EVALUATION OF pH,BUFFERING CAPACITY AND FLOW RATE OF SALIVA IN CARIES FREE PEOPLE AND IN PEOPLE WITH DENTAL CARIES AMONG DIFFERENT AGE GROUPS

Type of manuscript : Research article

Running title : EVALUATION OF pH,BUFFERING CAPACITY AND FLOW RATE OF SALIVA IN CARIES FREE PEOPLE AND IN PEOPLE WITH DENTAL CARIES AMONG DIFFERENT AGE GROUPS .

Aparnaa.M
Undergraduate student
Saveetha dental college ,Saveetha university, Chennai, India

Corresponding Author
Dr.Arvina
Senior Lecturer
Department of Periodontics
Saveetha Dental college
Saveetha University
162,Poonamallee High Road
Chennai – 600077
Tamil Nadu, India

Abstract :

BACKGROUND:

The saliva plays important roles in the maintenance of the oral health because it can prevent bacterial invasion, growth and metabolism through different mechanisms . Also, it can modulate the bacterial adhesion to teeth and attenuate the deleterious effects of the production of metabolites by oral microbiota due to its organic and inorganic components, contributing for oral health maintenance . The aim of this research is to evaluate the salivary pH,buffering capacity and flow rate levels of saliva in caries free people and in people with dental caries among different age groups .

MATERIALS AND METHODS:

The saliva samples of 120 children were collected with the aid of micropipette with disposable tips and plastic cylinders for 5 minutes. Salivary flow was determined by dividing the volume collected by the time of aspiration. All saliva produced was stored into a sterile plastic cylinder .The measurement of pH was performed with the aid of a digital pH meter and buffering capacity of saliva was measured.

RESULT:

There was a significant decrease in the mean salivary flow rate, salivary pH and salivary buffer capacity in people with dental caries compared to caries free people among different age groups, The calculated p value is statistically significant (p value - <0.05) in case of salivary pH and buffering capacity , whereas it is not significant in case of salivary flow rate (p value - >0.04).

CONCLUSION:

The physicochemical properties of saliva, such as salivary flow rate, pH, buffering capacity and viscosity, has a relation with caries activity in people and act as markers of caries activity.

Keywords: Buffering capacity,saliva,pH,flow rate,samples.

INTRODUCTION:

Saliva, a heterogeneous fluid comprising proteins, glycoproteins, electrolytes, small organic molecules and compounds transported from the blood, constantly bathes the teeth and oral mucosa. Whole saliva represents a mixture of the secretions of the major (submandibular, sublingual, parotid) and minor (accessory) salivary glands, together with the gingival fluid.(1)Saliva is essential

for the maintenance of oral health and it is an important diagnostic biofluid.(1) It plays a pivotal role in protection and lubrication of oral mucosal tissues, remineralization of teeth, and alimentation.(2,3) The composition of saliva gives it many important physical and biochemical properties.(4)It is useful for diagnosis, prognosis, and management of patients with oral and systemic diseases.(1)There is increasing inclination toward using saliva samples for the diagnosis of oral and systemic diseases.(1)

Among the oral diseases, dental caries is the most common chronic disease of mankind.(1,2)It is the main oral health problem in industrialized countries.(3)Saliva definitely promotes oral health and hence lack of its secretion contributes to the disease process. The saliva which constantly moisturise the teeth and oral mucosa, functions as a cleansing solution, a lubricant, a buffer and an ion reservoir of calcium and phosphate, which are essential for the remineralization of initial carious lesions.(1,2) A basal unstimulated secretion is produced continuously to moisturize and lubricate the oral tissues for more than 90 percent of the day. (2,4)The normal resting salivary flow rate ranges from 0.25 to 0.35 milliliter per minute. Mechanical, gustatory, olfactory or pharmacological stimuli increase the production and secretion of saliva. Stimulated saliva represents 80 to 90 percent of daily salivary production, and the stimulated flow rate varies from 1 to 3 mL/minute(3).The salivary pH and the salivary buffering capacity are determined by the hydrogen bicarbonate balance in saliva. Salivary pH is approximately neutral, and buffering agents, such as inorganic phosphate in resting saliva and carbonic acid-bicarbonate system in stimulated saliva, help maintain neutrality(3).

Among the various protective functions of saliva, including diluting and cleaning the oral cavity, serving as a host defense, and buffering and enabling ion exchange, certain salivary characteristics outside the normal range of values may contribute to the caries process(4).Dental caries results from the dissolution of minerals from the tooth surface by organic acids formed from the bacterial fermentation of sugars.

Saliva maintains the integrity of oral hard and soft tissues and protects against immunologic bacterial, fungal and viral infections.(1)Saliva controls the equilibrium between demineralization and remineralization in a cariogenic environment. Salivary buffers can reverse the low pH in plaque and allow for oral clearance thus preventing demineralization of enamel. The flow rate and viscosity of saliva may also influence the development of caries.(4)

The purpose of this study was to evaluate the relationship between the physiochemical properties of saliva such as flow rate, pH, buffering capacity in caries-free people and in people with dental caries among different age groups .

MATERIALS AND METHODS :

The study population consisted of 120 healthy children aged 7–15 years that was further divided into two groups: Group 1(7–10) years caries free and active children ,Group 2 (11–15) years caries free and active children. Unstimulated saliva samples were collected from all groups. Flow rates were determined, and samples analyzed for pH, buffer capacity.
Inclusion criteria : (1) children should be free from systemic or local diseases that affect salivary gland secretions (such as submandibular duct canaliculi, asthma and diabetes) and (2) children should be consuming only municipal water (those consuming hard water were not included as hard water consumption predisposes to dental fluorosis).
Exclusion criteria were on any medication for current and past illness and oral status other than dental caries like ulcers, oral tumors, herpetic lesions. Prior consent was obtained from the respective school authorities and from the parents through the school to conduct the study. The examinations were carried out in the subjects own surroundings that is, the school. The examination for dental caries was made according to the dentition status, and treatment.

Salivary analysis:

Collection of saliva and Estimation of salivary flow rate :

Twelve milliliters of stimulated whole saliva was collected for the study. Saliva was stimulated by making the children to chew the paraffin wax for about 1 minute before spitting the saliva and the sample collection was carried out in the day time between 10 am and 12 pm, 2 h after breakfast. Then, the children were made to sit comfortably in a ventilated and well-illuminated room, and were instructed to spit the saliva, which was collected for exactly 5 min in a pre-weighed graduated cylinder. A note was made of this value. Saliva collection was then continued till 12 mL of saliva was accumulated in the cylinder.Salivary flow rate was calculated in gms/ml which is almost equivalent to ml/min.

Collection of salivary samples

Estimation of pH & buffering capacity:-

• pH of saliva was measured by using digital pH me-ter.
• Buffering capacity of saliva {by Ericsson method 1959}:- 0.5 ml of saliva was added to 1.5 ml of 5mmol/l HCl. The mixture was vigorously shaken and then centrifuged for one minute and allowed to stand for 10 min when the final pH of supernatant was measured by using manual pH meter (6)

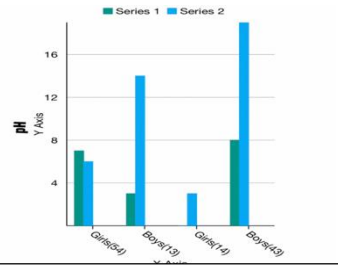
Salivary pH and Buffering capacity analysis

STATISTICAL ANALYSIS:

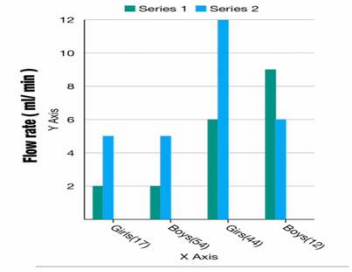
Statistical analysis of the data was performed with Student's t-test. Differences with P-values were considered to be not statistically significant.

RESULTS :

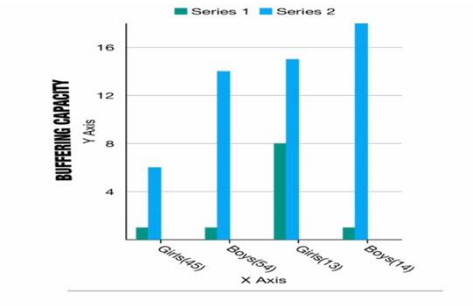
On observation from the below graphs , the salivary parameters like flow rate, pH and buffering capacity are slightly decreased in caries active children compared to caries free children



COMPARISON OF pH BETWEEN CARIES ACTIVE AND CARIES FREE INDIVIDUALS



COMPARISON OF FLOW RATE BETWEEN CARIES ACTIVE AND CARIES FREE INDIVIDUALS



COMPARISON OF BUFFERING CAPACITY BETWEEN CARIES ACTIVE AND CARIES FREE INDIVIDUALS

Salivary flow rate :

Group 1 :

The mean level of flow rate in Group 1 Caries Free children is (3.61 +/- 0.58) , the mean level of flow rate in Group 1 Caries Active children is (3.22+/-0.23) therefore , p- value for group 1 is 0.4433 , which is not Statistically significant (p value > 0.04) by the rule of one – tailed hypothesis.

Group 2 :

The mean level of flow rate in Group 2 Caries free children is (3.45+/- 0.2) and the mean level flow rate in Group 2 Caries active children is (3.25+/0.3) The mean level of flow rate is decreased in caries active children when compared to caries free children ,therefore the p – value is 0.467, it is not statistically significant (p value >0.04) by the rule of one – tailed hypothesis. (Table-1)

Groups				
Salivary flow rate	**Mean**	**SD**	**T -value**	**P value**
Dental caries free individuals				
Group 1 - age group (7-10 years)	3.61	0.58	1.432	(> 0.04)
Group 2 -age group (11- 15 years)	3.45	0.2	1.35	(>0.04)
Dental caries active children				
Group 1 - age group (7-10 years)	3.22	0.23	1.23	(>0.04)
Group 2 -age group (11- 15 years)	3.25	0.3	1.25	(>0.04

[Table-1]

Salivary pH :

Group 1 :

The mean level of pH in Group 1 Caries Free children is (7.6+/0.94) , the mean level of pH in Group 1 Caries active children is (6.24+/-0.13) ,as the p value is 0.000112 , therefore it is statistically significant (p value - <0.05) by the rule of one tailed hypothesis.

Group 2 :

The mean level of pH in Group 2 Caries free children is (7.13+/-0.13) and the pH in group 2 caries active children is (5.4+/-0.32)The mean level of pH is decreased in caries active children when compared to caries free children as the p value is 0.000306, and it is statistically significant (p value <0.05) by one tailed hypothesis .(Table-2)

Groups				
Salivary pH	**Mean**	**SD**	**T -value**	**P value**
Dental caries free individuals				
Group 1 - age group (7-10 years)	7.6	0.94	3.55	(<0.05)
Group 2 -age group (11- 15 years)	7.13	0.13	3.45	(<0.05)
Dental caries active children				
Group 1 - age group (7-10 years)	6.24	0.13	2.54	(<0.05)
Group 2 -age group (11- 15 years)	5.4	0.32	1.97	(<0.05)

[Table-2]

Salivary buffering capacity :

Group 1 :

The mean level of Buffering capacity in Group 1 Caries Free children is (5.9+/0.53), and the mean level of buffering capacity in Group 2 Caries active children is (2.7+/-0.33) , as the p - value is 0.006491, p value is statistically significant (p value <0.05)by one tailed hypothesis.

Group 2 :

The mean level of Buffering capacity in Group 1 Caries free children is (5.13+/-0.15) and the buffering capacity in Group 2 individuals is (3.5+/-0.21). Therefore the mean level of buffering capacity is decreased in caries active children when com-pared to caries free children , p value is 0.004283,and it is statistically significant (p value <0.05) by one tailed hypothesis . (Table-3)

[Table-3]

Groups				
Salivary Buffering capacity	**Mean**	**SD**	**T -value**	**P value**
Dental caries free individuals				
Group 1 - age group (7-10 years)	5.9	0.53	2.44	(<0.05)
Group 2 -age group (11- 15 years)	5.13	0.15	2.3	(<0.05)
Dental caries active children				
Group 1 - age group (7-10 years)	2.7	0.33	0.8	(<0.05)
Group 2 -age group (11- 15 years)	3.5	0.21	1.43	(<0.05)

DISCUSSION:

Human saliva is an oral fluid that has several functions involved in oral health and homeostasis, with an active protective role in maintaining oral health.(15)Saliva helps bolus formation by moistening food, protects the oral mucosa against mechanical damage and plays a role in the preliminary digestion of food through the presence of α-amylase and other enzymes. It also facilitates taste perception and also has a role in maintaining teeth enamel mineralization.(14)

Dental caries has been thought of as a multifactorial disease as it is not only influenced by dietary factors but host factors as well documented.(13) These defense systems include clearance, buffering, antimicrobial agents and calcium and phosphate delivery for remineralization, to name a few.(12) The interaction of protective and pathologic factors in saliva and plaque biofilm, as well as the balance between the cariogenic and non-cariogenic microbial populations that reside in saliva, decides the caries process.(10)The factors in saliva most frequently related to dental caries are: (a) aciduric/acidogenic bacteria and (b) rate of acid production in the presence of glucose. Other factors that have been suggested as being related to dental caries include (a) amount of saliva secreted in a given time and (b) acid-neutralizing ability (buffering capacity) of saliva.(14)

SALIVARY pH:

In the present study , there was a significant difference in the mean salivary pH among the study groups Group 1 and Group 2 in both the caries free and caries active children and it is Statistically significant (p value < 0.05) .There was a significant difference in the mean salivary pH among the study groups ($P < 0.0001$). Group I had a significantly higher mean salivary pH value than that of Groups II . The results obtained are in accordance with the studies performed by Prabhakar *et al*. in 2009(2)and Preethi *et al*. in 2010.(5)However, the results obtained in their studies were not significant. The salivary pH was only slightly reduced in caries-active children compared with caries-free children. Another study by Zhou *et al*. in 2007(6) showed that the pH of saliva from early childhood caries children was statistically higher than that in caries-free children.(12) In contrast, a study carried out by Thaweboon *et al*. in 2008(8) revealed that the mean values for salivary pH were similar in caries-free and rampant-caries children. Swerdlove in 1942(3)and Malekipour *et al*. in 2008(4) reported no relationship between the incidence of dental caries and the pH of normal resting saliva. Lamberts *et al*. in 1983(12) observed no relationship of salivary pH rise activity and caries experience in caries-free and caries-active subjects.

SALIVARY BUFFERING CAPACITY :

In the present study , there was a significant difference in the mean salivary buffering capacity among the study groups Group 1 and Group 2 in both the caries free and caries active children (p value <0.05) and it is Statistically significant (p value <0.05).There was a significant difference in the mean salivary buffering capacity among the study groups ($P < 0.0001$). Group I had a significantly higher mean salivary buffering capacity than that of Groups II and III. However, no significant difference was seen between the mean salivary buffering capacities of Group II and Group III. The results obtained are in accordance with the studies performed by Prabhakar *et al*. in 2009(2) and Preethi *et al*. in 2010.(1) However, the results obtained in their studies were not significant.(5) The salivary buffering capacity was only slightly reduced in caries-active children compared with caries-free children. Another study by Zhou *et al*. in 2007(5)showed that the buffering capacity of saliva from early childhood caries children was statistically higher than that in caries-free children. A study performed by Malekipour *et al*. in 2008(6)showed similar results, although the difference was not statistically significant.(6)

SALIVARY FLOW RATE :

In the present study , there was only a small amount of significant difference in the mean salivary flow rate among the study groups Group 1 and Group 2 in both the caries free and caries active children (p value > 0.04) and it is not Statistically significant . Whereas there was a significant difference in the mean salivary flow rate among the study groups ($P < 0.0001$). Group I had a significantly higher mean salivary flow rate than that of Groups II and III. Similarly, Group II had a significantly higher mean salivary flow rate than Group III. Lopez *et al*. in 2003(5) reported a salivary flow rate of 0.27 ± 0.14 in a group of children aged 5-12 years. The results obtained are in accordance with the studies carried out by Preethi *et al*. in 2010(1).and Prabhakar *et al*. in 2009.(5)However, the results obtained in their studies were not statistically significant. The salivary flow rate was only slightly reduced in caries-active children compared with caries-free people. In contrast, a study performed by Thaweboon *et al*. in 2008(8)revealed that the mean values for salivary flow rate were similar in caries-free and caries active people. The salivary flow rate did not influence the presence of caries.(20)

CONCLUSION:

Dental caries is a complex and dynamic process where a multitude of factors influence and initiate the progression of disease. One of the most important factor which influences the development of dental caries is saliva. Alterations in the physicochemical properties of saliva such as decreased salivary pH, buffering capacity play a major role in the development of caries, whereas salivary flow rate contribute only a small role in caries development. These results re-emphasize that there is a relationship of the various physiochemical properties of saliva, such as salivary flow rate, pH and buffering capacity along with the caries activity in the oral cavity.

REFERENCES:

1.Edgar WM. Saliva: Its secretion, composition and functions. Br Dent J 1992; 172: 305-310
2. Amerongen AVN, Bolscher JGM, Veerman ECI. Sali-vary proteins: Protective and diagnostic value in cari-ology. Caries Res 2004; 38: 247-253.
10. Kedjarune U, Migasena P, Changbumrung S, Pong-paew P and Tungtrongchitr R. Flow rate and composi-tion of whole saliva in children from rural and urban Thailand with different caries prevalence and dietary intake. Caries Res 1997; 31: 148-154.
3. Lumikari Lenander.M, Loimaranta,V. Saliva and Den-tal Caries. Adv Dent Res 2000: 14: 40-47.
4.Mandel ID. The functions of saliva. J Dent Res 1987; 66(special issue): 623-662. 13. Johansson I, Lenander-Lumikari M, Saellstrom A. Saliva composition in Indian children with chronic protein-energy malnutrition. J Dent Res 1994; 73(1): 11-19.
5. Tenovuo J, Lehtonen OPJ,Aaltonen AS, Vilja Pand Tuohimma P. Antimicrobial factors in whole saliva of human infants. Infection and Immunity.1986; 51: 49-53
6.Prior RL,CoaG.In vivo total antioxidant capacity: Comparission of different analyticalmethods. Free Radical Biology and Medicine 1999; 27: 1173-1181.
7.Moore S, Calder KA,Miller NJ and Rice Evans CA. Antioxidant activity of saliva and periondontal disease. Free Radical Research 1997; 26: 209-234.
8.Tulunoglu O,Demirtas S,Tulunoglu I.Total antioxidant levels of saliva in children related to caries, age and gender. Int J Paed Dent 2006; 16: 186-191.
9.Mc-Donald RE, Avery DR, Stookey GK. Dental caries in the child and adolescents. In: Mc-Donald RE, Avery DR, Dean JA, editor. Dentistry for the child and ado-lescent. 8th ed. New Delhi: Elsevier; 2005.p. 203-235.
10.Shafer W.G, Hine M.K and Levy B.M. ED. A text book of oral pathology 5th Ed . Philadelphia W. B. Saunders company.1993; 567-658.
11.Lavelle. L.B. Christopher Applied oral physiology, 2nd edition. Butterworths and Co (publishers) LTD 1988. Saliva. P.128-141.
12. John T, Mc Devitt. Saliva as the next best diagnostic tool. Journal of Biochem.2006; 45(2): 23-25.
13. Battino M, Ferreior MS, Gallardo I, Newman HN, Bul-lon P. The antioxidant capacity of saliva. J Clin Perio-dontol 2002; 29: 189-194
14.Gopinath VK, Arzreanne. Saliva as a diagnostic tool for assessment of dental caries. Archives of Orofacial Sciences 2006; 1: 57-59.
15.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;39:893–9.
16.Shafer WG, Hine MK, Levy BM. Dental caries. In: Sivapathasundharam B, Raghu AR, editors. A Textbook of Oral Pathology. 5th ed. Philadelphia: WB Saunders Company; 1993. pp. 567–8.
17.Nikiforuk G. Clinical features and classification of dental caries. In: Nikiforuk G, editor. Understanding Dental Caries Etiology and Mechanisms: Basics and Clinical Aspects.New York: Karger; 1985. p. 10.
18. Amanthi Ganapathi ,Study Of Enzymatic Biomarkers To Evaluate Periodontal Severity , Pharm. Sci. & Res. Vol. 8(7), 2016, 696-699.
19. A study on Variations of salivary pH with Intake of food Gokul*, Vishnupriya, gayathri,Asian journal of pharmaceutical science and clinical research, Vol 9, Suppl. 1, 2016.
20. Salivary cariogenic microflora,buffer capacity,secretion rate and its relation to caries experience ,Nithin manchery,Joseph John j .contempt dent 2015;5(1):12-16