

# Salivary Buffering Capacity, Flow Rate and Calcium Levels in Children with and without Early Childhood Caries – a Comparative Study

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## ABSTRACT

**Background:** Early Childhood Caries (ECC) is a phenomenon that affects primary teeth in children below 6 years of age. Its severity could have a far-reaching impact on children with resultant effects on their quality of life. An assessment of a child's caries risk using saliva is a valuable non-invasive diagnostic tool utilised in preventing or reducing the impact of this condition.

**Objective:** To assess the buffering capacity, flow rate and calcium levels of saliva in children and their association with ECC.

**Methodology:** The study consisted of fifty (50) subjects aged 45 to 71 months recruited from Lagos University Teaching Hospital (LUTH) and staff primary school, Idi-Araba. Unstimulated saliva collected between 9 – 11 am was used to evaluate saliva's buffering capacity, flow rate and calcium levels in children with and without ECC.

**Results:** The subjects were aged below 71 months (with a mean age of  $56.66 \pm 7.17$  months). A major proportion (67.0%) of the component of the dmft was cavitated lesion related. The caries-free subjects had slightly higher mean rank values in salivary flow rate (MR = 27.52, U = 262, p = 0.325), slightly lower mean rank values in calcium level (MR = 24.64, U = 291, p = 0.677) and lower buffering capacity values than caries active subjects.

**Conclusion:** The properties of saliva such as buffering capacity, flow rate and calcium level were not significantly different between the study groups. Therefore, there is no association between buffering capacity, flow rate, calcium level, and ECC. This implies that more investigations are required to evaluate the protective effects of other salivary physiochemical factors like antioxidants, other than those investigated in this study.

**Keywords:** Buffering capacity, Flow rate, Calcium level, Early Childhood Caries, Saliva

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## INTRODUCTION

Early Childhood Caries (ECC) as defined by the American Academy of Pediatric Dentistry is the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger.<sup>1</sup> Prevalence of ECC in Nigeria varies from 4.7% - 21.2%;<sup>2,3</sup> and the reported prevalence in India and Italy was 44.4% and 19.0% respectively.<sup>4,5</sup>

Due to its high prevalence, ECC is a disease of public health importance and a burden to the parents, community and clinician.<sup>6</sup> Parents of children with ECC have reported a significantly lower oral health-related quality of life (QoL) than those without ECC.<sup>6</sup> They have also reported that they were not able to concentrate on their work. They needed to take several time off work due to frequent dentist visits and increased expenditure on drugs and treatment.<sup>6</sup> In the community, caries generates a significant increase in health, financial and social costs, increases the strain on the inadequate available dental care and reduces productivity due to loss of manpower and inefficient use of resources.<sup>7</sup>

The effect of ECC on the child is quite significant characterised by difficulty in sleeping and eating as a result of infection and pain leading to loss of school hours, orthodontic and aesthetic problems, and masticatory disturbance which ultimately leads to weight loss.<sup>7,8</sup> The effect of ECC on the clinician is related to the complicated and costly process of treating younger children, who may require the administration of pharmacological methods of behavioural management.<sup>8</sup> Fortunately, the disease process is reversible in its early stage that is before a cavity is formed.

The criteria used in detecting and diagnosing a carious lesion is pivotal in implementing early preventive measures that can be instituted in managing and preventing the progression of caries. The International Caries Detection and Assessment System (ICDAS) evaluates and detects the presence of early and cavitated carious lesions. The ICDAS also provides clinicians, epidemiologists and researchers with an evidence-based system that permits

standardized caries detection and diagnosis in differing environments and situations.<sup>9</sup>

Due to its reversibility and public health importance, several prevention measures are being investigated. One of such measure is the utilisation of oral structures and their products. Saliva is a mixture of secretions produced by the major and minor salivary glands and the gingival crevicular fluid.<sup>10</sup> This fluid plays an important role in maintaining oral homeostasis as it lubricates and protects the oral structures against micro-organisms and acts as a buffer by neutralizing acids produced in the oral cavity.<sup>11</sup> In addition, it acts as an oral cleanser which removes debris and food particles from the mouth.<sup>11</sup> Saliva is a calcium and phosphate-rich reservoir that helps repair incipient caries and prevents disease progression.<sup>11</sup> Studies have shown that patients with salivary deficiency have a higher risk of developing caries.<sup>11</sup>

Apart from the multiple roles and functions played by saliva in maintaining oral health, it also serves as a diagnostic tool in dentistry. Saliva is used in assessing an individual's caries or periodontal disease risk. As a caries risk assessment tool, it is used to measure the salivary counts of Streptococci mutans and Lactobacilli.<sup>11</sup> Assessing an individual's periodontal disease risk involves genetic screening of DNA from mouth swabs.<sup>11</sup> For these assessments, saliva is collected non-invasively at the chair side without the need for specialized equipment, a procedure tolerated by patients and accepted by their parents and is a cost-effective approach.<sup>10, 11</sup> An accurate caries risk assessment using saliva biochemical properties can aid the categorization of patients into different caries risk levels. This categorization of patients will aid in the institution of appropriate preventive and therapeutic management such as the use of salivary substitutes and professional fluoride application which will alter the chemistry of the mouth toward 'remineralization' of the affected teeth.<sup>11</sup> As a result of the crucial role saliva plays in maintaining oral homeostasis, it is important to investigate the salivary buffering capacity, flow rate and calcium levels in children and determine their association with Early Childhood Caries.

## MATERIALS AND METHODS

### Ethical clearance

Ethical clearance was obtained from Lagos University Teaching Hospital, Health Research and Ethics Committee (HREC) with the protocol number ADM/DCST/HREC/1501. Written consent forms and letters were sent to and obtained from the parents/guardians.

### Study Population and Design

This study was a case-control study. A non-probability sampling technique was used to select subjects for the study. These subjects comprised children 71 months and below and were matched for age and gender. These subjects were grouped<sup>12</sup> based on the presence or absence of caries into the caries-active group and caries-free group. The caries-free group were recruited from the Staff School, University of Lagos while the caries-active group were recruited from the Paediatric Dental clinic, LUTH, Idi-Araba in the Mushin Local Government Area of Lagos State.

### Determination of Sample Size

The sample size was calculated using a formula for comparing two (2) means.<sup>13</sup> The sample size ( $n_1, n_2$ ), mean value ( $\mu_1, \mu_2$ ) and standard deviation ( $S_1, S_2$ ) of pH in prototype study A<sup>14</sup> and B<sup>15</sup> were used. Sample size ( $N$ ) =  $((Z\alpha + Z\beta)^2 \times 2 \times \sigma^2) / ((\mu_1 - \mu_2)^2)$ .

$$\text{Variance } (\sigma^2) = \frac{S_1^2(n_1 - 1) + S_2^2(n_2 - 1)}{(n_1 + n_2) - 2}$$

$$\sigma^2 = \frac{(0.82)^2(45-1) + (0.32)^2(15-1)}{(45+15) - 2}$$

$$\sigma^2 = 0.5348$$

$$\text{Sample size } (N) = \frac{(1.96 + 1.65)^2 \times 2 \times 0.5348}{(7.43 - 6.59)^2}$$

$$N = 19.75496$$

A confidence level of 95% and margin of error of 5% was used. The minimum estimated sample size was 20. The size was adjusted considering an attrition rate of 25%. Sample size was therefore 25 in each group.

### Selection criteria

**Inclusion Criteria:** Healthy subjects who were 71 months of age and below. All subjects who had refrained from drinking, eating, or brushing their teeth for at least one hour prior to collecting the

samples. The subjects who returned with duly signed consent forms were recruited into the study.

**Exclusion Criteria:** Subjects who had physical, medical or surgical conditions that could alter saliva composition and subjects whose parents were uncertain about the time when patients last ate, drank or brushed their teeth.

### Procedure

Using an interviewer-administered questionnaire, the subject's demographic profile and information on past medical and dental history were collected. Clinical evaluation was done by the primary researcher with each subject seated on a well-lighted dental chair. Caries detection was based on the Merged codes recording system of the International Caries Detection and Assessment System (ICDAS).<sup>9</sup> Merged code recording system assesses dental caries as Initial stage decay - when a first or distinct visual change is observed in enamel; Moderate decay - when there is a localized enamel breakdown or an underlying dark shadow is observed from dentin; and Extensive decay - when there is a distinct or extensive distinct cavity with visible dentin.

A) Estimation of salivary flow rate  
Unstimulated saliva was collected between 9 am – 11 am to standardize the method and minimize the effects of circadian rhythms. Each subject was instructed to sit comfortably and drooled saliva passively into a graduated centrifuge tube through a funnel and avoided swallowing saliva during the time of collection. After 5 minutes, the amount of saliva collected was noted on the graduated centrifuge tube (Picture 1). The subject continued drooling until 10 ml of saliva was collected.<sup>16</sup>

Saliva flow rate (ml/min) was calculated as the total amount of saliva collected in 5mins divided by 5.<sup>17</sup> All samples were placed in an ice box to prevent changes in the composition of the saliva sample and taken to the laboratory immediately.

B) Buffering capacity estimation  
The buffering capacity was measured within thirty minutes after collection. Saliva buffering capacity was calculated using a modification of Maldupa method.<sup>18</sup> The initial pH of the saliva sample was measured with a pH meter (Jenway pH meter PHS 25) before the measurement of buffering capacity (Picture 2). Buffering capacity measurement

involved titrating 0.1 ml of 0.01 M Hydrochloric acid (HCl) into 2.5mls of saliva using a precise burette (Picture 3), the solution was mixed gently and left to settle for five seconds, then the pH was re-measured. The titration of acid into the saliva sample continued until the initial pH value decreased by one (1), two (2) and three (3) units. The total volume of acid titrated was noted for each unit change.

The buffering capacity (mmol/mlHCl/pH) was calculated using the formula

$$\text{Buffering capacity} = C \times V_a / \Delta\text{pH} \times V_s^{18}$$

C = concentration of HCl in mmol/ml,

$V_a$  = volume of HCl added in mls,

$\Delta\text{pH}$  = pH changes from initial pH,

$V_s$  = volume of saliva in mls.

### C) Estimation of calcium levels

Calcium level was determined using the Atomic Absorption Spectrophotometer (AAS)<sup>19</sup> (PerkinElmer A Analyst 200). This device has a hollow cathode lamp used for measuring calcium ions in solutions. The AAS was calibrated using a stock standard solution of calcium prepared by dissolving calcium carbonate ( $\text{CaCO}_3$ ) in 1000mls of 5% Nitric acid. 5mls of the saliva sample was placed in a conical flask and digested using 10mls of Aqua Regia. The solution was then made up to 25mls using distilled de-ionized water.

The digested saliva sample was then burnt in the hollow cathode lamp. The burning process compressed the solution to form aerosols thereby dissociating the ions in the sample from a ground state to an excited state. In the AAS, calcium was selected by the monochromator at a wavelength of 422.7nm and its content was displayed in milligrams per deciliter (mg/dl).

### Data analysis

Analysis of data was done using the Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM, Armonk, New York). Data cleaning and tests of normality were done. The data for buffering capacity was normally distributed while that for salivary flow rate and calcium levels were not normally distributed. The mean rank values of the salivary flow rate and calcium levels were determined for each group and compared using the Mann-Whitney U test. The mean and standard deviation of

the salivary buffering capacity of each group were determined. A comparison of the buffering capacity of the two groups was done using the Student t-test. Linear and Multiple regressions were used to assess the association between the predictor variables and the caries experience. The level of significance was set at 5% (0.05). Results were presented in tables.

### RESULTS

The study consisted of fifty (50) subjects aged 45 to 71 months equally grouped. The mean age of caries free subjects was  $55.48 \pm 7.57$  months and that of caries active subjects was  $57.84 \pm 6.76$  months (Table 1). In the caries active group, the dmft value was 94 and the mean dmft was  $3.76 \pm 2.44$ . Using the ICDAS system, the major proportion (67.0%) of the dmft were cavitated lesions with 13.0% as non-cavitated lesions (Table 2). Out of the 25 subjects with ECC, 2 had only non-cavitated lesions, 21 had only cavitated lesions while 2 had both cavitated lesions and non-cavitated lesions (Figure 1). The mean rank value for flow rate (27.21) and calcium levels (26.77) of saliva was slightly higher in the male subjects studied (Table 3).

Subjects in the caries-free group had a higher mean rank salivary flow rate value (27.52) relative to those in the caries-active group (23.48), though not significant ( $p = 0.325$ ). Subjects in the caries-free group had a lower mean rank value for salivary calcium level (24.64) relative to those in the caries-active subjects (26.36), though the difference was not statistically significant ( $p = 0.677$ ) (Table 3).

The initial pH values of saliva recorded while determining buffering capacity in the laboratory showed a significantly higher value in the caries free subjects ( $7.36 \pm 0.20$ ) than in caries active subjects ( $7.21 \pm 0.26$ ) ( $p = 0.027$ ). The buffering capacity recorded at 1 and 2 units of pH changes ( $5.51 \pm 1.41$  and  $4.20 \pm 0.90 \times 10^{-3}$  mmol/mlHCl/pH) respectively was lower in the caries-free group than in the caries-active ( $5.90 \pm 1.37$  and  $4.60 \pm 0.88 \times 10^{-3}$  mmol/mlHCl/pH) with p value 0.325 and 0.120 respectively. At 3 units of pH change, the buffering capacity recorded for the caries-free group ( $3.62 \pm 0.84$ ) was further lower than those in the caries active group ( $4.15 \pm 0.94$ ). This difference was statistically significant ( $p = 0.040$ ) (Table 4).

Linear regression showed significant variability ( $p = 0.001$ ) between initial pH and caries experience (Table 5). The coefficient of determination ( $R^2$ ) of 0.601 showed that 60% of the total variability in the

caries experience (dmft) was explained by the combined set of predictor variables (flow rate, calcium, initial pH, buffering capacity at 1, 2 and 3

unit changes). Multiple regression also showed significant predictive values with initial pH (95% C.I = -16.507 - -2.209, p = 0.012) (Table 6).

**Table 1: Demographic characteristics of the subjects**

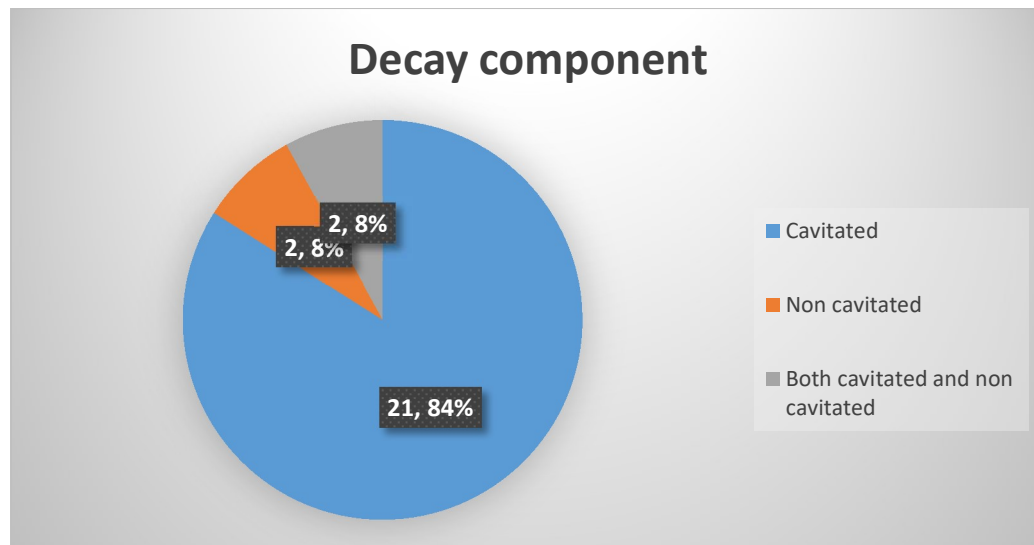
Factors	Caries free (n=25 )	Caries active (n=25 )	p value
Age (months)	Mean (SD) 55.48 ± 7.57	57.84 ± 6.76	0.25
	Frequency (%)		
Age (months) 45 – 54	13 (52.0%)	10 (40.0%)	
55 – 64	8 (32.0%)	11 (44.0%)	
65 – 71	4 (16.0%)	4 (16.0%)	
Gender Female	12 (48.0%)	12 (48.0%)	
Male	13 (52.0%)	13 (52.0%)	

**Table 2: Distribution of decayed, missing and filled teeth (dmft) in the caries active group using the ICDAS scoring system**

Dmft	No of teeth n = 94 (%)
decayed: cavitated	63 (67.0)
non -cavitated	12 (13.0)
Missing	0(0.0)
Filled	19 (20.0)
Total teeth	94 (100.0)

dmft = decayed, missing and filled teeth, ICDAS = The International Caries Detection and Assessment System

**Figure 1: Distribution of subjects with decayed component using the ICDAS system**



\*Some participants had both cavitated and non-cavitated lesions

**Table 3: Mean salivary flow rate, calcium level according to gender and caries experience**

Variables	Flow rate (mls/min)	Calcium (mg/dl)
	Mean Rank	
Male (n=26)	27.21	26.77
Female (n=24)	23.65	24.13
p value	0.385	0.522
U	267.5	279.0
z score	- 0.869	- 0.641
Caries free (n=25)	27.52	24.64
Caries active (n=25)	23.48	26.36
p value	0.325	0.677
U	262	291
z score	- 0.985	- 0.417

**Table 4: Mean salivary buffering capacity according to gender and caries experience**

Variables	Buffering Capacity Initial pH	Buffering Capacity ( $\times 10^{-3}$ mmol/mlHCL/pH)		
		1 unit change	2 units change	3 units change
Male (n=26)	7.27 $\pm$ 0.24	5.98 $\pm$ 1.37	4.42 $\pm$ 0.78	3.86 $\pm$ 0.74
Female (n=24)	7.30 $\pm$ 0.25	5.40 $\pm$ 1.39	4.37 $\pm$ 1.03	3.92 $\pm$ 1.09
p-value	0.624	0.150	0.837	0.808
Caries free (n=25)	7.36 $\pm$ 0.20	5.51 $\pm$ 1.41	4.20 $\pm$ 0.90	3.62 $\pm$ 0.84
Caries active (n=25)	7.21 $\pm$ 0.26	5.90 $\pm$ 1.37	4.60 $\pm$ 0.88	4.15 $\pm$ 0.94
p-value	0.027*	0.325	0.120	0.040*

\* Significant =  $p < 0.05$ **Table 5: Linear regression analysis of individual predictor variables on caries experience**

Variables (Predictors)	dmft (Outcome)	$\alpha$	B	95% Lower bound	Confidence Interval Upper bound	p Value
Flow rate (mls/min)		2.871	-2.265	- 4.800	0.271	0.079
Calcium (mg/dl)		2.293	-0.179	- 0.658	0.300	0.457
Buffering Capacity	Initial pH	36.404	-4.741	- 7.459	- 2.022	0.001*
Buffering Capacity	1 unit change	2.524	-112.903	- 643.551	417.745	0.671
( $\times 10^{-3}$ mmol/mlHCL/pH)	2 unit change	0.538	304.886	- 513.459	1123.232	0.457
	3 unit change	-1.019	745.786	- 27.125	1518.698	0.058

$Y = \alpha + \beta X_i$ ; dmft = decayed, missing and filled teeth;  $\alpha$  = constant/ intercept;  $\beta$  = regression coefficient; Y = Outcome variable (dmft);  $X_i$  = Individual predictor variable; p Value =  $< 0.05$

Table 6: Multiple regression analysis of all predictor variables on caries experience

Variables (Predictors)	dmft (Outcome)	$\alpha$	B	95% Lower Bound	Confidence Interval Upper bound	p Value	R <sup>2</sup>
		9.351					0.601
Flow rate (mls/min)			- 1.701	- 3.959	0.557	0.136	
Calcium (mg/dl)			- 0.266	- 0.641	0.110	0.160	
Buffering Capacity	Initial pH		- 9.358	- 16.507	- 2.209	0.012	
Buffering Capacity ( $\times 10^{-3}$ mmol/mlHCL/pH)	1 unit change		- 961.884	- 2075.348	151.580	0.088	
	2 unit change		1933.964	- 2289.170	6157.097	0.360	
	3 unit change		- 384.506	- 3758.122	2989.110	0.819	

$Y = \alpha + \beta X_1 + \dots + \alpha + \beta_k X_k$ ; dmft = decayed, missing and filled teeth;  $\alpha$  = constant/ intercept;  $\beta$  = regression coefficient; R<sup>2</sup> = coefficient of determination; Y = Outcome variable (dmft); X<sub>i</sub> = Individual predictor variable; k = number of individual predictor variables; p Value = < 0.05

## DISCUSSION

Saliva plays a vital role in maintaining integrity of the dental hard tissues and soft tissues.<sup>19</sup> It prevents loss of dental hard tissue from processes like abrasion, attrition, erosion and dental caries by acting as a buffer and cleanser.<sup>11, 19</sup> In addition, it helps to maintain the integrity of the soft tissues by lubricating the oral mucosa.<sup>11, 19</sup> Therefore, the assessment of the protective properties of saliva is essential for risk assessment and would offer an efficient way to promote better oral health. In this study, we found that unstimulated saliva is a suitable tool for the analysis of the composition and properties of saliva. This is because it was easy to collect, analyze and was also a source of amusement in the subjects. This finding is similar to those reported in literature.<sup>10, 14, 20</sup>

The mean dmft observed among the caries-active group in this study was similar to that reported by Sakeenabi and Hiremath<sup>21</sup> but lower than that reported by Bagherian and Asadikaram.<sup>14</sup> The difference may be associated with the variations in diet and socioeconomic status in the study populations. Out of the 25 subjects with ECC, 2 had only non-cavitated lesions, 21 had only cavitated lesions while 2 had both cavitated lesions and non-cavitated lesions. Similarly, a study by Folyan *et al*<sup>2</sup> reported that out of 73 subjects with ECC, 45 had non-cavitated lesions and 43 had cavitated lesions. This highlights the importance of evaluating and detecting the presence of incipient caries using

ICDAS, so early preventive measures can be instituted in managing ECC.

The caries free subjects had slightly higher mean rank values in flow rate when compared to the caries active subjects. Similarly, Preethi *et al*<sup>10</sup> and Dogra *et al*<sup>22</sup> showed a non-significant increase and several studies<sup>23-25</sup> reported a significant increase in salivary flow rate in the caries free subjects. This similarity could be due to the cleansing effect attributed to the flow rate of saliva resulting in an increased likelihood of caries occurring in a state of reduced salivary flow rate. In contrast, Ahmadi-Motamayel *et al*<sup>20</sup> reported a slightly higher salivary flow rate in caries active group which was attributed to the recruitment of healthy children with no underlying systemic disease that could have altered the flow rate in caries free and caries active groups.

The mean rank value of calcium was slightly higher in the caries active subjects, a finding similar to those by Bagherian and Asadikaram<sup>14</sup> and Ahmadi-Motamayel *et al*.<sup>20</sup> This could be due to the presence of proline-rich proteins in saliva that bind to calcium thereby inhibiting the precipitation of calcium on the oral structure so that although saliva is supersaturated with calcium, formation of hydroxyapatite does not occur leading to demineralization of dental tissues.<sup>[14]</sup> This is at variance with Preethi *et al*<sup>10</sup> who showed a slightly lower mean value, Dogra *et al*<sup>22</sup> who showed a significantly lower calcium level in caries active subjects and Leone and Oppenheim<sup>[26]</sup> who reported

that a moderate correlation existed between the low levels of calcium concentration in the saliva with caries susceptibility.

This study showed a significantly higher mean value in the initial pH in caries free group which is similar to reports by Annapoorna and Dipayan<sup>23</sup> and Pyati *et al.*<sup>25</sup> This finding may be because a less acidic oral environment is favourable to prevent caries initiation and progression. This finding suggests that the pH of caries free subjects would need to fall through a higher gradient to reach the critical point of 5.5, making the initiation of caries more difficult than in the caries active group.

The mean value of the buffering capacity was lower in the caries free subjects, a finding similar to the report by Jayaraj and Ganesan.<sup>27</sup> This is different from Preethi *et al.*<sup>10</sup> and Dogra *et al.*<sup>22</sup> who showed a non - significantly higher value of buffering capacity while Leone and Oppenheim<sup>26</sup> reported no association between buffering capacity and caries. In this study, the amount of hypochloric acid needed to cause a unit change of the pH of saliva in the caries free subjects was lower when compared to the caries active subjects. These variations suggest that individual systems involved in the buffering process may be responsible for the neutralization of acids produced in the oral cavity. These factors include bicarbonate, phosphate and protein systems which are the three major systems contributing to buffering capacity of saliva. In addition, plaque a firm tenaciously adherent microbial biofilm covering the tooth surface which is rich in acidogenic microorganisms may also be responsible for caries initiation and progression. It is therefore crucial to investigate these factors.

#### CONCLUSION

The properties of saliva such as buffering capacity, flow rate and calcium level were not significantly different between the study groups which implies that there is no association between these variables and ECC. Therefore more investigations are required to evaluate the protective effects of other salivary physiochemical factors like antioxidants, other than those investigated in this study.

#### Limitations

The paucity of scientific literature on the use of the ICDAS system (which assesses both non-cavitated and cavitated lesions in contrast to most studies that used the WHO diagnostic criteria which only assesses cavitated carious lesions) thus militated against a more robust literature review and discussion.

#### Recommendations

We would recommend further evaluation of saliva's various properties, e.g., fluoride, Immunoglobulin A, antioxidant capacity and their relationship with ECC using a larger sample size. The buffering capacity of plaque also needs to be evaluated as it is the microbial biofilm directly covering the tooth surface and it contains acidogenic microorganisms.

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#### Conflict of interest

None declared

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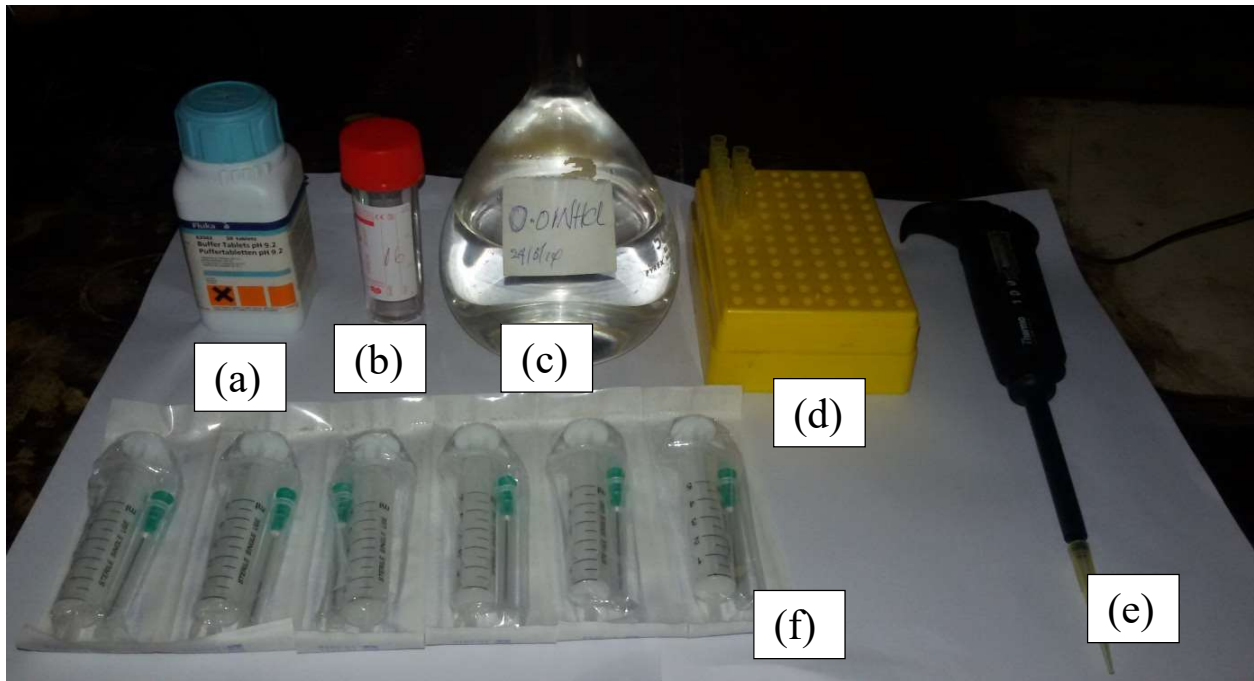


PICTURE 1



PICTURE 2

Picture 1 shows a female subject drooling saliva into a graduated centrifuge tube with the aid of a funnel. Picture 2 showing the pH meter for pH and buffering capacity estimation.



Picture 3 showing (a) A container of standard buffer 4.0; (b) Universal bottle; (c) Conical flask containing 0.01M HCl; (d) A rack of micropipette tips; (e) A micropipette and (f) A row of 5ml syringes.