Comparative Quantitative Assessments of Salivary Ion Activity Product for Hydroxyapatite and Buffering Capacity in Children with Different Caries Experience

Siddharth Anand*/ Updesh Masih **/ Ramakrishna Yeluri ***

Aim: *If a relation exists between salivary I_{PHA}, buffer capacity and caries experience, then this relationship could be used as screening chair side test for caries risk assessment.* ***Study design:*** *One hundred ninety seven children aged 4 to 6 years were examined. Data was collected by interview and clinical examination. They were divided into low, moderate and high caries experience group of 20 children each. Two ml of each sample was used to measure the pH value with pH meter. Regarding the buffering capacity, freshly prepared hydrochloric acid (HCl) was titrated into saliva and pH was recorded. The collected saliva samples were sent to Laboratory for measurement of calcium and phosphorus. I_{PHA} was calculated and the negative logarithms of I_{PHA} were used to determine the enamel solubility. The correlation between salivary I_{PHA}, buffering capacity and caries experience were evaluated.* ***Results:*** *There was a significant relation between pH, log I_{PHA} and dental caries experience, it could be considered as a predictor of dental caries.* ***Conclusion:*** *pH measurement after HCl titration in saliva could be used as chair side screening test for the assessment of caries risk.*

Key words: *ion activity product for hydroxyapatite (I_{PHA}), buffering capacity and hand held pH meter*

INTRODUCTION

Dental caries, a common chronic infectious disease of the oral cavity, is considered as a complex phenomenon and is involved with different important factors including salivary characteristics, tooth surface morphology and oral hygiene.[1] Different biochemical characteristics of saliva such as salivary flow rate, buffering capacity, inorganic component, dental remineralisation, initial digestion of starch, antimicrobial actions as well as proteins may affect the development of dental caries. [2,3,4]

*Siddharth Anand,BDS, MDS, Department of Pedodontics and Preventive Dentistry, K. D. Dental College and Hospital, Mathura, India.

** M Updesh Masih,.DDS, Professor, Department of Pedodontics and Preventive Dentistry, K. D. Dental College and Hospital, Mathura, India.

*** Ramakrishna Yeluri MDS, Professor and Head, Department of Pedodontics and Preventive Dentistry, Treethankar Dental College and Hospital, Moradabaad, India.

Send all correspondence to
Siddharth Anand,
Dept. of Pedodontics & Preventive Dentistry,
K. D. Dental College and Hospital,
Mathura, Uttar Pradesh, India.
Phone. +917677350641.
Fax: 0565-2530764
E-mail: drsiddharthanand@yahoo.co.in

The buffer capacity of both unstimulated and stimulated saliva involves three major buffer systems: the bicarbonate, the phosphate, and the protein buffer systems. Since most of the salivary buffering capacity operates during food intake and mastication is due to the bicarbonate system sufficient saliva flow provides the oral cavity with the neutralizing components.[5] According to Ericsson, there is an inverse relationship between buffer capacity and caries experience.[6] On a population level, salivary flow rate and buffer effect show an inverse correlation with caries susceptibility.[7]

It is expected that saliva might be effective on enamel maturation and remineralisation,[8,9] with respect to its high level of calcium and phosphate.[10,11] However, spontaneous precipitation of these ions from saliva to tooth structure cannot occur.[3] It seems that the function of this protein relatively depends on the salivary pH and buffering capacity.[12] To show the degree of saturation of the solutions, many authors used the ion activity product for hydroxyapatite (I_{PHA}) calculated by employing the equation $I_{PHA}= \{Ca^{2+}\}^5 \{PO_4^{3-}\}^3 \{OH-\}$. [13,14,15] Different factors such as pH, buffering capacity, and temperature have shown, in an in-vitro experiment, have an effective on the I_{PHA} value;[16] however, it seems that there is a relationship between the values of I_{PHA} and demineralisation and remineralisation of the enamel.[17]

Specific changes in saliva, such as a lower pH and decreased buffering capacity, may also contribute to increased susceptibility to dental caries.[16] Although a colour code chart is commercially available as a test for saliva buffering capacity, there are possibly cases where the level of the buffering capacity is difficult to classify. Recently, a quantitative saliva buffering capacity test has been

introduced to dental practice which uses a hand-held pH meter that allows a more objective analysis.[18,19,20] The quantitative evaluation of saliva buffering capacity helps dentist and child patient to know about caries risk and its progression which is lacking in the colorimetric paper strip type tests. However, individual pH changes, even with the quantitative saliva test, cannot define when enamel may demineralise or determine different degrees of caries susceptability.[16]

The identification of high risk children is essential for planning of oral health treatment and prevention of future diseases. It will also help institutions when planning outreach activities to focus on communities that are most vulnerable to dental caries, one of the pillars for the success of a community service program.[21]

To the best of our knowledge, there is no study available in the literature evaluating the relationship between the quantitative assessments of I_{PHA}, buffering capacity of saliva and their relation to tooth caries experience in children. Therefore it seems necessary to conduct a study to compare the I_{PHA}; reflecting salivary calcium and phosphate concentration, [17] which may balance enamel remineralisation, as well as buffering capacity; that may affect the function and the quantity of ion activity product for hydroxyapatite, regarding the caries risk and age.

The research protocol was reviewed and approved by the institutional ethical committee of K.D. Dental College and Hospital, Mathura, Uttar Prdesh, India and affliated to Dr. B.R. Ambedkar University, Agra, Uttar Prdesh, India and the guidelines are as per Helsinky declaration. This was an ex vivo study and the parameters evaluated were: Dental caries experience, Salivary pH, Salivary Buffering capacity, Salivary ion activity product for hydroxyapatite (I_{PHA}) before and after hydrochloric acid (HCl) titration.

A total of 197 children aged 4 to 6 years (primary dentition period) were examined among the out patient department of Pedodontics and Preventive Dentistry and various government schools, Mathura, Uttar Pradesh, India and 60 children including both sexes who were fulfilling the inclusion criteria were selected for this study. Prior to the initiation of this study, parents or legally responsible persons received detailed information about the study and signed a pre-informed consent form, permitting the participation of their children. All the children were instructed regarding oral hygiene procedures during the course of this study.

Exclusion Criteria - Volunteers who had positive history of illness or treatments which could cause alternation in salivary rate and composition including diabetes, rickets, osteitis deformans, periodontal disease, history of radiotherapy or chemotherapy, dehydration, using antibiotics in recent two weeks and mouth breathers. The teeth that were lost or restored due to trauma, orthodontic treatment or esthetic reasons and whose permanent first molar were erupted at the age of six were excluded from this study.

Data was collected by interview and clinical examination. Participants were divided into low, moderate and high caries experience group which involved 20 children equally in each group (Low caries experience (dfs < 1), Moderate caries experience (dfs 5-10), High caries experience (dfs >10).

Saliva collection and salivary buffering capacity test

Saliva collection was done between 9 a.m. to 11 a.m. The subjects were abstain from eating, drinking or brushing 2 hours prior to sampling. The unstimulated saliva was collected into a sterile disposable container. Two ml of each sample was used to measure the pH value within 30 seconds after placement of hand held pH – sensitive electrode (pH 107, CE, RoHS, Mainland, China). Regarding the buffering capacity, freshly prepared 1.5 ml of 5 mmol/L hydrochloric acid was titrated into the 0.5 ml saliva in another sterile container and the pH was recorded with hand held pH meter in the Department of Biochemistry to obtain a pH titration curve for each sample. After every reading, the hand held pH electrode was cleaned in distilled water and was dried with absorbent paper. To avoid the false positive reading of the hand held pH meter, it was cross checked by dipping it in the standard buffer solution of pH 7 (AK2 AF52395, Merck limited, Worli, Mumbai, India). The collected saliva samples were then transported to the Pathology Laboratory, within 2 hours for further measurement of calcium and phosphorus in each sample before and after HCl titration.

Calculation of ion activity product for hydroxyapatite (I_{PHA})

The definition of the I_{PHA} is described by the following equation: $I_{PHA} = [Ca^{2+}]^5 [PO_4^{3-}]^3 [OH^-]$, where each of the parentheses indicates activity of each ion. As described in the equation, I_{PHA} is a comprehensive parameter which involves the influence of calcium and phosphate concentrations and the pH of saliva on de and remineralization. This parameter governs the rate of de and remineralization of enamel, i.e., the greater the value of I_{PHA}, the greater the rate of remineralization potential. As it is clear that the pH + pOH are 14, after measurement of the pH of the samples, pOH was used to calculate the concentration of $[OH^+]$. I_{PHA} was calculated by placement of the concentration of calcium^{2+}, phosphate^{3-} and OH^- in the following equation: $I_{PHA} = [Ca^{2+}]^5 [PO_4^{3-}]^3 [OH^-]$, in which any value in the curly brackets shows the ion activity in saliva. The negative logarithms of I_{PHA} (p I_{PHA}) were used for convenience in order to determine the enamel solubility. After data collection, the correlation between salivary I_{PHA}, buffering capacity and caries experience were evaluated.

Statistical Analysis

Wilcoxon signed ranks test, Spearman's rho correlation co-efficient test and Kruskal Wallis test were used to compare dental caries experience with buffering capacity and salivary ion activity product for hydroxyapatite before and after hydrochloride titration using SPSS software version 14. The significance level for all the statistical tests utilized in this study was set at $p < 0.05$ %.

RESULTS

Table – 1 Comparison of various variables within low, moderate and high caries experience group. (Result of Wilcoxon Signed Ranked Test)

Caries experience group	Wilcoxon signed ranked test	Calcium after-Calcium before (Difference)	Phosphate after-Phosphate before (Difference)	pH after – pH before (Difference)	Log I_{PHA} after - Log I_{PHA} before (Difference)
Low caries experience	**Z test**	-0.709	-3.883	-3.927	-3.920
	p- value	0.478	**0.000*** **	**0.000*** **	**0.000*** **
Moderate caries experience	**Z test**	-0.560	-3.808	-3.928	-3.883
	p- value	0.575	**0.000*** **	**0.000*** **	**0.000*** **
High caries experience	**Z test**	-0.149	-3.845	-3.922	-3.920
	p- value	0.881	**0.000*** **	**0.000*** **	**0.000*** **

Table – 2 Correlation of each variable within low caries experience group before and after HCl titration. (Result of Spearman's rho correlation co-efficient test)

	Variable		P (mmol/L)		pH		Log I_{PHA} (M)	
			Before	After	Before	After	Before	After
Spearman's rho	**Ca before (mmol/L)**	**Correlation coefficient**	-0.078		-0.011		-0.528	
		p – value	0.745		0.962		0.017*	
	Ca after (mmol/L)	Correlation coefficient		0.230		0.105		-0.577
		p – value		0.329		0.661		0.008**
	P before (mmol/L)	Correlation coefficient			-0.355		-0.415	
		p – value			0.125		0.069	
	P after (mmol/L)	Correlation coefficient				0.286		-0.391
		p – value				0.222		0.088
	pH before	**Correlation coefficient**					0.208	
		p – value					0.379	
	pH after	Correlation coefficient						0.007
		p – value						0.977

Table – 3 Correlation of each variable within moderate caries experience group before and after HCl titration. (Result of Spearman's rho correlation co-efficient test)

	Variable		P (mmol/L)		pH		Log I_{PHA} (M)	
			Before	After	Before	After	Before	After
Spearman's rho	**Ca before (mmol/L)**	**Correlation coefficient**	0.133		-0.257		-0.281	
		p – value	0.575		0.274		0.231	
	Ca after (mmol/L)	Correlation coefficient		0.345		0.482		-0.200
		p – value		0.136		0.032*		0.398
	P before (mmol/L)	**Correlation coefficient**			-0.093		-0.096	
		p – value			0.695		0.686	
	P after (mmol/L)	Correlation coefficient				0.520		-0.035
		p – value				0.019*		0.885
	pH before	**Correlation coefficient**					0.347	
		p – value					0.134	
	pH after	Correlation coefficient						0.205
		p – value						0.386

Table – 4 Co-relation of each variable within high caries experience group before and after HCl titration. (Result of Spearman's rho correlation co-efficient test)

Variable			P (mmol/L)		Ph		Log I_{PHA} (M)	
			Before	After	Before	After	Before	After
Spearman's rho	**Ca before (mmol/L)**	**Correlation coefficient**	0.301		-0.500		-0.720	
		p – value	0.198		0.025*		0.000***	
	Ca after (mmol/L)	Correlation coefficient		-0.211		-0.447		-0.561
		p – value		0.373		0.048*		0.010*
	P before (mmol/L)	Correlation coefficient			-0.111		-0.487	
		p – value			-0.641		0.029*	
	P after (mmol/L)	Correlation coefficient				-0.392		-0.349
		p – value				0.088		0.132
	pH before	**Correlation coefficient**					0.437	
		p – value					0.054	
	pH after	Correlation coefficient						0.369
		p – value						0.109

Table – 5: List of various variables between low, moderate and high caries experience group. (Results of non – parametric Kruskal Wallis tests)

Variable	Group	N	Before HCl titration	After HCl titration
Calcium (mmol/L)	**Low**	20	33.48	33.65
	Moderate	20	30.28	33.05
	High	20	27.75	24.80
Phosphate (mmol/L)	**Low**	20	37.50	34.22
	Moderate	20	29.85	30.78
	High	20	24.15	26.50
pH	**Low**	20	31.65	22.68
	Moderate	20	34.48	32.68
	High	20	25.38	36.15
Log I_{PHA} (M)	**Low**	20	29.15	22.40
	Moderate	20	30.45	33.98
	High	20	31.90	35.12

Table 1 showing comparison of all the variables in low, moderate and high caries experience group. This table shows a highly significant result in phosphate, pH and log IPHA before and after HCl titration in all the three groups.

Table 2 showing correlation between different variables in low caries experience group and a significant correlation was seen between calcium and log IPHA before and after HCl titration.

Table 3 showing correlation between different variables in moderate caries experience group and a significant correlation was seen between calcium, phosphate and pH after HCl titration.

Table 4 showing correlation between different variables in high caries experience group and highly significant relation was seen between calcium and log IPHA before HCl titration. A significant relation exists between calcium and pH after HCl titration. There is a significant correlation of log IPHA with phosphate before titration and with calcium after titration with HCl.

Table 5 showing the parametric values of all the variables in low, moderate and high caries experience group before and after HCl titration.

DISCUSSION

Dental caries is characterized, at least in its initial stages, by the dissolution of the inorganic salts of the hard dental tissues. Solubility of enamel depends largely on the concentration of calcium and phosphate ions in the surrounding medium. The buffering power of the saliva is, by definition, its ability to counteract pH changes. Previous studies, such as the one reported by Karshan,[22] stated that calcium and phosphorous content of saliva is low in caries active persons.

Wiktorsson *et al* [23] and Powell *et al* [24] showed that saliva has different effects on dental caries regarding to its inorganic materials, including calcium, phosphate and so on. Salivary buffer capacity can be measured in the resting or stimulated states. Similar to the previous study conducted by Varma *et al*,[25] total or rest saliva was used in the present study, because of its long time contact with the teeth. Total saliva, compared to the limited secretion of stimulated saliva; is imparted in our mouth for approximately 14 hours a day.[26] Aiuchi [16] and Vahedi[1] conducted a study to evaluate the relationship between salivary calcium and phosphate concentrations, I_{PHA} concentrations with dental caries only in adults population.

The relationship between salivary buffer capacity, log I_{PHA} and dental caries experience showed an excellent correlation in this study. On the other hand, the hand held pH meter used in this study can determine the pH of 0.1 ml of saliva, making it possible to determine the buffering capacity. As enamel dissolves, the pH as well as the concentrations of calcium and phosphate increase until saturation with respect to hydroxyapatite ends the dissolution. Although this correlation was demonstrated at a pH range from pH 2.92 to 7.52 when 0.05 mmol/L HCl was titrated into the saliva sample, the normal pH range of human stimulated saliva is generally between pH 3.0 to 8.25. [6]

Aiuchi [16] reported that the mean value of log I_{PHA} was 40 and related to the salivary buffering capacity. In the present study, the mean log I_{PHA} was 37.13. Compared to the previous study conducted by Margolis et al,[13] the result of the present study showed a lower amount of log I_{PHA}. This difference may be contributed to the different variants such as age of the participants whom the salivary samples were collected from, as well as the initial pH of saliva.

Three different buffering factors including bicarbonates, phosphates and proteins are responsible for salivary buffering.[27] In this study, Ericson method was used to evaluate the salivary buffering capacity. According to this method, salivary pH after HCl titration was considered as an appraisal factor for buffering capacity.[6] The results of the present study were similar to the findings of Aiuchi *et al* [16] who showed that there is significant difference in buffering capacity of saliva according to dental caries risk and were different from Monezgo *et al*,[28] Gabris *et al*,[29] and Vahedi *et al* [1] who proved that there is no significant difference in buffering capacity of saliva according to dental caries risk.

The results of this study were similar to the results of Ruiz et al,[30] who concluded that buffering capacity could be deliberated as a predictive factor for dental caries experience. In this study, the saliva pI_{PHA} obtained from different levels of buffer capacity clearly demonstrated variation in enamel dissolution as an indicator of the cariogenic potential.

Table - 5 showed result of Kruskal Wallis test in low caries experience group, pH was decreased (31.65 to 22.68), in moderate caries experience group, pH value was moderately increased (34.48 to 32.68) and in high caries experience group, pH was increased (25.38 to 36.15). There was statistically significant relation between caries experience and pH level before and after HCl titration. Even log I_{PHA} value showed statistically significant result between different caries experience group. In low caries experience group, log I_{PHA} value was decreased (29.15 to 22.40), in moderate caries experience group, log I_{PHA} value was moderately increased (30.45 to 33.98) and in high caries experience group, log I_{PHA} value was increased (31.90 to 35.12). In low, moderate and high caries experience group, there was no difference in the calcium level before and after HCl titration ($p>0.05$). But in low, moderate and high caries experience group, calcium was inversely proportional to phosphate, i.e. as the calcium increased, phosphate decreased and vice-versa.

There was a significant reduction in phosphate level in low caries experience group before and after HCl titration (37.50 to 34.22). In moderate caries experience group, the phosphate level is moderately increased (29.85 to 30.78) and in high caries group, the phosphate level was increased (24.15 to 26.50).

The correlation of each variable in low caries experience group (Table – 2) before HCl titration showed negative relation between calcium, phosphate and log I_{PHA} level but calcium and log I_{PHA} showed statistically significant difference ($p< 0.05$). For moderate caries experience group (Table – 3) before HCl titration, calcium was positively related to phosphate but this relation was not statistically significant ($p>0.05$). In high caries experience group (Table – 4) before HCl titration, calcium was negatively related to pH and log I_{PHA} level and showed statistically significant difference ($p<0.05$).

The correlation of each variable in low caries experience group after HCl titration showed positive relation between calcium, phosphate and negative relation between log I_{PHA} level but only log I_{PHA} showed statistically significant difference ($p< 0.05$). For moderate caries experience group after HCl titration, calcium and phosphate was positively related to pH and showed statistically significant difference ($p<0.05$). In high caries experience group after HCl titration, calcium was negatively related to log I_{PHA} level and showed statistically significant difference ($p<0.05$).

A high saliva buffer capacity may result in an elevated surface pH of the enamel crystal, resulting in favorable conditions for mineral intake.[6] This implies that for the chair side buffer capacity test, a speedy and simple caries risk assessment can be accomplished using a handy type pH meter by titrating 0.05 mmol/L HCl directly into the saliva samples. This quantitative saliva buffering capacity test may contribute to the promotion of oral health, especially for identifying pediatric patients with the risk factors of low buffering capacity and low initial pH as a screening test for caries risk assessment.

According to the results of the present study, since there is a significant relation between pH, log I_{PHA} and dental caries experience, it could be considered as a predictor of dental caries, however performing further research in a wider pediatric population, regarding age, sex, race and geographic area is suggested.

CONCLUSIONS

In the present study, an attempt had been made to relate calcium, phosphorus, pH and log I_{PHA} in saliva for the prediction of dental caries activity. Although individual salivary I_{PHA}, pH, calcium, phosphorous and buffering capacity vary in the results of the present study, they might be reliable for analysis of dental caries experience. The measurement of pH with hand held pH meter after 0.05 mmol/L HCl in 0.5 ml of saliva of children aged 4-6 years could be used as a screening tests at the chair side for the assessment of caries risk.

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