

Comparative Study of Saliva Flow Rate, pH and Buffer Capacity in Caries-Free and Caries-Active Ugandan Adolescent Students

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ABSTRACT:- Saliva is an essential factor in the development and prevention of dental caries. This is dependent on its quantity and composition, thus variations in the physicochemical properties of saliva may predispose an individual to caries. This study aimed to compare the saliva flow rate, pH and buffering capacity in caries free and caries active adolescents. This comparative cross sectional study comprised of one hundred and five healthy adolescent students aged 11-19 years, divided into three groups based on their dental caries status. Each group consisted of 35 participants; Group I (DMFT=0), group II (DMFT=1-3) and group III (DMFT ≥4). Both unstimulated and stimulated saliva were collected and the flow rates, pH and buffering capacity estimated using the GC Saliva-Check Buffer Kit (GC Corporation, Tokyo, Japan). The data was statistically analysed using Chi-Square test and ANOVA. The intergroup comparison was carried out using the Turkey's HSD Posthoc test. Statistically significant value was inferred at $p < 0.05$. The mean unstimulated saliva flow rate of adolescents without caries (1.39 ± 1.12) was significantly higher than those with high caries activity (0.81 ± 0.38) with mean difference (Std Err) of $0.586(0.17)$ ($p = 0.003$). The mean stimulated saliva pH values of adolescents without caries (7.7 ± 0.20) was significantly higher than those with moderate caries activity (7.5 ± 0.20) with mean difference (Std Err) of $0.194(0.052)$ ($p = 0.001$). The three groups had comparable mean stimulated saliva flow rates ($p = 0.702$) and buffer capacity points ($p = 0.304$). The properties of saliva such as unstimulated flow rate and pH may be useful in caries risk assessment.

Keywords:- Saliva, flow rate, pH, buffer capacity, caries free, caries active

I. INTRODUCTION

Saliva is an essential factor in the maintenance of oral health including the prevention of dental caries [1]. The ability of saliva to affect caries development is dependent upon the quantity and composition of the secretions, thus alterations in these factors may affect caries status [1, 2]. The anti caries functions of saliva include its mechanical flushing action, inhibiting demineralization, enhancing remineralization and the antimicrobial function [2-5]. In clinical practice, saliva can be evaluated by its physicochemical properties, such as flow rate, buffering capacity, hydrogen-ion concentration (pH) and consistency. Variations of these saliva factors may increase the risk of caries in an individual [6-8].

Several studies especially in Caucasian populations have investigated the correlation between the physicochemical properties of saliva and dental caries status [7, 9-14]. Most of the studies, for example [7, 11, 12] have observed an association between these factors and dental caries status. Associations of saliva parameters and dental caries status coupled with other factors have led to the development of new predictive models for the prevention and management of dental caries [15-18]. Such models emphasize the role of saliva as a risk factor for dental caries. Subsequently, the saliva parameters of pH, buffer capacity and flow rate have been devised as diagnostic tools in comprehensive caries risk assessment models [3, 8].

Within the African perspective, there is scanty literature in determining the normal values for these saliva parameters. Equally, there is not enough literature to determine the relationship of these factors with dental caries status [19, 20]. Yet, it is probable that there may be variations in the mean normal values for saliva parameters within Caucasian or African population due to difference in environmental factors and dietary habits. There has not been much research on this subject in the context of Uganda. However, the most recent national oral health survey indicates that there is high (90%) prevalence of dental caries in several communities of Uganda [21]. Several African countries have reported similar observations in the recent past [22, 23]. This

statistic thus emphasizes the need for developing new measures for prevention and management of dental caries and it is upon this that this study is premised.

This study set out to determine the relationship between dental caries status against the saliva parameters of flow rate, pH and buffer capacity.

II. MATERIALS AND METHODS

Study design and study location

The present comparative and cross-sectional study was carried out over a period two weeks from January to February 2018 at two secondary schools in Uganda. A total of one hundred and five adolescent students between the age of 10 and 19 years participated in the study. This investigation was part of a cross sectional survey that evaluated the prevalence and factors associated with dental caries among adolescents attending two secondary schools in Uganda.

Sample size calculation

The sample size was estimated using the formulae: $N=f(\alpha\beta) \times 2 \times SD^2 / (d^2)$; where $f(\alpha,\beta) = 10.5$ for 90% power with 5% significance, SD is the standard deviation of the main event of interest (i.e. buffer capacity), d is the clinically important effect size. According to one study, [14] among caries free and caries active adolescents, they reported a significant difference in buffer capacity between the groups with a mean buffer capacity of 41.22 ± 16.99 for the caries active group [14]. Assuming $f(\alpha\beta) = 10.5$ for 90% power with 5% significance, the total sample size was 34 participants per group, to which an adjustment of one participant was made to cater for any errors.

Subjects and selection method

One Hundred and five study participants were recruited by convenience sampling from the adolescent students who had participated in the cross-sectional survey. The students were divided into three groups each consisting of 35 participants basing on their dental caries status as follows:

Group I (n=35) - caries-free participants (DMFT score=0);

Group II (n=35) - participants with moderate caries activity (DMFT score=1-3); and

Group III (n=35) - participants with high caries activity (DMFT score ≥ 4)

Inclusion criteria

The inclusion criteria were: (1) adolescents free from systemic or local diseases that affect salivary gland secretions (such as submandibular duct canaliculi, asthma and diabetes), (2) they did not smoke, and (3) they did not use any medications or mouth rinses for the last two weeks.

Exclusion criteria

The exclusion criteria were: (1) adolescents who were physically and medically compromised, (2) adolescents who were using any medications or using mouth rinses for the last two weeks, and (3) the inability to cooperate and/or to follow the instructions given regarding the saliva collection.

Data collection

Clinical examination

The clinical examination was carried out to score the caries status of the participants by four examiners. The caries status was scored using the decayed-missing-filled teeth (DMFT) index according to World Health Organization criteria [24]. The examiners were trained and calibrated according to WHO basic methods for oral health surveys [24].

Saliva sampling

Saliva was collected as per the protocol stipulated on the GC Saliva-Check Buffer kits (GC Corporation, Tokyo, Japan). These methods are similar to saliva collection techniques described by Navazesh and Kumar, 2008, and are used at University of Southern California School of Dentistry. The methods have been found to be convenient [25]. Saliva collection procedures were carried out with the participants seated comfortably in a quiet environment in the early hours of the day between 10 am and 12 noon. Subjects were instructed not to eat for one hour prior to saliva collection.

Collection of unstimulated saliva: Unstimulated mid-morning whole saliva sample was collected for each adolescent. After a few minutes of relaxation on sitting on a chair, the students were trained to avoid swallowing and collect saliva into a clean calibrated container. The saliva was allowed to drool into the container continuously for five minutes. The volume of unstimulated saliva collected for the five minutes was measured and recorded excluding the froth. This volume was used to calculate unstimulated saliva flow rate (ml/min.).

Collection of stimulated saliva: The stimulated saliva sample were collected in our study by spitting method that appeared to be the most reproducible. Stimulated whole saliva samples were collected to determine the salivary flow rate, pH and buffer capacity. The participants were asked to chew a supplied piece of wax and

were told to swallow all the saliva that accumulated during the first 30 seconds. Thereafter, the students were requested to continue chewing the wax for an additional five minutes, expectorating every 15-20 seconds all the saliva which collected in the mouth into a sterile calibrated cup. The volume of saliva collected in the calibrated cup was measured and recorded excluding the froth. This volume was used to calculate stimulated saliva flow rate (ml/min)

Determining stimulated saliva pH and buffer capacity

Buffer capacity and pH were measured using the respective test strips from the GC Saliva-Check Buffer Kit (GC Corporation, Tokyo, Japan) following the manufacturer’s instructions. The measurements were taken and recorded by one examiner.

Buffering capacity: A sufficient amount of stimulated saliva was drawn from the collection container using a pipette and one drop of saliva was dispensed onto each of the three test pads of the test strip. After 2 minutes, the observed colour was recorded for the three test pads on comparison with available buffer capacity testing chart. The combined total buffer capacity points for each saliva sample were then calculated by adding the points the three test pads. Buffering capacity of stimulated saliva was considered to be very low if the value of test was 0-5, low for 6-9 and normal/high for a value of 10-12.

Saliva pH: Stimulated saliva pH values were measured by dipping pH strip into the saliva samples for 10 seconds and comparing with a reference chart.

Ethical clearance

Ethical clearance was obtained from Makerere University, School of Health Sciences Institutional Review Board (SHSREC REF: 2017-039) as well as Uganda National Council of Science and Technology. Permission to carry out the study was obtained from the district and school authorities. Informed consent was obtained from participants aged ≥18 years and the parents or guardians of those aged 11 to 17 years. Informed assent was also obtained before the study. The nature of the study and the participants’ right to accept or refuse to take part in the study were dully explained to the participants in accordance with the Helsinki Declaration [26].

Statistical analysis

Data were analysed using STATA software version 16.0. Qualitative data were presented as numbers and percent while quantitative data were presented as mean ± standard deviation. Chi-square test was used for comparison between groups for qualitative data. One- way ANOVA with post hoc Tukey’s test were performed to evaluate significant intergroup differences between dependant and independent variables. P<0.05 was considered as statistically significant.

III. RESULTS

Distribution of study participants

Table 1 shows the distribution of the study participants by gender and dental caries status. Hundred and five participants with a mean age of 15.21±2.08 were included in the study divided in three groups: caries-free: DMFT=0 (n=35), moderate caries activity: DMFT=1-3 (n=35) and high caries activity: DMFT above 4 (n=35). The three groups were matched to gender (Table 1).

Table 1: Distribution of the study participants based on dental caries status and gender.

Characteristic	Caries Status			P value
	DMFT=0 (n=35)	DMFT=1-3 (n=35)	DMFT ≥4 (n=35)	
Gender				
Male	17	14	18	0.608
Female	18	21	17	
Overall	35	35	35	

DMFT - Decayed–missing–filled teeth; n-number;
p < 0.05 (chi-squared): caries status groups vs gender

Unstimulated and stimulated saliva flow rate

The stimulated salivary flow rate among the study participants (n=105) was between 0.6mls/min–3.2 mls/min with a mean (SD) flow rate of 1.97±0.60 mls/min while the mean (SD) unstimulated saliva flow rate was 1.08±0.75mls/min. Table 2 shows the mean saliva flow rate values over the groups for caries status. There was a significant difference in the mean unstimulated saliva flow rate for adolescents in relation with dental caries status (p=0.004). The mean unstimulated saliva flow rate of adolescents without caries (1.39±1.12) was

significantly higher than those with moderate caries activity (1.04±0.40) or high caries activity (0.81±0.38) (p = 0.004). The three groups had comparable mean stimulated saliva flow rates (p=0.702) (Table 2).

Table 2: Unstimulated and stimulated saliva flow rates over the caries category for the adolescents

Caries status	Unstimulated saliva flow rate (ml/min)	Stimulated saliva flow rate (ml/min)
	mean±SD	mean±SD
Group I (DMFT=0)	1.39±1.12	2.03±0.59
Group II(DMFT=1-3)	1.04±0.40	1.91±0.52
Group III (DMFT≥4)	0.81±0.38	1.98±0.69
Probability	0.004†	0.702†

SD-standard deviation [95% confidence-interval]; n- number; %-percentage;
 †p < 0.05 (Anova): groups for DMFT =0 vs DMFT 1-3 and Dmft 4 ≥ above

Inter group comparison of the unstimulated saliva flow rate was carried out using the Tukey HSD. The results between caries free group (DMFT=0) and group III individuals (DMFT≥4) was found to be significant (p=0.003), but the results between caries free group (DMFT=0) and group II (DMFT=1-3) (p=0.110), group III (DMFT≥4) and group II (DMFT=1-3) (p=0.356) were not significant [Table 3].

Table 3: Intergroup comparison of unstimulated saliva flow rate using the Tukey HSD test

Multiple comparison (Turkey HSD) post hoc					
Dependent Variable	Group (I)	Group (J)	Mean difference (I-J)	Std Error	p value
Unstimulated saliva flow rate	Group I (DMFT=0)	Group II (DMFT=1-3)	0.349	0.172	0.110
	Group I (DMFT=0)	Group III (DMFT≥4)	0.586	0.172	0.003
	Group III (DMFT≥4)	Group II (DMFT=1-3)	0.237	0.172	0.356

Std Error- standard error

Saliva pH and buffer capacity

Table 4 shows the mean values for saliva pH and buffer capacity points over groups for caries status. The table also shows the proportions of the participants categorised by buffer capacity over the caries status. There was a significant difference in the stimulated saliva pH for the three groups (p=0.001). The mean stimulated saliva pH values of adolescents without caries (7.7±0.20) was significantly higher than those with moderate caries activity (7.5±0.20) or high caries activity (7.5±0.30) (p = 0.001). The three groups had comparable mean buffer capacity values (p=0.304) (Table 4).

Table 4: The values of the saliva pH and buffer capacity over the caries category for the adolescents

Caries Status	Stimulated saliva pH	Buffer capacity	Categories of buffer capacity		
			Normal/High (10-12 points)	Low (6-9 points)	Very low (0-5 points)
	mean±SD	mean±SD	n (%)	n (%)	n (%)
Group I(DMFT=0)	7.7±0.20	10.17±3.22	26(74.29)	5(14.29)	4(11.43)
Group II(DMFT=1-3)	7.5±0.30	8.89±3.86	20(57.14)	10(28.57)	5(14.29)
Group III (DMFT≥4)	7.5±0.20	9.37±3.39	21(60.00)	9(25.71)	5(14.29)
Probability	0.001†	0.304†			0.589*

SD-standard deviation [95% confidence-interval]; n- number, %-percentage;

†p < 0.05 (Anova): groups for DMFT=0 vs DMFT 1-3 and Dmft 4 ≥ above

*p < 0.05 (chi-squared): comparing proportions of participants with normal buffer capacity vs Low and very low buffer capacity

The inter group comparison of the saliva pH using the Tukey HSD yielded significant results between caries free (DMFT=0) students and group II (DMFT=1-3) ($p=0.001$), but difference between the result for students in both groups with dental caries (group III and group II) was not significant ($p=0.327$) [Table 5].

Table 5: Intergroup comparison of stimulated saliva buffer capacity and pH using the Tukey HSD test

Multiple comparison (Turkey HSD) post hoc					
Dependent Variable	Group (I)	Group (J)	Mean difference (I-J)	Std Error	P value
pH	Group I (DMFT=0)	Group II (DMFT=1-3)	0.194	0.052	0.001
	Group I (DMFT=0)	Group III (DMFT≥4)	0.120	0.051	0.058
	Group III (DMFT≥4)	Group II (DMFT=1-3)	0.074	0.052	0.327

Std Error- standard error

IV. DISCUSSION

In the present study, the caries free adolescents had a significantly higher unstimulated salivary flow rate and pH values compared to caries active adolescents. The mean stimulated saliva flow rate and buffer capacity points were comparable for the caries active and caries free adolescents in the study.

In this study, the unstimulated saliva flow rate correlated with dental caries severity while no significant correlation between caries activity and stimulated salivary flow rate were established. These findings are in support of the recommendation that unstimulated whole saliva flow rate is the proposed test of choice to detect a reduced salivary flow, it's the major determinant of salivary clearance. As the unstimulated saliva flow rate may be reduced, even if the stimulated whole saliva is unaffected [27-29]. The decreased mean unstimulated saliva flow rate observed among the caries active groups compared to the caries free group in this study is in agreement with previous studies [9, 19, 30, 31]. In general, the lesser the flow rate of saliva, the poorer the cleansing action on tooth surfaces, hence the greater the microbial attacks and greater the risk of dental caries.

The normal salivary flow rate values are approximately (0.25-0.35 ml/min) for unstimulated saliva and (1-3ml/min) for the stimulated saliva [1]. On comparing the mean saliva flow rate values for this study and reported mean values in Caucasian populations of similar age [1, 10, 32-34]; the values for this study are higher. Similarly high mean saliva flow rate values have been reported by the few studies done in African populations [20, 31]. Adeniji, Jeboda and Salado [20] observed high saliva flow rate values for Nigerian adolescents and young adults aged 20-25 years and, they attributed the high values in their African population to difference in climate and diet. The tropical climate leads to consumption of more water in comparison with the temperate climate. This, coupled with intake of a highly fibrous diet, leads to a lot of masticatory actions and increased salivary gland size in the Africans. Probably explaining the higher saliva flow rate values for the studied African populations in comparison to Caucasians. However, Oluwadaisi, Oziegbe and Akinsomisoye [19] observed mean saliva flow rates values similar to the mean values for Caucasians among 6-12 year old Nigerian children.

In this study salivary pH values were found to be significantly higher in caries-free group compared to caries active adolescents ($p < 0.001$). Other studies are in agreement with this study and they found a negative correlation between salivary pH and dental caries [9, 30, 35]. While several studies have reported contrasting results where no correlation was observed between saliva pH and dental caries [10, 19].

Buffering agents in saliva try to bring the pH back to the normal range as fast as possible when the oral cavity is exposed to food items whose pH differs from normal pH (6.5-7.5) of saliva. In our study, buffer capacity values were comparable for caries active groups and caries- free group. This result differs from other studies that found saliva buffer capacity values had an inverse relationship with presence of dental caries [9, 19, 30, 31]. On contrast, this result is agreement with Pandey et al, 2015 [10].

V. CONCLUSION

Caries risk assessment still remains an interesting research area, given its complexity. However, this research set out to compare some saliva parameters against dental caries status. It has been observed that the caries free adolescents had significantly higher unstimulated saliva flow rates and pH values than caries active

adolescents. However, there was no significant correlation between caries status and buffer capacity or stimulated saliva flow rate.

These results thus resonate well with previous studies that the various saliva parameters such as salivary flow rate, pH may act as markers of caries status. While these results cannot be generalised due to the sample size selected for this study, there is a need for undertaking a larger study so as to determine the mean values in regard to specific age groups and gender for the Ugandan population.

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