Role of unstimulated salivary flow rate, pH and buffer capacity on dental caries of children: Findings from community based cross-sectional study

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Research Article

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Abstract

Background: Dental caries development is a complex and dynamic process which is influenced by wide array of factors, of which dietary habits and salivary physicochemical biomarkers are of most significance. Salivary markers such as flow rate, pH and buffering capacity play a major role in the development of caries. In this study the relationship between these salivary marker among caries free and caries active was measured.

Methods: In this analytical cross sectional study, multistage stratified sampling technique was used, 700 private and public school children of different socioeconomic class, between six to sixteen years of age were selected. The status of dental caries was determined through dental examination using DMFT index. Unstimulated whole saliva was collected through Passive Drool Method and the selected physiochemical properties were assessed. The mean difference between caries free and caries active groups was noted and to identify significant risk factors for caries logistic regression analysis was performed.

Results: Comparison of salivary parameters between the two groups (caries free, caries active) showed that, there is significant decrease in mean value for salivary flow rate and buffering capacity in caries active group i.e., $p<0.001$. Risk factors were found to be significantly high in caries active group with flow rate (OR=20.06; $p< 0.001$), pH (OR=1.82; $p= 0.90$) and buffer capacity (OR=8.76; $p=0.79$).

Conclusion: Research showed that participants with active caries have lower resting salivary flow rates than caries free subjects. Caries free participants had a normal range of salivary pH and better buffering capacity than participants with active caries.

Background

Saliva is the principal defensive element in the oral cavity(1). It is a unique biofluid plays vital role in safeguarding oral health, by protecting normal oral cavity from dental diseases and act as a diagnostic fluid for various systemic diseases(2). The constituents of saliva comprises of both organic and inorganic components providing a biological homeostasis within oral cavity(1, 3) Essential function such as lubrication and protection of both soft and hard oral tissues against dehydration, penetration, ulceration, and potential carcinogens(1) is dependent on the composition and adequate flow of saliva(4). Maintenance of tooth integrity depends on salivary mechanical cleansing and enamel remineralization(5). Salivary flow reduction affects orodental health(4) and diminished salivary flow can cause diverse unspecific symptoms, therefore the establishment of patients' saliva flow is imperative in oral medicine and dentistry(6). Generally accepted values for unstimulated saliva range from 0.25 to 0.35 ml/min and for stimulated saliva it ranges from 1.0 to 3.0 ml/min(7, 8). Saliva flow in oral cavity is not constant it varies among individual and within an individual over time(9). Unstimulated whole saliva (UWS) is a combination of secretions within oral cavity in the absences of any exogenic stimuli like, tastants and chewing, UWS is noticeable different among age and gender(10, 11).

For the clinical determination of caries risk, analysis of various factors is crucial as dental caries is influenced by multiple factors like enamel solubility, saliva flow rate, pH, buffering effect of saliva, type of bacteria, dietary habits and oral hygiene(12, 13). Some of the most prevalent and critical pathological conditions of the teeth and the oral cavity are strongly dependent on variation in salivary pH (14). The buffering action of saliva is a significant defense mechanism(15, 16), controlled through three main buffer systems in human saliva: the bicarbonate, phosphate and protein buffer system(14). pH of oral cavity is maintained effectively by saliva via its buffer capacity by regulating dental biofilm pH and enamel surface integrity(17). The condition, which influences the saliva flow rate, pH and buffer system in saliva, thus control the susceptibility of a person to conditions like, dental caries(18).

Dental caries has been categorized as most frequently occurring oral disease(19). A major public health problem, strongly associated with sugar consumption and cariogenic bacteria(20). Dental caries and erosion both are characterized by dissolution of inorganic salts of tooth substance(21, 22) The effect of saliva on dental caries in individuals without any diseases state or any chronic salivary gland dysfunction is not well understood, and it is essential to explore these salivary parameters in healthy individuals to understand their role in dental caries development.

Caries have been described as dietobacterial disease as it cannot take place without the presence of fermentable carbohydrates(23). However, results of recent studies have shown that genetic and salivary factors may explain more than 50 percent of the variance in caries experience among people(24, 25). The mechanism of damaging effect of caries involve highly localized drop in the pH at the plaque tooth interface due to the metabolic activity of pathogenic plaque highly concentrated with mutans streptococci and lactobacilli, which produce organic acids mainly lactic acids(26). Oral clearance plays a vital part in the protection of tissues by dilution of sugars and acids present in the oral cavity(27, 28). Flow rate is considered most significant because of its caries protecting property as it influences the buffering capacity by controlling the release of organic component in saliva(29). That is why the purpose of this study was to determine the relationship between the physicochemical properties of saliva such as flow rate, pH and buffering capacity with dental caries.

Methods

Study design and setting:

This was an analytical cross sectional study, conducted at the Oral Biology department of Dr Ishrat Ul Ebad Khan Institute of Oral Health Sciences; Dow University of health Sciences.

Sample size Calculation:

Taken margin of error 0.05 the sample size was calculated using standard deviation of 0.58. The total sample size comes out to be N = 516. A surplus sample of 30% non-response rate was added to it. Therefore, a total number of subjects rounded of to 700 for dental examination and for salivary analysis.

Sample Selection:

The study samples were collected from randomly selected public and private schools of Karachi. Three towns of Karachi city were selected based on socioeconomic status, one private and one public school was included from each selected town. School children, pre-adolescent, and adolescent from both gender were examined to assess their caries activity. 700 school students were selected for the study and their saliva samples were collected. Multistage stratified cluster random sampling technique was used for the selection of samples. Using the town listing of City district Government; cluster of towns in Karachi with identical socioeconomic status were made. Applying lottery method one town from each cluster was randomly selected. From each selected town then a public and private school was selected using random number generator from MS Excel program.

The criteria on the bases of which subjects were selected for this study; public and private school children available at the time of data collection and provided consent, healthy subjects between the ages of 6 to 16 years, having mixed and permanent dentition with at least four permanent teeth present for mixed dentition, and at least 20 number of teeth present in permanent dentition. Habitual smoker and betel nut chewer, subjects wearing any prosthesis within oral cavity, with any oral or systemic diseases or having radio or chemo therapeutic drug treatment were excluded from the study.

Ethical approval

for the study was obtained from Institutional Review Board of Dow University of Health Sciences (Ref: IRB-293/DUHS-11). Prior to the data collection, written permission was taken from Directorate of Education, Karachi region (DSE/H.Q/7459-60), to conduct the research and to collect samples from these Schools. Further, approval for subjects and sample collection was also taken from the principals of respective schools and written assent were taken from students' parents. The students and the school authorities were briefed about the study and the assent forms were distributed. After taking permission from schools, data was collected in two visits:

Oral examination was performed for assessing caries status during first visit. The examined participants were placed into two major study groups based on their DMFT/dmft score:

1) Group I: Participant without any carious lesion and having DMF/ dmft score equal to zero (0) were placed in Caries free (CF) group

2) Group II: Participant having DMFT/ dmft score of at least (≥ 4), were included in the Caries active (CA) group,

Oral Examination for Caries Activity:

Single trained examiner conducted the clinical examination of all the subjects. To detect caries a dental mirror was used, using the WHO caries assessment criteria; DMFT/dmft index (30).

Collection of Unstimulated Saliva:

Passive Drool Method was used for unstimulated whole saliva collection. To remove any bias due to circadian rhythm all saliva samples were collected between 9 am to 11 am. Participants were instructed to abstain from consuming any food or beverage an hour prior to sample collection. The participants were asked to sit relax into chair with head bowed down. Then asked to place the collection tube closed to lower lip and let the saliva dribble in the graduated collecting tube kept in ice containing plastic glass near the lower lip. Time was noted at the start of this drill. Saliva was collected for 10 minutes. The collected saliva is then transported to laboratory kept in icebox.

Salivary Analysis:

Salivary Flow Rate Estimation:

Flow rate was calculated by dividing the collected amount with time required for collection and recording in milliliter per minute (ml/min). The measurement of whole unstimulated salivary flow scored as: low (< 0.25ml/min), normal (0.26-0.37ml/min) and high (> 0.37ml/min).

pH Measurement:

Digital pH meter (Model 3510 Jenway) was used to measure saliva pH. pH of each saliva sample was measured 3 times and mean value was recorded as result. The measurement of whole unstimulated salivary pH was scored as: low (< 6.6), normal (6.7–7.9) and high (> 7.9).

Buffering Capacity of Saliva:

Ericsson method for measuring buffering capacity was used (31)with 0.5 ml of saliva titrated by 1.5ml of 5mmole/l HCl (Merck), then the shaken mixture was centrifuged for a minute. After resting for 10 minutes the pH of this mixture is measured using digital pH meter and the variation in pH is recorded. The measurement of salivary Buffer capacity was scored as: low (< 4.5), medium (4.5–5.5) and high (> 5.5).

3.13 Data Analysis Procedure:

Statistical Package for the Social Sciences software v.18.0 was used for statistical analysis. Descriptive statistics were analyzed and presented as mean, standard deviations, frequencies, and percentages. Mann Whitney U test was used to compare caries free, and caries active groups. Level of significance set at 0.05. To estimate the relationship of selected physiochemical properties with caries free and caries active groups, risk prediction model was estimated using logistic regression. Results are expressed in crude and adjusted odds ratio.

Results

Demographic distribution and descriptive statistics of study populations are explained in Table 1, study population was comprised of N = 700 (350 boys and 350girls). The number of subjects selected from three socioeconomic classes and distribution of these subjects in private and public schools. Participants

were examined to evaluate their dental caries status. Based on dmft/DMFT score they were then stratified into caries free (CF; N = 350) and Caries Active (CA;N = 350)groups.

UW Saliva flow rate and pH were reported as low, normal, and high values; with low flow rate reported in 15.6%, 46.7% have normal salivary flow and 37.7% higher value of salivary flow. Similarly, pH value of UWS was found to be low in 13.9%, normal in 56.1% and high in 30% of the subjects. The buffer capacity of UWS also showed range of value from low to high. 18.1% subjects showed lower value, 75.7% had values in medium range and 6.1% of subjects has higher value of buffering capacity (Fig. 1).

Table 1
Distribution of Demographic Factors, DMFT/dmft and Caries Activity

Age	**Gender**		**Total**
	Boys (350)	**Girls (350)**	
6–8 Years	90 (25.7)	91 (26)	181(25.8)
9–12 Years	124 (35.4)	123 (35.1)	247 (35)
13–16 Years	136 (38.8)	136 (38.8)	272(38.8)
Socioeconomic Class	**School**		**Total**
	Public (350)	**Private (350)**	
Upper	116 (33.1)	116 (33.1)	232 (33.1)
Middle	117 (33.4)	117 (33.4)	234 (33.4)
Lower	117 (33.4)	117 (33.4)	234 (33.4)
Caries Activity	**DMFT Score**	**Frequency**	**Total**
Caries Free	0	350 (50)	350(50)
Caries Active	4	191 (27.28)	350(50)
	5	145 (20.71)	
	6	10 (1.4)	
	7	4 (0.57)	
	Total	700 (100)	700(100)
Results are expressed in N (%)			

Comparison of Salivary Parameters between the Groups:

The mean DMFT/dmft score in CA group was 4.480 ± 0.68. Mean UWSFR in CF group was found as 0.391 ± 0.07ml/min while in CA group it was observed to be 0.30 ± 0.081ml/min. Mean salivary pH in CF

group was 6.99 ± 0.23 while in CA group it was observed to be 6.85 ± 0.27. Mean salivary buffering capacity in CF group was 5.12 ± 0.31 while in CA group it was observed to be 4.86 ± 0.38 (Table 2). There is significant difference between the mean values of the CF and CA group indicate that the two groups had appreciable difference with CF group have higher mean values than CA group (Fig. 2).

Table : 2: Comparison of Salivary Parameters in Caries Free and Caries Active Groups

Variables		Caries Free n = 350	Caries Active n = 350	Significance
DMFT	Mean ± SD	-	4.48 ± 0.68	-
	Range	-	4–7	
Flow Rate	Mean ± SD	0.388 ± 0.076	0.30 ± 0.081	< 0.001*
	Range	0.20–0.70	0.14–0.60	
pH	Mean ± SD	6.99 ± 0.23	6.85 ± 0.27	< 0.001*
	Range	5.31–7.32	5.49–7.31	
Buffering Capacity	Mean ± SD	5.12 ± 0.31	4.86 – 0.38	< 0.001*
	Range	4.21–5.87	4.12–6.70	

Risk assessment model for dental caries:

Univariable analysis showed, that subjects with low salivary flow rate were 30 times more prone for dental caries than subjects with high salivary flow rate (OR = 30.30; p < 0.001), for the subjects even with normal flow rates they are 4 times more prone to dental caries than subjects with high flow rate (OR = 4.03; p < 0.001). For pH, 4 times higher likelihood for dental caries occurrence was observed in subjects with low salivary pH as compared to subjects with high salivary pH (OR = 4.54; p < 0.00). Subjects with normal salivary pH value were 1.3 times more prone for having dental caries as compared to the subject with high salivary pH (OR = 1.3; p < 0.001). For buffer capacity, 19.85 times higher likelihood for dental caries occurrence was observed in subjects with low salivary buffer capacity as compared to subjects with high salivary buffer capacity (OR = 19.85; p < 0.001). Subjects with normal salivary buffer capacity value were 1.4 times more prone for having dental caries as compared to the subject with high salivary pH (OR = 1.45; p = 0.24). Adjusted model using multivariate analysis depicted that in the presence of other significant factors in crude model, flow rate, pH and buffer capacity were sorted out to be significant. Among all the factors, low flow rate and low buffer capacity was the riskiest factor for dental caries in our setup (Table 3).

Table 3
Crude and Adjusted model for the Prediction of dental caries

Salivary Parameters	CF	CA	Chi-Square	Crude		Adjusted	
				OR	P-Value	OR	P-Value
Flow Rate							
Low	10(9.2)	99(90.8)	< 0.001	30.30	< 0.001*	20.06	< 0.001*
Normal	141(43.1)	186(56.9)		4.03	< 0.001*	2.63	< 0.001*
High*	199(75.4)	65(24.6)		1.0	< 0.001*	1.0	< 0.001*
pH							
Low	23(23.7)	74(76.3)	< 0.001	4.54	< 0.001*	1.82	0.907
Normal	204(51.9)	189(48.1)		1.31	< 0.001*	0.97	0.069
High*	123(58.6)	87(41.4)		1.0	0.118	1.0	0.110
Buffer Capacity							
Low	12(9.4)	115(90.6)	< 0.001	19.85	< 0.001*	8.76	0.793
Medium	309(58.3)	221(41.7)		1.48	0.244	1.10	< 0.001*
High*	29(67.4)	14(32.6)		1.0	< 0.001*	1.0	< 0.001*
***Reference category in logistic model.							

Discussion

Present study investigates the salivary properties that influence the development of dental caries. This study measured salivary flow among school going children between the ages of 6 to 16 years. Results showed significant variability in unstimulated salivary flow, with lower reference limit found to be 0.14ml/min. Previous reports showed the similar trend in children and adolescent population (32–34). A positive correlation of buffer capacity with flow rate was found, with the calculated buffering capacity mean value (4.99 ± 0.37) found to be comparable to previous studies as most of the subjects showed good buffering capacity values(11) .

Mean values for unstimulated salivary flow in CF individual was 0.38 ± 07ml/min while in CA individuals were 0.30 ± 0.08ml/min. When the results of this study were compared between the two groups, the

resting salivary flow rates were observed to be higher in the CF group than in the CA group at statistically significant level $p < 0.001$. Analogues results were reported previously(35, 36)where resting salivary flow rate was increased highly significantly in CF individuals. It was also like the other observations where the results of various studies showed that alterations in saliva properties play an essential role in protection against dental caries (37–39).

It is observed that the rate of oral clearance is high when the flow rate is faster(4). Reduced salivary flow was associated with high caries experience, reflecting the decrease of oral clearance capacity and protective salivary components. A normal salivary flow rate provides strong protection against the development of dental caries (40). Logistic regression analysis showed that subjects with decreased saliva flow rate have high likelihood of developing caries. These findings are supported by studies conducted by other researchers where they reported that hyposalivation lead to dental caries (38, 41). As proven by the current study an unstimulated salivary flow rate of less than 0.30 ml/min is considered a potential risk factor.

The analysis of the salivary pH showed that the majority 56% of participants had pH within normal ranges, few samples showed value of pH reaching critical limit. Highly significant ($p < 0.001$) association reported between mean value of pH for CF and CA groups. Previous studies however showed no significant difference between salivary pH and mean caries score(39, 42).

The result of the multivariate analysis showed that even with slightly negative association, there was still increased odds of developing dental caries in individuals with slightly lower value of salivary pH or even if it is falling under normal range. Result indicated that salivary buffering capacity has a statistically significant linear relation with dental caries. These results are similar to prior studies which concluded that increased in caries activity due to decreased in buffering capacity (18, 42, 43) .

Conclusion

Positive association was observed between saliva flow rate and dental caries status as subjects with active caries have a significantly lower resting salivary flow rate. As the objective here to find the association between dental caries and physicochemical salivary biomarkers including flow rate, pH and Buffer capacity.

Strength of Study

This study assisted in better understanding the relationship of dental caries with selected physicochemical marker of saliva (flow rate, pH and buffering capacity). This was a novel concept using large sample size, young healthy subjects, and provides the base line values of these salivary markers in Pakistani population.

Limitations of Study

As the approached used for dental caries was purely visual and no dental radiographs were used, there is possibility of underestimation of burden of caries. Climate condition like humidity, temperature, and level of hydration of subject can also affect the values of salivary parameters. Flow rate, pH, and buffer capacity, which is proven to be positively associated with dental caries.

Recommendations:

Measuring salivary flow rate, pH and buffer capacity should be included as part of the routine dental examination, this will assist in assessing the individual's susceptibility to dental caries. Dentists may be able to improve care plan with early detection and control of dental caries. Future longitudinal studies are needed to learn more about relation of saliva and dental caries. Other potential predictors and cofounders in regression for multilevel modelling should be included in future studies. A further insight is needed on the bacterial etiology of dental caries along with salivary component.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available as ethics approval was granted on the basis that only the researchers involved in the study could access the identified data but are available from the corresponding author on reasonable request.

Abbreviations

CA =Caries Active

CF =Caries Free

SFR =Salivary Flow rate

UWSFR =Unstimulated whole salivary flow rate

pH =log of Hydrogen ion

BC =Buffer Capacity

HCl = Hydrochloric acid

DMFT=Decay Missing Filled teeth

OR = Odds Ratio

SPSS = Statistical Package for the Social Sciences

Declarations

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Contributions

ASA was the principal investigator and has contributed to concept, design, data acquisition, analysis, and interpretation, and drafted the manuscript. IA has contributed to the conception, design, and interpretation of results. MAQ has contributed to the conception, critically revised the manuscript. MH has contributed to revising the manuscript critically and contributed to statistical analyses. ZM has contributed to manage the data, statistical analysis and results compilation. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. This analytical cross sectional research was approved by the Institutional Review Board of Dow University of Health Sciences (Ref: IRB-293/DUHS-11). Permission was taken from Directorate of School Education, City of Karachi (DSE/H.Q/7459-60) to conduct the research and to collect samples from these Schools. Further, approval for subjects and sample collection was also taken from the principals of selected schools and written informed consent were taken from participants and parents (for under 16years participants).

Consent for publication

Not applicable.

Competing interests

Thera have no competing interests (financial and non-financial) related to the article.

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Figures

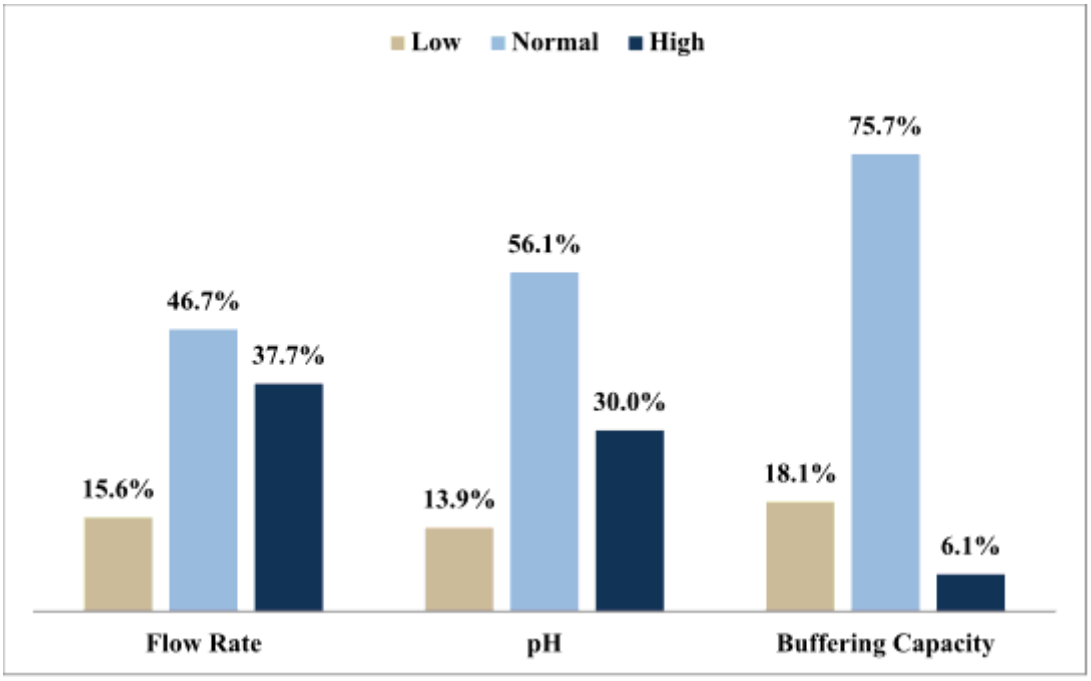

Figure 1

Percentage Distribution of Salivary Parameters in Low, Normal and High Value

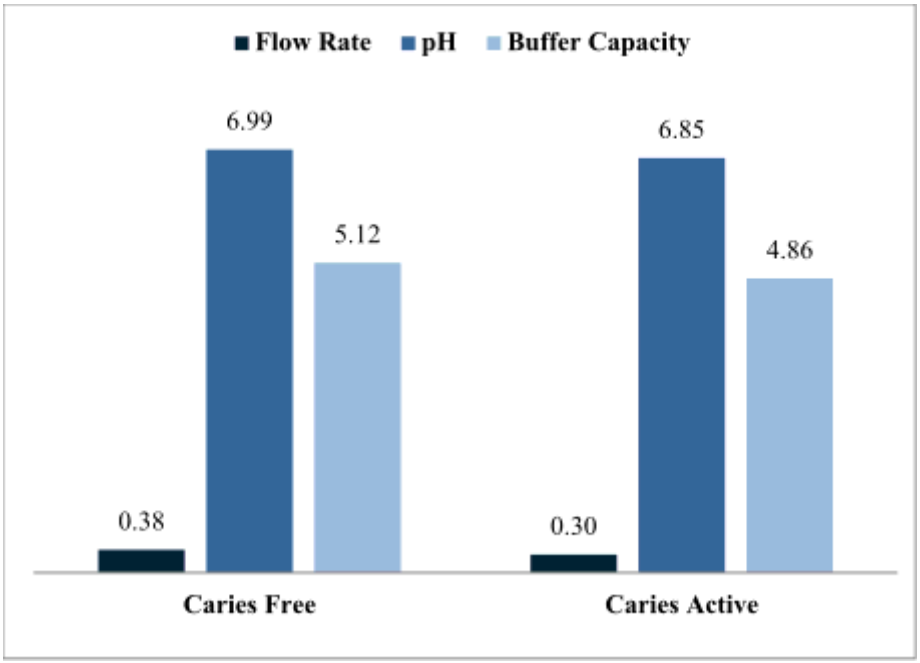

Figure 2

Comparison of Mean Flow Rate, pH And Buffer Capacity in Caries Free and Caries Active Groups