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Dentistry Section

Assessment of Salivary pH, Flow Rate, Buffering Capacity and Alpha-amylase Enzyme Activity in Caries-free and Caries-active Children: A Cross-sectional Study

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ABSTRACT

Introduction: It is generally accepted that the caries process is largely controlled by a natural protective mechanism in saliva. The pH, flow, buffering, and remineralising capacity of saliva are recognised as critical factors that affect and regulate the progression and regression of the caries process. Salivary Alpha-Amylase (SAA) is an enzyme in saliva that has a high affinity for binding to oral streptococci and plays a role in carbohydrate digestion, potentially leading to enamel demineralisation.

Aim: To estimate and associate the pH, flow rate, buffering capacity, and SAA levels in the saliva of children with and without caries.

Materials and Methods: A cross-sectional study was conducted in the Department of Paediatric and Preventive Dentistry at Rajarajeswari Dental College and Hospital in Bengaluru, Karnataka, India where 50 children were included. They were divided into two groups: Group A (Caries-active) and Group B (Caries-free), each consisting of 25 children. Unstimulated saliva samples were collected and analysed using a pH meter and Ultraviolet (UV) spectrophotometer, with parental consent and after obtaining ethical clearance. The Mann-Whitney Test was used to compare mean salivary amylase scores between the two groups. An Independent Student's t-test was used to compare the mean initial pH values, buffering capacity values, and Salivary Flow Rate (SFR) between the two groups. Spearman's correlation test was conducted, and the level of significance was set at $p<0.05$.

Results: The mean age of the children in the caries-active group was 5.52±0.71 years, while in the caries-free group it was 5.40±0.76 years. Assessment and correlation were conducted between caries scores and all parameters in the study group. Salivary pH, flow rate, and buffering capacity showed a statistically significant negative correlation with caries activity (rho=-0.60, -0.53, -0.38), whereas alpha-amylase showed a statistically significant strong positive correlation with caries activity and caries score. Comparison of the mean values of all parameters based on the number of caries (<6 and >6) showed statistically significant results at $p<0.05$.

Conclusion: Based on the results, it can be concluded that SAA levels were increased in children with Early Childhood Caries (ECC), showing a significant positive correlation with caries activity.

Keywords: Early childhood caries, Physiochemical properties, Salivary alpha-amylase

INTRODUCTION

The restoration of primary dentition with extensive carious lesions is a complex clinical challenge with several dimensions [1]. Early loss of primary teeth is most commonly caused by inappropriate oral hygiene, dental injuries, and tooth decay. Tooth decay continues to be the main causative factor behind the high rate of loss [2]. The ECC is defined as the presence of one or more decayed, missing, or filled surfaces in any primary tooth in children younger than 71 months [3]. It poses a significant public health problem that negatively affects children's physical and mental health, as dental decay can lead to pain, reduced growth and development, speech disorders, and premature tooth loss, resulting in chewing problems, loss of self-confidence, and potential harm to permanent dentition. The World Health Organisation (WHO) lists dental caries as the third most common chronic, non infectious disease after cancer and cardiovascular disease [4].

Saliva is a complex biological fluid that contains chemicals, hormones, enzymes, and antimicrobial elements, as well as electrolytes [5]. It functions similarly to serum and reflects the body's physiological status [6]. Any measurable and quantifiable biological entity that can be used as an indicator for health-related assessments is referred to as a biomarker. Salivary diagnostics is a dynamic and developing field that incorporates proteomics and molecular diagnostics, which aids in diagnosing various systemic and oral diseases using salivary biomarkers [7].

The natural protective mechanism inherited in saliva largely controls the caries process. The pH, flow, buffering capacity, and remineralising ability of saliva are recognised as critical factors that affect, and in some ways regulate, the progression and regression of the caries process [7].

The SAA, also known as ptyalin, is a protein enzyme that hydrolyses the alpha bonds in large insoluble polysaccharides like starch, converting them into soluble forms, with maltose as the final product. It is one of the most crucial components of saliva, manifesting various biological functions linked to a high affinity for binding to oral streptococci and facilitating carbohydrate digestion. The enzyme has a binding site for the enamel surface and potential sites for the attachment of bacterial adhesives, thus playing an essential role in enamel demineralisation [8].

The UV spectroscopy is based on the amount of light and its wavelength absorbed by a sample, with components in the sample solution detected very specifically [9]. There is a lack of literature associating the levels of SAA and pH with different genders, ages,

and various caries scores. The aim of present study was to estimate and correlate the pH, flow rate, buffering capacity, and SAA in the saliva of children with and without caries.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Paediatric and Preventive Dentistry at Rajarajeswari Dental College and Hospital in Bangaluru, Karnataka, India on 50 children aged 3-6 years, with and without ECC over a period of six months, from December 2023 to May 2024. They were divided into two groups: caries-active (children with caries) and caries-free (children without caries), with 25 participants in each group. Institutional Ethical Clearance was obtained for this study (Ref no RRDCH/IEC22/19), and it was registered under the Clinical Trial Registration of India (CTRI) with Registration no CTRI/2023/02/049529.

Patients were selected using a random sampling technique, and allocation was made using a computer-generated random assignment sequence. A study proforma was designed, which included demographic details, chief complaints, and recordings of all subjective and objective symptoms, as well as clinical and radiographic findings. The experimental group (Caries-Active) consisted of 25 patients with Severe Early Childhood Caries (S-ECC), while the control group (Caries-free) involved 25 patients without caries who met the study criteria after obtaining informed consent from their parents or guardians.

Inclusion criteria: Children aged 3-6 years, children with four or more-active carious lesions (caries-active), children with no caries (caries-free), and parents who provided consent for the study were included.

Exclusion criteria: Children who received fluoride topical applications within the last month, children requiring special health care needs, those with pulpally involved teeth, children on antibiotic therapy in the past three months, children with genetic diseases or syndromes, and those on any medications were excluded from the study.

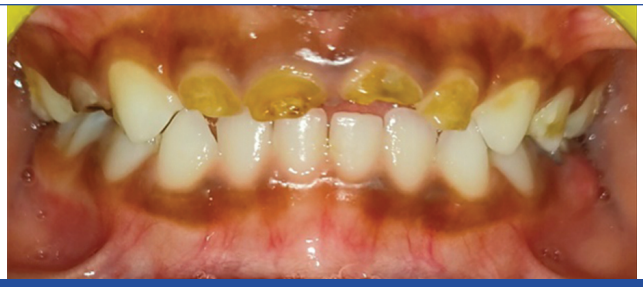
Sample size estimation: The sample size for present study was estimated using GPower software v. 3.1.9.4 (Franz Faul, Universität Kiel, Germany). Considering the effect size to be measured (d) at 80% for two-tailed hypotheses, and based on results from a previous study by de Farias DG et al., (2003), the power of the study was set at 80% with an alpha error of 5%. The total sample size needed is 50. Thus, each group comprised 25 samples (25×2=50 samples) [10].

Study Procedure

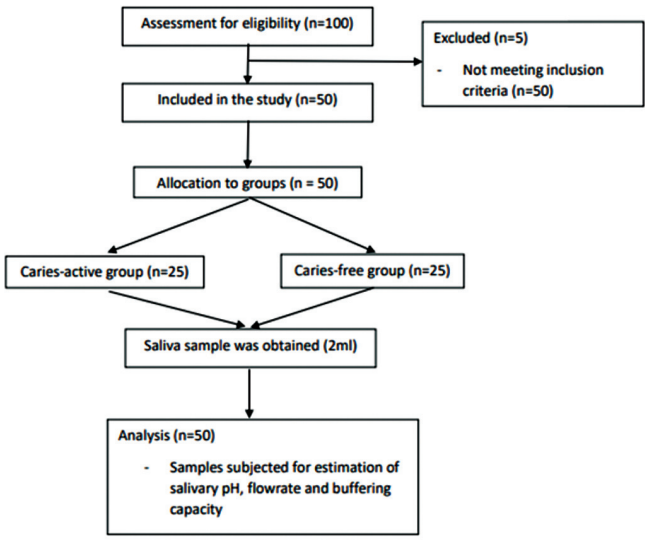
Caries were recorded using the DMFT index WHO form (Decayed, Missing teeth due to caries, Filled teeth). The children were classified according to the presence of the disease (DMFT≥4) or absence of the disease (DMFT=0). According to the International Caries Detection and Assessment System (ICDAS), patients with ICDAS numbers 5 and 6 were considered severe Early Childhood Caries (ECC) for the study group, while those with an ICDAS, score of '0' were included in the control group.

A 2 mL sample of unstimulated saliva was collected using the spit method [Table/Fig-1]. The study design is depicted in a flow chart [Table/Fig-1,2]. Following an hour of saliva collection, the samples were transferred to the laboratory and kept at -80°C in a hermetically sealed container filled with ice. Each sample was assigned a number, and this process continued until the laboratory study was completed. To avoid bias, the sample investigator was blinded.

All 50 samples were subjected to pH measurements using a pH meter to determine pH and buffering capacity in saliva [Table/Fig-3]. All samples underwent analysis with Dinitrosalicylic Acid (DNS) reagent and a UV spectrophotometer to determine the SAA levels.



[Table/Fig-1]: Intraoral photograph showing early childhood caries.



[Table/Fig-2]: Flowchart depicting study design.

[Table/Fig-3]: Digital pH meter.

Estimation of flow rate: Saliva was allowed to accumulate in the floor of the mouth, and the subjects were then asked to spit into a sterile saliva-collecting vial. The SFR was estimated immediately after the collection of samples and expressed as mL/min [11].

pH analysis: To determine pH, the saliva was placed in a clean beaker, and the pH electrode was immersed in the sample. The values were recorded using a pH meter (PP50, Sartorius, Germany) [11].

Estimation of buffering capacity: After assessing salivary pH, 1 mL of the saliva sample was transferred to a closed container, and 10 µL of 0.1 N Hydrochloric acid (HCl) was added using a micropipette. The sample was gently shaken to mix the saliva and HCl homogeneously. The electrode was then immersed in the sample, and a stable reading was recorded for salivary buffering capacity. This procedure was repeated for 10 µL, 50 µL, and 100 µL

titrations of 0.1 N HCl, and the respective readings were recorded for all samples to determine the range of salivary buffering capacity. The collected data was subjected to statistical analysis [3].

Estimation alpha amylase enzymatic activity: For the estimation of SAA activity, 1% starch and DNS reagents were prepared. In total, 25 samples were used, with the first test tube labeled '0' as the blank. The remaining samples were placed in test tubes labeled 1 through 25.

At a time, five test tubes were prepared. To each test tube, 500 µL of the saliva sample was added, while only glucose was added to the blank (0) tube. For SAA estimation, a clean sterile test tube was used to combine 100 µL of starch with 500 µL of saliva sample, and this mixture was set aside for incubation at room temperature for five minutes. Then, 500 µL of DNS was added to each tube [Table/Fig-4], and the tubes were incubated in a boiling water bath for 30 minutes.

[Table/Fig-4]: Adding 500 µL of prepared DNS reagent to all the test tubes.

After incubation, the test tubes were cooled to room temperature, and 3 mL of distilled water was added to each. Subsequently, 3 mL of the resulting reaction mixture from each tube was transferred to a quartz cuvette, and absorbance was measured at 540 nm using a UV-VIS Nanodrop spectrophotometer (ND2000C; Thermo, USA) [Table/Fig-5] [12].

[Table/Fig-5]: Solution kept in a UV-spectrophotometer.

STATISTICAL ANALYSIS

Statistical analysis was performed using GPower v. 3.1.9.4 software. The Mann-Whitney test was utilised to compare the mean salivary amylase scores between the two groups, also considering age group and gender within the control and study groups. The independent Student's t-test was used to compare the mean initial pH values, buffering capacity values, and SFR between the two groups, also based on age group and gender. Spearman's correlation test was conducted to assess the relationship between caries scores, salivary amylase levels, initial pH values, buffering capacity values, and SFR in the study group. The level of significance was set at $p<0.05$.

RESULTS

The mean age of the children in the caries-active group was 5.52±0.71 years, while in the caries-free group it was 5.40±0.76 years. Gender distribution among the study participants revealed that 60% (n=15) of the caries-active group were males and 40% (n=10) were females. In the caries-free group, 68% (n=17) were males and 32% (n=8) were females.

The salivary pH was higher in the caries-free group (7.26±0.18) and was statistically significant (p=0.03). The flow rate was also greater in the caries-free group (1.63±0.12 mL/min) and was statistically significant (p<0.001). Similarly, the buffering capacity level was higher in the caries-free group (5.69±0.47) and was statistically significant (p=0.03). In contrast, the salivary amylase levels were significantly higher in the caries-active group (58.46±19.71 U/mL) and were statistically significant (p<0.001) [Table/Fig-6].

Parameters	Groups	n	Mean±SD	Mean diff	p-value
Initial pH	Caries-free	25	7.26±0.18	0.17	0.03*
	Caries-active	25	7.09±0.31		
Flow Rate (mL/min)	Caries-free	25	1.63±0.12	0.77	<0.001*
	Caries-active	25	0.86±0.11		
50 µL buffering capacity (mEq/pH/mL)	Caries-free	25	5.69±0.47	0.47	0.03*
	Caries-active	25	5.23±0.93		
Amylase levels (U/mL)	Caries-free	25	28.45±20.41	-30.00	<0.001*
	Caries-active	25	58.46±19.71		

[Table/Fig-6]: Comparison of mean values of different parameters b/w caries-free and caries-active using Mann-Whitney test/Independent Student's t-test.
*Statistically significant

Spearman's correlation test of salivary pH, flow rate, and buffering capacity showed a significant negative correlation with caries scores in the caries-active group (rho=-0.60, -0.53, -0.38), whereas SAA showed a significant positive correlation with caries scores in the caries-active group (rho=0.64, p=0.001), as shown in [Table/Fig-7].

Groups	Variable	values	Amylase (U/mL)	Initial pH	BF_50µl (mEq/pH/mL)	Flow Rate (mL/min)
Study	Caries scores	Rho	0.64	-0.60	-0.38	-0.53
		p-value	0.001*	0.001*	0.04*	0.006*

[Table/Fig-7]: Spearman's correlation test to assess the relationship between caries scores, amylase levels, buffering capacity, initial pH and flow rate in the study group.
*Statistically significant

A comparison of all the parameters based on the number of carious teeth, categorised as having more than or less than 6 caries, was conducted using the Mann-Whitney Test. Amylase showed a significantly lower mean score in children with fewer than 6 caries (49.80±12.18) (p=0.01). In contrast, pH, flow rate, and buffering capacity exhibited a higher mean score of (7.25±0.24, 0.91±0.07, 5.57±0.62) in the group with fewer than 6 caries, and the results were statistically significant at p<0.05 [Table/Fig-8].

A comparison of all parameters based on different age groups (4-5 and 5-6 years) and genders was performed using the Independent Student's t-test and Mann-Whitney test, and the results were not statistically significant [Table/Fig-9,10].

Parameters	Caries	N	Mean±SD	Mean diff	p-value
Amylase (U/mL)	<6 no.	14	49.80±12.18	-19.67	0.01*
	>6 no.	11	69.47±22.38		
50 µl - Buffering capacity (mEq/pH/mL)	<6 no.	14	5.57±0.62	0.77	0.04*
	>6 no.	11	4.80±1.09		
Initial pH	<6 no.	14	7.25±0.24	0.37	0.002*
	>6 no.	11	6.89±0.27		
Flow Rate (mL/min)	<6 no.	14	0.91±0.07	0.11	0.01*
	>6 no.	11	0.80±0.12		

[Table/Fig-8]: Comparison of mean values of different parameters based on the number of caries teeth using Mann-Whitney Test/Independent Student's t-test.
*Statistically significant

Parameters	Groups	Age (years)	N	Mean±SD	Mean Diff	p-value
Salivary-amylase levels (U/mL)	Control	4-5	11	30.60±22.99	3.83	0.96
		5-6	14	26.77±18.85		
	Study	4-5	9	57.57±15.52	-1.38	0.85
		5-6	16	58.95±22.18		
pH	Control	4-5	11	7.21±0.18	-0.08	0.27
		5-6	14	7.29±0.18		
	Study	4-5	9	7.12±0.27	0.04	0.76
		5-6	16	7.08±0.34		
Buffering capacity (conc.=50 µL) (mEq/pH/mL)	Control	4-5	11	5.65±0.46	-0.07	0.71
		5-6	14	5.73±0.49		
	Study	4-5	9	4.89±1.21	-0.54	0.17
		5-6	16	5.42±0.69		
Salivary flow rate (ml/min)	Control	4-5	11	1.63±0.13	-0.01	0.86
		5-6	14	1.64±0.12		
	Study	4-5	9	0.87±0.10	0.01	0.82
		5-6	16	0.86±0.12		

[Table/Fig-9]: Comparison of mean Salivary-amylase levels, pH, buffering capacity and salivary flow rate based on different age groups in each group using Mann Whitney Test.

Parameters	Groups	Gender	n	Mean±SD	Mean diff	p-value
Salivary-amylase levels (U/mL)	Control	Males	17	30.84±22.23	7.44	0.73
		Females	8	23.39±15.97		
	Study	Males	15	59.14±21.59	1.70	0.87
		Females	10	57.44±17.57		
pH	Control	Males	17	7.27±0.16	0.04	0.57
		Females	8	7.23±0.22		
	Study	Males	15	7.11±0.34	0.05	0.68
		Females	10	7.06±0.29		
Buffering capacity (conc.=50 µL) (mEq/pH/mL)	Control	Males	17	5.71±0.46	0.04	0.86
		Females	8	5.67±0.52		
	Study	Males	15	5.26±0.90	0.07	0.85
		Males	10	5.19±1.00		
Salivary Flow rate (mL/min)	Control	Males	17	1.63±0.12	-0.01	0.88
		Females	8	1.64±0.12		
	Study	Males	15	0.84±0.11	-0.05	0.27
		Females	10	0.89±0.10		

[Table/Fig-10]: Comparison of mean salivary amylase levels, pH, buffering capacity and salivary flow rate based on gender in each group using Mann-Whitney Test.

DISCUSSION

In the present study, the pH, flow rate, and buffering capacity were decreased in caries-active children compared to caries-free children, which was statistically significant. Generally, higher pH and flow rate lead to faster clearance, and greater buffering capacity results in lesser microbial attacks. Normal Stimulated Flow Rate (SFR) imparts a robust protective effect against dental caries [11]. There was also a significant negative correlation between salivary pH, flow rate, buffering capacity, and DMFS/dfs scores (pH r=-0.60, p<0.05; flow rate r=-0.53, p<0.05; buffering capacity r=-0.38, p<0.05).

Salivary alpha-amylase (SAA) is a metalloenzyme produced by the serous cells of the parotid and other salivary glands that hydrolyzes starch to glucose and maltose, resulting in products that can be transformed into acids, leading to dental caries [13]. In this study, the evaluation and correlation of SAA enzymatic activity levels in caries-active and caries-free children showed that SAA levels increased in caries-active children compared to caries-free children, with statistical significance at p<0.05 and a strong positive correlation. Similar studies concluded that SAA levels were elevated in caries-active children relative to caries-free children [8,13-15]. In contrast, other studies indicated that SAA levels correlate with the severity of dental caries, with lower levels associated with greater severity [16-18].

One possible explanation for these results is that when the amylase-binding protein is bound to the cell wall and released, it interacts with alpha-amylase, amylase-binding protein A (AbpA), and glucosyltransferase G (GtfG) to form a complex. Alpha-amylase remains enzymatically-active within this complex, allowing the hydrolysis of dietary starch to maltose, which is then transported to cells via an ATP-binding Cassette (ABC) transporter. These carbohydrates are ultimately metabolised for energy production, with lactic acid as an end product, leading to tooth demineralisation [18,19].

Literature suggests that amylase binds with oral bacteria in dental plaque, facilitating dietary starch hydrolysis, which consequently leads to acid formation in dental plaque. This process accelerates demineralisation and the progression of dental caries [20].

Studies have shown that the number of caries correlates with the physicochemical properties of saliva, whereby an increase in caries leads to changes in these properties [15,16]. In the present study, a comparison of salivary pH, flow rate, and buffering capacity based on the number of carious teeth—categorised as more or less than six—revealed that as the number of caries increased, pH, flow rate, and buffering capacity decreased, which was statistically significant at p<0.05. These findings are consistent with other studies conducted earlier [20,21]. In contrast, some studies reported no relationship between the incidence of dental caries and the pH of saliva [22-24].

The present study also found that as the number of caries increased, salivary alpha-amylase (SAA) levels also increased, which was statistically significant at p<0.05. A similar study by Kor M et al., indicated a positive correlation between SAA level and DMFT [25]. However, a contrasting study by Gyergyay R et al., reported no correlation between DMFT and amylase activity [26].

According to our estimates, nearly one in two preschool children experiences early childhood caries (ECC) in India. A study by Devan I et al., also reported that nearly one in two preschool children suffers from ECC, with various factors contributing to caries incidence, including the child's age, parents' occupation, education, and socioeconomic conditions [27].

In the present study, the comparison of salivary pH, flow rate, buffering capacity, and alpha-amylase enzyme activity across different age groups showed that as age increased, pH and flow rate decreased, whereas buffering capacity and SAA levels increased, though these results were not statistically significant. Similar findings were observed by Vacaru RP et al., who found that SAA increased with age [17]. In contrast, studies by Nasiru WO and Pandey P et al., concluded that both pH and flow rate increase with age [20,28], while buffering capacity decreases [20]. This could be because older children may find it easier to expectorate saliva than younger

age groups. Increased flow rate with age may also result from more developed salivary glands in older children. However, caries prevalence increases with age, and other cariogenic factors, such as poor oral hygiene, may also contribute to increased caries [20].

A gender-wise analysis showed that males have a higher prevalence of ECC compared to females. In this study, the comparison of salivary pH, flow rate, buffering capacity, and SAA levels across different gender groups showed no statistically significant differences. Similar results have been observed in studies that found no statistically significant results based on age-wise distribution [3,8,15]. In contrast, other studies have reported statistically significant differences in flow rate, buffering capacity, and SAA between genders, with girls showing greater susceptibility to caries compared to boys [17,20,29]. Additionally, pH levels were found to be lower in boys compared to girls, with this difference being statistically significant [29].

Three factors can explain the prevalence of caries in females compared to males: the earlier eruption of teeth in girls, which causes longer exposure to a cariogenic environment in the mouth; women consuming more snacks and candy; and the effects of pregnancy and hormonal influences. Additionally, genetic factors and nutrition significantly influence the differences in caries prevalence between the two genders [13]. The increase in mean stimulated flow rate (SFR) observed among females may be due to decreased caries activity compared to their male counterparts. SFR is known to have an anticariogenic effect and better cleansing properties [28,30].

Nowadays, there is increased interest in non-invasive and personalised dentistry. Molecular assays that utilise salivary biomarkers can be an effective tool for detecting caries in the early stages and assessing a patient's caries risk. The current study identified different molecular signatures that can be used for caries diagnosis, prognosis, risk assessment, and disease monitoring. SAA (salivary alpha-amylase) can serve as a predictive biomarker in pediatric dentistry.

Limitation(s)

The main limitations of this study include being conducted at a single center and lacking dietary history. The sample should have included children from various ethnic backgrounds and socioeconomic statuses to determine the precise relationship between saliva's physicochemical characteristics and dental caries activity in different populations. Additionally, including dietary history would help establish the connection between dietary habits and the severity of early childhood caries (ECC).

CONCLUSION(S)

It can be concluded that pH, flow rate, and buffering capacity decreased in children with ECC, whereas SAA levels increased, showing a significant positive correlation with caries activity. Significant differences were observed in all parameters when compared with the number of caries, but no significant differences were found when comparing age and gender. Understanding the role of SAA in the pathogenesis of dental caries provides valuable information on how we can use this biomarker to help prevent caries.

REFERENCES

[1] Martin AG, Shivashakarappa PG, Arumugam S, Sundaramurthy N. Posts in Primary Teeth–Past to Present: A Review of Literature. Int J Clin Paediatr Dent. 2021;14(5):705.

[2] Murshid SA, Al-Labani MA, Aldhorae KA, Rodis OM. Prevalence of prematurely lost primary teeth in 5–10-year-old children in Thamar city, Yemen: A cross-sectional study. J Int Soc Prev Community Dent. 2016;6(2):126.

[3] Jayaraj D, Ganesan S. Salivary pH and buffering capacity as risk markers for early childhood caries: A clinical study. Int J Paediatr Dent. 2015;8(3);167-71.

[4] Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ. 2005;83(9):661-69.

[5] Schipper RG, Silletti E, Vingerhoeds MH. Saliva as research material: Biochemical, physicochemical and practical aspects. Arch Oral Biol. 2007;52(12):1114-35.

[6] Choudhry AA, Sharma P, Mohapatra T, Prasad M, Kumar P. Assessment of salivary lipid profile parameters in healthy and type 2 diabetes mellitus patients. J Clin Diag Res. 2021;15(6):01-05.

[7] Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DT. Salivary biomarkers: Toward future clinical and diagnostic utilities. Clin Microbiol Rev. 2013;26(4):781-91.

[8] Monea M, Vlad R, Stoica A. Analysis of the salivary level of alpha amylase as a risk factor for dental caries. Acta Medica Transil Vanica. 2018;23(1):93-95.

[9] Mettler Toledo. UV/VIS spectrophotometry - Fundamentals and applications. Analytical Sonnenbergstrasse 74 CH-8603 Schwerzenbach, Switzerland 2015:1-50.

[10] de Farias DG, Bezerra AC. Salivary antibodies, amylase and protein from children with early childhood caries. Clin Oral Investig. 2003;7(3):154-57.

[11] Preethi BP, Reshma D, Anand P. Evaluation of flow rate, pH, buffering capacity, calcium, total proteins and total antioxidant capacity levels of saliva in caries free and caries active children: An in vivo study. Indian J Clin Biochem. 2010;25(4):425-28.

[12] Jain A, Jain R, Jain S. Determination of Salivary Amylase Activity. In: Basic Techniques in Biochemistry, Microbiology and Molecular Biology. Humana, New York, NY: Springer Protocols Handbooks; 2020.

[13] Preetha K, Tejasri K, Vijaya Nirmala S, Rajan ST. An evaluation of role of salivary alpha-amylase in the caries activity among young individuals. IJMSCR. 2019;2(3):54-57.

[14] Ahmadi-Motamayel F, Goodarzi MT, Jamshidi Z, Mahdavinezhad A, Rafieian N. Evaluation of salivary and serum alpha amylase level in dental caries of adolescence. Braz Dent Sci. 2016;19(2):40-46.

[15] Mojarad F, Fazilollahifar S, Poorolajal J, Hajilool M. Effect of alpha-amylase on early childhood caries; A matched case-control study. Braz Dent Sci. 2013;16(1):41-45.

[16] Parsaie Z, Rezaie P, Azimi N, Mohammadi N. Relationship between salivary alpha amylase enzyme activity, anthropometric indices, dietary habits, and early childhood dental caries. Int J Dent. 2022;2022:2617197.

[17] Vacaru RP, Didilescu AC, Constantinescu I, Mărunțelu I, Tănase M, Stanciu IA, et al. Salivary enzymatic activity and carious experience in children: A cross-sectional study. Children. 2022;9(3):343.

[18] Borghi GN, Rodrigues LP, Lopes LM, Parisotto TM, Steiner-Oliveira C, Nobre-dos-Santos M. Relationship among α amylase and carbonic anhydrase VI in saliva, visible biofilm, and early childhood caries: A longitudinal study. Int J Paediatr Dent. 2017;27(3):174-82.

[19] Nikitkova AE, Haase EM, Scannapieco FA. Taking the starch out of oral biofilm formation: Molecular basis and functional significance of salivary α-amylase binding to oral streptococci. Appl Environ Microbiol. 2013;79(2):416-23.

[20] Nasiru WO. Salivary flow rate, buffering capacity and dental caries among 6-12-year-old school children, age and gender in Nigeria: A comparative study. IOSR J Dent Med Sci. 2019;18(1):72-79.

[21] Pyati SA, Naveen Kumar R, Kumar V, Praveen Kumar NH, Parveen Reddy KM. Salivary flow rate, pH, buffering capacity, total protein, oxidative stress and antioxidant capacity in children with and without dental caries. J Clin Paediatr Dent. 2018;42(6):445-49.

[22] Thaweboon S, Thaweboon B, Nakornchai S, Jitmaitree S. Salivary secretory IgA, pH, flow rates, mutans streptococci and Candida in children with rampant caries. Southeast Asian J Trop Med Public Health. 2008;39(5):893-99.

[23] Swerdlove CK. Relation between the incidence of dental caries and the pH of normal resting saliva. J Dent Res. 1942;21(1):73-81.

[24] Lagerlof F, Oliveby A, Ekstrand J. Physiological factors influencing salivary clearance of sugar and fluoride. J Dent Res. 1987;66(2):430-35.

[25] Kor M, Pouramir M, Khafri S, Ebadollahi S, Gharekhani S. Association between dental caries, obesity and salivary alpha amylase in adolescent girls of Babol City, Iran-2017. Dent Shiraz Univ Med Sci. 2021;22(1):27-32.

[26] Gyergyay R, Kovacs B, Bratu DC, Szekely M, Kovács M, et al. Effect of dental prophylaxis on the salivary cortisol levels and alpha-amylase activity in children. Rev Chim. (Bucharest). 2015;66(12):212-14.

[27] Devan I, Ramanarayanan V, Janakiram C. Prevalence of early childhood caries in India: A systematic review and meta-analysis. Indian J Public Health. 2022;66:S3-11.

[28] Pandey P, Reddy NV, Rao V, Saxena A, Chaudhary CP. Estimation of salivary flow rate, pH, buffer capacity, calcium, total protein content and total antioxidant capacity in relation to dental caries severity, age, and gender. Contemp Clin Dent. 2015;6(1):65-71.

[29] Ahmadi-Motamayel F, Goodarzi MT, Jamshidi Z, Mahdavinezhad A, Rafieian N. Evaluation of salivary and serum alpha amylase level in dental caries of adolescence. Brazilian Dental Science. 2016;19(2):40-46.

[30] Gudkina T, Brinkmane A. caries experience in relation to oral salivary cariogenic microflora, buffer capacity and secretion rate in 6 year olds and 12 year olds in Riga Stomatologija, Baltic Dent Maxillofac J. 2008;10(2):76-80.

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