilli count in resting saliva among high caries risk patients, as observed in a previous study. [2] The sample size (n) was calculated from the following formula.

$$n = \frac{z^2 pq}{d^2}$$

where,

z = 2 (assuming the distribution is normal and confidence limit is 95%)

p = the prevalence of Lactobacilli among high caries experience patients (in %) = 70

q = 1- p (in %) = 30

d = Admissible error in estimation = 15% of p (standardised) = 15% of 70 = 10.5

Substituting the values in the formula, the total number of samples is approximately 76 cases and when divided into three equal groups (based on dental caries experience), will give a sample size of approximately 25 subjects per group Based on the above calculations, to compensate for dropouts if any, a sample size of 30 subjects per group was used in this study thus totalling to 90 subjects. The study was conducted for a period of six months from 3rd August 2009 to 3rd February 2009 at Smt. Pushpa Shamnur Mahalingappa Residential School, a residential school located in Davangere city with strength of 300 students aged 12-13 years, was selected for the study. From this school, the children fulfilling all the inclusion and exclusion criteria were selected.

Inclusion criteria:

1. School children in age group of 12 years.

2. Minimum of 20 natural teeth.

Exclusion criteria:

1. Children with orthodontic appliances.

2. Recent antibiotic therapy

3. The presence of any systemic illness.

Those children who fulfilled the above criteria were screened for caries status using mouth mirror and explorer. Caries status of each child was scored by using DMFT index. They were assigned into three groups based on their DMFT scores (≥ 6 High risk, 1-5 Medium risk and <1 Caries free). [5] As per the sample size estimation, a total of 90 children were included in this study with 30 students in each group.

Written informed consent was obtained from the Principal of the residential school before the beginning of examination by discussing with them the purpose of the study, the advantages and the disadvantages associated with the study.

Collection of Saliva

On the day of collection, participating children were instructed not to eat or drink anything for at least one hour before the collection of saliva sample. To control the circadian variations, samples were collected between 8:00 am – 9:00 am. All necessary armamentarium required for saliva collection were assembled before the saliva collection procedure. Children were asked to rinse their mouth with water thoroughly 10 minutes before collection of saliva to avoid the contamination of food debris. Then they were made to sit on a chair. For collection of resting saliva, the children were instructed to let saliva collect in the floor of the mouth without swallowing it for atleast 1 min, and then to expectorate into the sterile graduated measuring cylinder with the help of a sterile funnel. This procedure was continued for a period of 5 minutes.

Estimation of salivary flow rate [6]

The flow rate of resting and stimulated saliva was determined by the following formula: Salivary flow rate = $\frac{\text{Total amount of saliva expectorated in 5 min (in ml)}}{5}$

Salivary flow rate was expressed in ml / min.

The salivary flow rate was determined and classified as follows

1: Very low < 0.1 ml/min.

2: Low: 0.1-0.25 ml/min.

3: Normal: 0.25-0.35 ml/min.

4: High: >0.35 ml/min.

Estimation of salivary buffering capacity

pH of saliva was measured by using manual pH meter. The estimation of buffering capacity was carried out as per the method described by Ericsson (1959) modified for smaller volumes[7].

Based on final pH of saliva

1: Low: < 4.1.

2: Intermediate: 4.1-5.5.

3: High: > 5.5.

Microbial analysis of saliva[8]

The saliva sample was homogenized manually by stirring using a stirrer. Hundred microliters of saliva was diluted with 1 ml of sterile peptone water to obtain 1:10 dilution of saliva. 100 µl of the diluted saliva

was further added to 1 ml. of sterile peptone water to obtain a dilution of 1:100. This procedure was repeated again to obtain a dilution of 1:1000. This dilution of saliva was used for microbial analysis.

Mutans streptococci were cultured on Mitis Salivarius Bacitracin agar and Lactobacilli on Rogosa SL agar, which are the selective media for culture of these organisms. The MSB agar plates were incubated for 48 hours at 37° C, anaerobically using candle jar. The Rogosa SL agar plates were incubated for 48 hours at 37°C, aerobically in the incubator. After 48 hours of incubation, the colonies were identified and counted using an electronic counter.

Based on Colony Forming Units (CFUs) per ml. of saliva

1: $<10^5$

2: $\geq 10^5$ - $<10^6$

3: $\geq 10^6$

Statistical analysis:

Data collected by experiments were computerized and analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0.

Results were expressed in frequencies. Nonparametric tests namely Kruskal Wallis ANOVA test and Mann Whitney U test were used for testing the statistical significance.

Kruskal Wallis ANOVA test was used for comparison of salivary factors in relation to the caries experience groups and as well as comparison of salivary factors at various time intervals. The intra-group analysis in relation to caries experience groups and different time intervals were analysed using Mann Whitney U test.

The variation of salivary factors between males and females were analysed using Mann Whitney U test.

For all tests a p-value of 0.05 or less was considered for statistical significance.

RESULTS

This 24 week follow up study to assess the variation in salivary variables was conducted among 90 children, aged 12-13 years, studying in a residential school in Davangere city.

Table 1 shows the comparison of resting salivary flow rate amongst subjects with different caries experience at different time intervals. None of the subjects had a resting salivary flow rate of < 0.1ml/min. In caries free group, none of the subjects had low salivary flow rate (0.1 – 0.25 ml/min) and on the contrary, in high caries experience group, none of the subjects had a high flow rate (> 0.35 ml/min.). It was observed that there is a statistically significant difference in resting salivary flow rates in different caries experience group at all time intervals ($p<0.001$). The significance was observed between all the three groups. The subjects with no caries showed significantly high flow rate in comparison to caries active subjects ($p<0.001$). Significant difference also existed in flow rates among medium and low caries group at all time intervals ($p<0.001$).

Table 2 shows the comparison of resting salivary buffering capacity amongst subjects with different caries experience at different time intervals. It was observed that there was a statistically significant difference in resting salivary buffering capacities in different caries experience group at all time intervals. The subjects with no caries showed significantly high buffering capacity in comparison to subjects with medium and high caries risk. However there was no significant difference in buffering capacities among medium and low caries group at any of the time intervals.

Table 3 shows the comparison of resting salivary Mutans streptococci counts amongst subjects with different caries experience at different time intervals. It was observed that there was a statistically significant difference in resting salivary Mutans streptococci count amongst subjects with different caries risk at all time intervals. The subjects with high caries risk showed significantly high Mutans streptococci counts in comparison to subjects with medium risk and caries free subjects. There was also a significant difference in Mutans streptococci counts among medium and low caries group at all the time intervals. None of the caries free subjects had a high count of Mutans streptococci ($\geq 10^6$ CFU/ml). Only one subject of high caries group at baseline had a

count of Mutans streptococci (< 10^5 CFU/ml).

Table 4 shows the comparison of resting salivary Lactobacilli counts amongst subjects with different caries experience at different time intervals. It was observed that there was a statistically significant difference in resting salivary Lactobacilli counts amongst subjects with different caries risk at all time intervals. The subjects with high caries risk showed significantly high Lactobacilli counts in comparison to subjects with medium risk and caries free subjects. There was also a significant difference in Lactobacilli counts among medium and low caries group at all the time intervals.

Table 5 shows the comparison of resting salivary flow rate among subjects with different caries risk at different time intervals. Table 6 shows the comparison of buffering capacity of resting saliva among subjects with different caries risk at different time intervals. Table 7 shows the comparison of Mutans streptococci counts in resting saliva among subjects with different caries risk at different time intervals. Table 8 shows the comparison of Lactobacilli count in resting saliva among subjects with different caries risk at different time intervals. It was observed that over a period of 24 weeks, none of the caries risk groups studied showed statistically significant difference in relation to any of the salivary variables assessed.

DISCUSSION

It is known that good oral health is an integral component of good general health. Although enjoying good oral health includes more than just having healthy teeth, many children have inadequate oral and general health because of active and uncontrolled dental caries. Dental caries is a disease pro-

TABLE 1: Comparison of resting salivary flow rate amongst subjects with different caries experience at different time intervals

CARIES EXPERIENCE		RESTING SALIVARY FLOW RATE AT DIFFERENT TIME INTERVALS															
		BASE LINE				6 WEEKS				12 WEEKS				24 WEEKS			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
HIGH		0	17	13	0	0	16	14	0	0	15	15	0	0	17	13	0
MEDIUM		0	1	27	2	0	0	28	2	0	0	28	2	0	0	27	3
FREE		0	0	4	26	0	0	4	26	0	0	1	29	0	0	3	27
Kruskal Wallis ANOVA	$\chi 2$	62.875				63.470				70.616				65.093			
	p value	0.001(HS)				0.001(HS)				0.001(HS)				0.001(HS)			
Mann Whitney U test		F>M (p<0.001) M>H (p<0.001) F>H (p=0.001)				F>M (p<0.001) M>H (p<0.001) F>H (p=0.001)				F>M (p<0.001) M>H (p<0.001) F>H (p=0.001)				F>M (p<0.001) M>H (p<0.001) F>H (p=0.001)			

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free
Flow rate: 1= <0.1 ml/min., 2= 0.1-0.25 ml/min.,3=0.25-0.35 ml/min., 4= >0.35 ml/min.

cess that afflicts a large proportion of the world's population. The etiology and pathogenesis of dental caries is multifactorial. Numerous host, agent and environmental factors play a role in the development of dental caries.[9]

The concept of the prediction of human dental caries risk has existed for many years. Salivary research has thus become an important field of dentistry and oral biology.[10]

Caries status of each child was scored using DMFT index. The age group of 12-13 years was selected for the study for the following reasons:

1. 12 years is one of the index age groups as recommended by the World Health Organisation.

2. It is the global monitoring age for international comparisons and monitoring of disease trends.[11,12]

3. It is one of the key ages or risk ages to be considered for caries risk assessment for screening in schools.[3]

Although various factors directly or indirectly affect the variables that are under study, diet appears to be one of the major factors that can have a profound influence on the variables. Strong evidence indicates that the association of Lactobacilli and Mutans streptococci with caries development is linked directly to carbohydrate consumption which, in turn, is one of the indispensable factors in caries development. [13] Hence a single residential school was selected for the study and for the same reason only students staying in the school hostel and not day-scholars were included as study samples. Under rest-

TABLE 2: Comparison of resting salivary buffering capacity amongst subjects with different caries experience at different time intervals

CARIES EXPERIENCE		RESTING SALIVARY BUFFERING CAPACITY AT DIFFERENT TIME INTERVALS											
		BASE LINE			6 WEEKS			12 WEEKS			24 WEEKS		
		1	2	3	1	2	3	1	2	3	1	2	3
HIGH		9	13	8	10	11	9	7	13	8	8	13	9
MEDIUM		9	13	8	6	16	8	7	14	7	7	12	11
FREE		2	9	19	3	9	18	1	1	2	2	11	17
Kruskal Wallis ANOVA	χ^2	12.46			8.612			8.776			6.378		
	p value	0.002(HS)			0.013(S)			0.012(S)			0.041(S)		
Mann Whitney U test		F>M (p=0.002) F>H (p=0.002			F>M (p=0.014) F>H (p=0.009)			F>M (p=0.001) F>H (p=0.014)			F>M (p=0.04) F>H (p=0.015)		

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free.
Buffering capacity: 1= < 4.1, 2= 4.1-5.5,3= > 5.5.

ing conditions without the exogenous stimulation associated with feeding, there is slow flow of saliva which keeps the mouth moist and lubricates the mucous membrane. This unstimulated flow, which is present majority of the time, is very important for the health and well being of the oral cavity and also imparts strong protective effect against dental caries.[14,15]

Several methods of collecting saliva are available. They are: draining method, spitting method, suction method and swab method. According to Navazesh and Christensen spitting method appeared to be most reproducible. [16] Hence this method of saliva collection was employed in this study.

In this study, it was observed that there was a statistically significant difference in resting salivary flow rates in all the three different caries experience groups. The results of this study are similar to the studies conducted by Hoolbrook et al in Iceland[17], Sawair et al in Jordan[18] and Gopinath et al in Malaysia[15]. However, huge amount of literature is available citing the association of xerostomia and increased dental caries prevalence that indicates the association between salivary flow rate and dental caries.[19]

There was no significant association between salivary flow rate and dental caries experience in studies reported by Parvinen T et. al in Hungary[20] and Bretz et. al. in Brazil[21]. However, according to Tenovuo, salivary flow rate is the most important single parameter, when considering possible associations with caries activity, and might be important as

TABLE 3: Comparison of resting salivary Mutans streptococci counts amongst subjects with different caries experience at different time intervals

CARIES EXPERIENCE		RESTING SALIVARY MUTANS STREPTOCOCCI COUNT AT DIFFERENT TIME INTERVALS											
		BASE LINE			6 WEEKS			12 WEEKS			24 WEEKS		
		1	2	3	1	2	3	1	2	3	1	2	3
HIGH		1	7	22	0	4	26	0	3	27	0	3	27
MEDIUM		6	23	1	3	25	2	2	26	2	2	26	2
FREE		26	4	0	27	3	0	27	3	0	27	3	0
Kruskal Wallis ANOVA	χ^2	71.904			72.200			74.516			59.545		
	p value	<0.001(HS)			<0.001(HS)			<0.001(HS)			<0.001(HS)		
Mann Whitney U test		M>F (p<0.001) H>M (p<0.001) H>F (p=0.002)			M>F (p<0.001) H>M (p<0.001) H>F (p=0.002)			M>F (p<0.001) H>M (p<0.001) H>F (p=0.002)			M>F (p<0.001) H>M (p<0.001) H>F (p=0.002)		

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free.
Mutans streptococci count (in CFU/ml): 1= $<10^5$, 2= $\geq 10^5$ - $<10^6$, 3= $\geq 10^6$.

a 'threshold' limit at the individual level. [22]

In this study, it was observed that there was a statistically significant difference in resting salivary buffering capacity in all the three caries experience groups. The high and medium caries experience groups had significantly higher resting and stimulated salivary buffering capacity in relation to the caries free group. The results are in accordance with numerous studies conducted by Farsi in Saudi Arabia[2], Tukia-Kumala et. al. in Finland[4], Holbrook in Iceland[17], Gopinath et. al. in Malaysia[15] and Malekipour et. al. in Iran. [23] In all these studies the dental caries prevalence was significantly higher in caries active groups than caries free groups.

The results are however in contrast with the results of studies conducted by Gabris et. al. in Hungary[24] and Preethi et. al. in Davangere. [25] The reason for this observed contrast in results could be due to the fact that other factors like microflora, diet and retention of food might have dominated the buffering capacity to initiate caries, which is a multifactorial disease, as justified by the author in the Davangere study.

Due to their positive numerical association with human caries and the linkage of this association to carbohydrate consumption, counts of Lactobacilli and Mutans streptococci may, potentially, serve not only as a caries risk predictor but also as an indicator

TABLE 4: Comparison of resting salivary Lactobacilli counts amongst subjects with different caries experience at different time intervals

CARIES EXPERIENCE		RESTING SALIVARY LACTOBACILLI COUNT AT DIFFERENT TIME INTERVALS											
		BASE LINE			6 WEEKS			12 WEEKS			24 WEEKS		
		1	2	3	1	2	3	1	2	3	1	2	3
HIGH		1	5	24	0	6	24	0	5	25	0	5	25
MEDIUM		5	15	10	4	20	6	5	19	6	4	20	6
FREE		14	13	3	13	14	3	12	14	4	12	14	4
Kruskal Wallis ANOVA	$\chi 2$	32.812			38.427			37.198			37.596		
	p value	<0.001(HS)			<0.001(HS)			<0.001(HS)			<0.001(HS)		
Mann Whitney U test		M>F (p<0.001) H>M (p=0.005) H>F (p=0.002)			M>F (p<0.001) H>M (p=0.015) H>F (p=0.002)			M>F (p<0.001) H>M (p=0.045) H>F (p=0.002)			M>F (p<0.001) H>M (p=0.043) H>F (p=0.002)		

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free.
Lactobacilli count (in CFU/ml): 1= $<10^5$, 2= $\geq 10^5$ - $<10^6$, 3= $\geq 10^6$.

of carbohydrate consumption, another caries-risk factor.[26]

In this study, the Mutans streptococci counts and Lactobacillus counts were thus evaluated in saliva. Mutans streptococci were cultured using Mitis salivarius bacitracin agar and Lactobacilli in Rogosa agar, which are the selective media for the growth of, Mutans streptococci and Lactobacilli respectively. Studies reveal that the results obtained by this culture plate method, as used in this study, correlate well with the dipslide methods[4], yet another method used for the same.

It was observed that there was a statistically significant difference in resting salivary Mutans streptococci count and Lactobacillus count amongst subjects with different caries risk at all time intervals. The subjects with high caries risk showed significantly high Mutans streptococci and Lactobacillus in comparison to subjects with medium risk and caries free subjects. There was also a significant difference in Mutans streptococci counts and Lactobacilli among medium and low caries group at all the time intervals. The results obtained are in accordance with a vast majority of the studies conducted to analyse this relation.[4,24,27-36]. None of the subjects in caries free group showed ≥ 10^6 CFU/ml counts of Mutans streptococci.

However few studies conducted by Farsi in Saudi Arabia[2] and Garnath et. al. in Sweden[26] show no significant relation between dental caries experience and Mutans streptococci and Lactobacilli. In the study conducted by Garnath et al., the authors attribute the reason for the results to a probably skewed distribution subjects in dmfs classes for lower bacterium classes than for higher bacterium classes.[26]

In relation to the evaluation of salivary variables at different time intervals, it was observed that over a period of 24 weeks, none of the caries risk groups studied showed statistically significant difference in salivary factors at the different time intervals. The observation was in accordance with a study conducted by Tukia-Kumala et. al. in Finland[4], among same age group subjects over a period of 9 months.

TABLE 5: Comparison of resting salivary flow rate amongst subjects at different time intervals

TIME INTERVAL		RESTING SALIVARY FLOW RATE								
		HIGH CARIES			MEDIUM CARIES			CARIES FREE		
		2	3	4	2	3	4	2	3	4
BASE LINE		17	13	0	1	27	2	0	4	26
6 WEEKS		16	14	0	0	28	2	0	4	26
12 WEEKS		15	15	0	0	28	2	0	1	29
24 WEEKS		17	10	0	0	27	3	0	3	27
Kruskal Wallis ANOVA	χ^2	2.204			0.795			0.366		
	p value	0.531(NS)			0.851(NS)			0.947(NS)		

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free
Flow rate: 1= <0.1 ml/min., 2= 0.1-0.25 ml/min., 3= 0.25-0.35 ml/min., 4= >0.35 ml/min.

TABLE 6: Comparison of resting salivary buffering capacity amongst subjects at different time intervals

TIME INTERVAL		RESTING SALIVARY FLOW RATE								
		HIGH CARIES			MEDIUM CARIES			CARIES FREE		
		1	2	3	1	2	3	1	2	3
BASE LINE		9	13	8	9	13	8	2	9	19
6 WEEKS		10	11	9	6	16	8	3	9	18
12 WEEKS		7	13	10	7	14	9	1	11	18
24 WEEKS		8	13	9	7	12	11	2	11	17
Kruskal Wallis ANOVA	$\chi 2$	0274			0.786			0619		
	p value	0.965(NS)			0.853(NS)			0.892(NS)		

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free.
Buffering capacity: 1= < 4.1, 2= 4.1-5.5, 3= > 5.5.

TABLE 7: Comparison of resting salivary Mutans streptococci counts amongst subjects at different time intervals

TIME INTERVAL		RESTING SALIVARY MUTANS STREPTOCOCCI COUNTS								
		HIGH CARIES			MEDIUM CARIES			CARIES FREE		
		1	2	3	1	2	3	1	2	3
BASE LINE		1	7	22	6	23	1	26	4	0
6 WEEKS		0	4	26	3	25	2	27	3	0
12 WEEKS		0	3	27	2	26	2	27	3	0
24 WEEKS		0	3	27	2	26	2	27	3	0
Kruskal Wallis ANOVA	$\chi 2$	1.053			1.859			0.701		
	p value	0.788(NS)			0.602(NS)			0.873(NS)		

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free.
Mutans streptococci count (in CFU/ml): 1= $<10^5$, 2= $\geq 10^5$ - $<10^6$, 3= $\geq 10^6$.

TABLE 8: Comparison of resting salivary Lactobacilli counts amongst subjects at different time intervals

TIME INTERVAL		RESTING SALIVARY LACTOBACILLI COUNTS								
		HIGH CARIES			MEDIUM CARIES			CARIES FREE		
		1	2	3	1	2	3	1	2	3
BASE LINE		1	5	24	5	15	10	14	13	3
6 WEEKS		0	6	24	4	20	6	13	14	3
12 WEEKS		0	5	25	5	19	6	12	14	4
24 WEEKS		0	5	25	4	20	6	12	14	4
Kruskal Wallis ANOVA	$\chi 2$	0.481			0.880			0.262		
	p value	0.923(NS)			0.830(NS)			0.967(NS)		

HS – Highly significant, S – Significant, NS – Not significant
Caries Experience: H – High, M- Medium, F – Caries Free.

CONCLUSION

There was a significant variation of the salivary factors studied namely salivary flow rate, buffering capacity, Mutans streptococci counts and Lactobacilli counts among all the three caries experience groups (High, medium and caries free) in resting saliva.

When the individual variations of salivary factors were analysed over a period of 24 weeks, although variation of salivary factors existed among all the caries experience groups at all time intervals studied, the variation was not statistically significant. The study thus indicated that the salivary variables studied are a valuable diagnostic tool to assess caries experience and predict caries risk.

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