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Salivary Flow Rate, Buffering Capacity and Dental Caries Among 6-12year Old Schoolchildren, Age and Gender in Nigeria: A Comparative Study.

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Abstract
Introduction
Despite the decrease in the prevalence of dental caries globally, its impact on children can be life threatening. These impacts have been found to constitute health problems which affect their quality of life[. 2] Saliva plays an important role in protecting the teeth from dental caries.
Aim
To compare the flow rate and buffering capacity of saliva in dental caries active and caries free subjects.
Design
A comparative study that included a representative samples of one hundred and sixty healthy children who were selected from 10 public primary schools in Ibadan and were divided into 80 caries active group and 80 caries free group. Unstimulated saliva samples were collected inside a graduated measuring tube fitted into a funnel over a period of 5minutes by spitting and salivary flow rates were measured. Buffering capacity of each saliva sample was determined by titration of 3ml of 0.005 mol/L hydrochloric acid (HCL) solution with 1ml of saliva, and the buffering was measured using a pH meter. The data was statistically analysed using student t test, ANOVA and Turkey's HSD Posthoc test.
Result
Mean ±SD age of study participants was 9.6 (2.6) years. The mean ±SD DMFT/dmft of age 6-8 years and 9-12 years were 0.6±0.10/1.54±1.84 and 1.21±1.53/1.09±1.23 respectively. The mean ±SD salivary flow rate and salivary buffering capacity were 1.0±0.5mls/min and pH 6.3±0.7. Caries free participants had highest mean SFR(1.1±0.5mls/min) and SBC (pH 6.4±0.6) respectively. The majority (70.6%) and 77.6% of the participants had SFR greater than 0.7mls/min and SBC greater than pH> 6.0 respectively. A steady and slight reduction in the mean SFR and SBC was observed as the DMFT/dmft increases (p>0.05).
In conclusion, the study demonstrated lower salivary flow rate and salivary buffering capacity among caries active participants when compared to caries free participants.

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I. Introduction

Dental caries is a post eruptive pathological process of external origin involving demineralization of dental hard tissues progressing to cavity formation[1]. Despite the decrease in the prevalence of dental caries globally, its impact on children can be life threatening and these impacts have been found to constitute health problems which affect their quality of life[2.]

The aetiology and pathogenesis of dental caries was initially depicted as the interaction between cariogenic bacteria, fermentable carbohydrates and the susceptible tooth but recently, many modifying factors including saliva have been recognised[3].

The role of saliva in dental caries prevention depends on its viscosity, its composition and its antibacterial properties. More importantly, the role of its mechanical cleansing properties which depends on its flow rate and its neutralization of acids produced by caries bacteria properties which depends on its buffering capacity cannot be under emphasised[4].Various authors in developed countries have reported increase in dental caries incidence in people with impaired salivary flow rate and impaired buffering capacity[5,6.]. There is paucity of information in Nigeria on the relationship between salivary flow rate, salivary buffering capacity and dental

caries among Nigerian children. Therefore, the aim of this study is to compare the flow rate and buffering capacity of saliva in active dental caries and dental caries free children.

II. Materials and methods

The study included a representative sample of one hundred and sixty healthy children aged 6 to 12year old from 10 randomly selected public primary schools in Ibadan North Local Government, Oyo State.Ethical approval Number UI/EC/13/0121 was obtained from the University of Ibadan/University College Hospital, Ibadan, Ethical Review Committee. Permission to undertake the study was obtained from Oyo State Universal Basic Education Board (SUBEB) and Head teachers of the primary schools.

Informed consent forms written in English language as well as Yoruba the local language of inhabitants of Ibadan North local government, Oyo state were also given to children within the age group to be delivered to their parents/guardians.

Only children with cooperative behaviour whose parents / guardian consented to participate in the study and who had no systemic / local diseases which may affect salivary secretion as well as children who had no oral exposure to food 1 hour before the commencement of the saliva collection were included in the study.

At the respective schools, according to WHO method of caries assessment, intra oral examinations were done outdoor under natural lightening condition and dental caries was assessed using sterile mirrors and dental probes. Eighty (80) children who had caries were selected by balloting into the caries active (DMFT/dmft=1 and above) group and 80 caries free children were selected from among the caries free (DMFT/dmft =0) group by balloting. (8 caries active and 8 caries free children were randomly selected per school). Caries experience was expressed as number of decay (Dd) teeth, teeth missing due to caries (Mm) and filled (Ff) teeth (DMFT/dmft) (Oral Health Surveys, 1987).

Using the Erricsson /Spitting method, unstimulated saliva samples were collected inside a graduated measuring tube fitted into a funnel over a period of 5minutes. The samples from caries free were labelled CF and samples from caries active were labelled CA. Salivary flow rates were measured directly from the graduated tubes and calculated per minutes.

Buffering capacity of each saliva sample were determined by titration of 3ml of 0.005mol/L hydrochloric acid (HCL) solution with 1ml of saliva, and the buffering capacity was measured using a pH meter. The saliva flow rate and buffering capacity were classified according to WHO classifications. The Salivary Flow rates (SFR) are classified into Low (≤ 0.3ml/min), Normal (0.3-0.7ml/min) and High (≥0.7ml/min). The buffering capacity was classified into Low (pH <4.0), Normal (pH 4.5-5.5) and High (pH > 6.0).

Mean (standard) of the relevant data was generated. Student t-test was used to compare means of 2 groups, while ANOVA and Turkey's HSD Posthoc tests were used to compare differences between the means for more than 2 groups at a 5% level of significance.

III. Results

The study included one hundred and sixty (160) children (of which 80 were caries free and 80 caries active)with age range 6-12 years and mean age of 9.6 (2.1) years. Eighty one (50.6%) of the study participants were female. DMFT/dmft among the caries active study groups were: 0.60(0.10)/1.54(1.84) among 6-8years and 1.21(1.53)/1.09(1.23) among 9-12years. Likewise, DMFT/dmft were 0.5(1.45)/0.93(1.46) among females and 0.87(1.28)/1.64(1.51) among males (Table i).

The salivary flow rate among the study participants (n=160) was between 0.28mls/min - 2.60mls/min with a mean (SD) flow rate of 1.0±0.5mls/min. Higher mean values were recorded among the caries free (DMFT/dmft=0) group with a range of 0.3mls/min - 2.6mls/min and mean (SD) value of 1.1±0.5mls/min. Majority (70.6%) of the study participants presented with salivary flow rate score of 0(> 0.7mls/min) with a higher percentage (76.3%) recorded among the caries free group (P =0.22).Likewise, higher percentage of females and children aged 9-12years in both study groups have salivary flow rate score > 0.7mls/min,with higher percentage recorded among the caries free (DMFT/dmft=0)groups.(Table ii)

The buffering capacity among the study participants (n=160) ranged from PH 3.8 and Ph 7.5 with mean buffering capacity of pH 6.3±0.7. Higher values were recorded among the caries free (DMFT/dmft=0) group with a mean (SD) value of (pH 6.4±0.6. One hundred and twenty four (77.6%) of the study participants had SBC of pH >6.0 with a higher percentage (78.9%) among the caries free group.SBC was slightly higher among the caries free males and children within the age range of 6-8years. (Table iii).

A steady and slight reduction in the mean SFR and SBC was observed as the caries activities (DMFT/dmft) increased. Children with DMFT/dmft of 5-6 have the least values of SFR and SBC. The difference was statistically significant (p=0.005) (Table iv)

Intergroup comparison between the study groups using posthoc analysis showed that the decrease in SFR and SBC were not statistically significant between groups with close DMFT/dmft values but were significant between groups with obvious difference in DMFT/dmft. (Table v).

IV. Discussion

The prevalence of dental caries was known to be higher in developed countries but lately, the prevalence is increasing in developing countries including Nigeria due to increased consumption of cariogenic diets. However, studies [7,8,9] have shown that dental caries prevalence is increasing in Nigeria.

Results from this study showed a mean DMFT/dmft of 0.60(0.10)/1.54(1.84) among 6-8yrs and 1.21(1.53)/1.09(1.23) among 9-12yrs. Likewise a mean DMFT/dmft of 0.50(1.45)/0.93(1.46) and 0.87(1.28)/1.64(1.51) were recorded among females and males respectively. The mean DMFT/dmft recorded in this study is higher when compared to previous studies[7, 8, 9] among similar age groups in Nigeria. The higher prevalence observed in the present study may be due to increase in consumption of cariogenic diets in this environment. Although not reported in this study, it was observed during data collection of this study that parents of public school children frequently provide their children with a token as lunch money, which most of the time can only buy available cheap sugar containing products around the school premises.

There was increase in the mean DMFT/dmft among the 9-12yrs age groups in this study. This could be due to the fact that, dental caries is a progressive disease, the prevalence which increases with age in any population, race or social status and probably also due to longer exposure time of the dentition to cariogenic causing factors.

Previous studies observed higher risk of developing caries among females than their male counterparts due to their early tooth eruption and longer time of exposure of their teeth to cariogenic process, [10,11.] However, the contrary was observed in this study where higher prevalence of dental caries was reported among males in both age groups. Since dental caries is a multi factorial disease, the role of poor oral hygiene and accumulation of plague with high acid concentration produced by cariogenic bacteria cannot be under estimated, and could be responsible for increase in the mean DMFT/dmft reported among males in this study. This is in agreement with Migale et.al[12] in southern Italy who reported a mean DMFT of 1.96 and 3.20 among the females and males of similar age groups to this study.

The dental caries process is controlled to a large extent by a natural protective mechanism inherent within the saliva. The most important caries protective functions of saliva are the cleansing and the neutralising effects of acids which is dependent on the flow rate and buffering capacity.

The Salivary flow rate among the overall participants in this study is between 0.28 – 2.60mls/min with a mean flow rate of 1.0 (0.5)mls/min. These results are in accordance with findings by Lawrence J. Walsh [13], who documented that the total flow rate for saliva (both stimulated and unstimulated) ranges between 0.5 – 1.5litres per day in an individual, and the average volume of unstimulated saliva present in the oral cavity is 1.0 ml/min.

The decreased in the mean salivary flow rate observed among the caries active groups than the caries free groups in this study is in agreement with previous studies [5,14,15]. These also support the idea that under resting conditions without exogenous stimulation that is linked with feeding, there is a slow flow of saliva, which only keeps the mouth moist and lubricates the mucous membrane. This unstimulated flow is what is secreted by the salivary glands majority of the time and is essential for providing the protection functions to the teeth against dental caries [16]. 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 [17]. In accordance with the WHO classification for SFR, higher percentage (70.6%) of the study participants has SFR. > 0.7mls/min, with greater percentage (76.3) recorded among the caries free groups. This is in agreement with some researchers [6,18,19] who reported similar results as in this study. Kaur et.al [18] and Gullery et.al [19] reported SFR of >0.7mls.min among 90% of the caries free groups in their study, likewise, Seekinabi and Hiremath [6] reported SFR of >0.7mls/min among 100% of their caries free participants. The increase in the mean SFR observed among females in this study may be accountable for their decrease in caries activities (DMFT/dmft) when compared to their male counterpart, as documented that SFR in any individual is known to have anticanogenic and better cleansing effect [20,21].

Despite an increase in caries activities (DMFT/dmft) recorded among the older age groups (9-12yrs) in this study, their mean SFR was still higher when compared to the 6-8years age groups, This could be due to the fact that, they may find it easy to expectorate saliva than the lower age groups. This also support Crossner C G,[22] in his study to evaluate saliva flow rate in children and adolescents , who reported an increase flow rate with increase age, thereby concluded that salivary glands seems to developed more in older age groups. However, since the progression of dental caries increases with age, other cariogenic factors such as poor oral hygiene may also be accountable for their increase caries activities. This result is in support of the other researchers [5,14] who reported that the mean salivary flow rate increases with increase in age in young adults.

The quantitative assessment of resistance to pH changes is referred to as buffering capacity. There is reasonably strong evidence to indicate that salivary buffering capacity protects the tooth from dental caries [23]. Low buffering capacity has been associated with caries development because of its impaired neutralization of plaque acids and reduced remineralization of early enamel lesions [21]. Oral cavity is quite frequently exposed to

components whose pH differs from normal pH of saliva (6.5-7.5) and these components may cause damage to teeth surface. Studies [21] have shown that patient with low or no caries activity had a resting salivary pH of around 6.5 -7.5. Those with extreme caries activity had a resting pH below critical pH 5.5

Results from this study showed the overall buffering capacity of pH 3.8-7.5 with the mean pH 6.3 (0.7 SD). The mean buffering capacity was slightly but not significantly higher among the caries free subjects. These results are in agreement with several researchers [14, 5, 21, 24] who found a slightly higher SBC among the caries free groups in their studies. Likewise, a recent study conducted by Bagherian and Gholanireza [25] to compared some salivary factors between children with and without early childhood caries showed that buffering capacity was not significant but higher (8.03±0.91) among children without early childhood caries when compared to (7.43 ±0.82) children with early childhood caries. Reports of other recent studies [18, 19, 26] also agreed with the results of this study.

Even though, salivary flow rate was lower among the lower age groups in this study, the buffering capacity of their saliva was higher, although not statistically significant than that of the older age groups. This explains the reason for the lower caries activities reported among this younger age group. From this report, it could be speculated that salivary buffering capacity probably plays an important role in the prevention of dental caries than the saliva flow rate. This report also agreed with Shrutii Chauhan et.al, [27] and several authors [4,5,6] who concluded from their study that Salivary buffering capacity increases with decrease age and may be used as an index for the development of dental caries.

The increase in buffering capacity despite an increase in caries activities reported among males in this study is in contrast to previous studies [18, 19] and it may be concluded that, gender has nothing to do with buffering capacity of saliva in the initiation and development of dental caries in this study group. Other factors like poor oral hygiene and cariogenic diet may be responsible for increase in their caries activities. This report is in agreement with Pandy Pallavi et.al [28] who reported higher buffering capacity among the males in their study to evaluate buffering capacity and dental caries in relation to age and gender.

Theoretically, greater ability of saliva to neutralize acid production in the oral cavity may help to restore pH balance in the caries free group more efficiently, and this is in accordance with the findings in this study, where majority (77.6%) of the study participants has buffering capacity of pH >6.0, followed by 21.8% with buffer score of pH 4.0 -6.0 with higher percentage reported among the caries free groups. This report was in agreement with Seeknabi and Hiremath[6], who reported that majority (75%) of participants in their study presented with buffering capacity of pH >6.0, followed by 23.7% with buffer score of pH 4.5-5.5. Kaur et.al [18] also reported similar results as in this study.

The determination of flow rate and buffering capacity of saliva helps to establish the integrity of tooth. In this study, salivary flow rate and salivary buffering capacity was shown to decrease with increase in caries activities (DMFT/dmft) of the study participants, and on intergroup comparison, there was a steady decrease in the values with slight variation observed amongst group i and ii and group iii and iv. Group iv had the lowest salivary flow rate and salivary buffering capacity and higher values of DMFT/dmft. This can be concluded that flow rate and buffering capacity of saliva had a strong relationship with dental caries.This report agreed with Chitharanjan Shetty [15] who concluded that flow rate and buffering capacity of saliva are contributing factors in maintaining the integrity of a tooth, hence a decrease in these factors can results in increase caries activities.

A negative correlation was reported between the two variables and dental caries, although not statically significant. This shows a strong relationship between the flow rate, buffering capacity and dental caries.This is in agreement with Tenovou [29] who also reported a negative correlation between flow rate, buffering capacity and caries activities in a population, thereby concluded that decrease in salivary buffering capacity leads to a drastic increase in caries susceptibility as the enamel dissolution by plague acids is left uncontrolled.

In conclusion, children in this study had demonstrated low caries activities in term of DMFT/dmft. Participants with lowest salivary buffering capacity and salivary flow rate have the highest caries activities.

Tables and illustrations:

DMFT/dmft	**Age of caries active participants**		**Gender of caries free participants**	
	6-8 yrs n=	**9-12yrs n=**	**Male n=**	**Female n=**
Mean(SD)DMFT	**0.60(0.10)**	**1.21(1.52)**	**0.87(1.28)**	**0.50(1.46)**
Mean (SD) dmft	**0.15(1.84)**	**1.09(1.23)**	**1.64(1.51)**	**0.93(1.46)**

Table 1: Mean (SD) DMFT/dmft of study participants by Age and Gender.

Caries Status	Mean Salivary flow rate (mls/min	P - value	Salivary Flow Rate score in mls/min			P – Value
			0 (>0.7)	1(0.3-0.7)	2 (<0.3)	
CA (80) CF (80)	1.0 ± 0.5 1.1 ± 0.5	0.22	52(65.0) 61(76.3)	28(35.0) 18 (22.4)	0(0.0) 1 (1.3)	0.22
Age range of study participants in years in relation to salivary flow rate						
CA 6-8 (33) 9-12 (47)	 0.8 ± 0.3 1.0 ± 0.4	 0.24	 18(54.5) 35(74.5)	 15(45.0) 12(25.5)	 0(0) 0(0)	 0.39
CF 6-8 (20) 9-12 (60)	 0.9 ± 0.5 1.2 ± 0.5	 0.39	 11(55.0) 49(81.7)	 9 (45.0) 10(16.7)	 0(0) 1(1.6)	 0.24
Gender of study participants in relation to salivary flow rate.						
CA F(41) M(39)	 1.0 ± 0.4 0.9 ± 0.4	 0.57	 28(68.3) 25(64.1)	 13(31.7) 14(35.9)	 0(0.0) 0(0.0)	 0.05
CF F(40) M(40)	 1.2 ± 0.5 1.1 ± 0.3	 0.39	 32(80.0) 28(70.0)	 8(20.0) 11(27.5)	 0(0) 1(2.5)	 0.36

TABLE II: Age and gender difference in the mean and range of salivary flow

Caries Status	Mean Buffering capacity in pH	P - value	Buffering Capacity scores in pH			P – Value
			>6.0	4.0-6.0	<4.0	
CA (80) CF (80)	6.2 (0.7) 6.4 (0.6)	0.59	61 (76.3) 61 (78.7)	19 (23.7) 16 (20.0)	0(0.0) 1 (1.3)	0.59
Age range of study participants in years in relation to salivary buffering capacity						
CA 6-8 (33) 9-12 (47)	 6.2 ± 0.6 6.3 ± 0.7	 0.79	 25(75.8) 32(68.1)	 8 (24.2) 15 (31.9)	 0(0) 0(0)	 0.05
CF 6-8 (20) 9-12 (60)	 6.6 ± 0.4 6.4 ± 0.7	 0.10	 20 (100.0) 47 (78.3)	 0(0) 12 (20.0)	 0(0) 1(1.7)	 0.55
Gender of study participants in years in relation to salivary buffering capacity						
CA F (41) M(39)	 6.2 ± 0.6 6.2 ± 0.7	 0.97	 28(68.3) 29(74.4)	 13 (31.7) 10 (25.6)	 0(0.0) 0(0.0)	 0.05
CF F(40) M(40)	 6.3 ± 0.8 6.6 ± 0.5	 0.35	 32(80.0) 35(87.5)	 8(20.0) 4(10.0)	 0(0) 1(2.5)	 0.55

CA (caries active), CF (caries free).

TABLE III: Age and gender difference in the mean and range of salivary buffering capacity in caries active and caries free groups.

DMFT/ Dmft	Freq (%)	Mean SFR ml/min	Mean Square	F value	P value	Mean SBC in pH	Mean square	F value	P value
Grp i (0)	80	1.10±0.48	0.843	4.398	0.005	6.44±0.63	1.119	2.883	0.038
Grp ii (1-2)	53	1.00±0.41				6.35±0.66			
Grp iii (3-4)	16	0.77±0.35				6.12±0.57			
Grp iv (5-6)	11	0.70±0.30				6.02±0.71			
Total	160	1.03±0.45				6.34±0.65			

TABLE IV: Comparing the flow rate , buffering capacity and caries activities in caries active and caries free groups.

Multiple comparison (Turkey HSD) post hoc					
Dependent	**(I)Group**	**(J)Group**	**I-J(Mean difference)**	**Std Error**	**P value**
Salivary Flow rate (mls/min)	**DMFT/dmft 0 (Grp 1)**	**1-2 (Grp ii)** **3-4 (Grp iii)** **5-6 (Grp iv)**	**0.062** **0340** **0.369**	**0.775** **0.119** **0.140**	**0.421** **0.005** **0.010**
	1-2 (Grp ii)	**0 (Grp i)** **3-4 (Grp iii)** **5-6 (Grp iv)**	**- 0.0062** **0.277** **0.306**	**0.077** **0.124** **0.145**	**0.421** **0.028** **0.036**
	3-4 Grp iii	**0 (Grp i)** **1-2 (Grp ii)** **5-6 (Grp iv)**	**-0.340** **-0.277** **0.028**	**0.119** **0.124** **0.171**	**0.005** **0.028** **0.867**
	5-6 Grp iv	**0 (Grp i)** **1-2 (Grp ii)** **3-4 (Grp iii)**	**-0.369** **-0.306** **-0.028**	**0.140** **0.145** **0.171**	**0.010** **0.036** **0.867**
Salivary Buffering Capacity (pH)	**0 (Grp i)**	**1-2 (Grp ii)** **3-4 (Grp iii)** **5-6 (Grp iv)**	**0.090** **0.420** **0.420**	**0.113** **0.176** **0.206**	**0.428** **0.018** **0.044**
	1-2 (Grp ii)	**0 (Grp i)** **3-4 (Grp iii)** **5-6 (Grp iv)**	**- 0.090** **0.330** **0.329**	**0.113** **0.183** **0/213**	**0.428** **0.074** **0.124**
	3-4 (Grp iii)	**0 (Grp i)** **1-2 (Grp ii)** **5-6 (Grp iv)**	**-0.420** **-0.330** **-0.001**	**0.176** **0.183** **0.251**	**0.018** **0.074** **0.998**
	5-6 (Grp iv)	**0 (Grp i)** **1-2 (Grp ii)** **3-4 (Grp iii)**	**-0.420** **-0.329** **-0.001**	**0.206** **0.213** **0.251**	**0.044** **0.124** **0.998**

TABLE V: Intergroup comparison of the values of flow rate and buffering capacity levels in caries free and caries active group

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