ORIGINAL RESEARCH

A comparative evaluation of physicochemical properties of saliva in habitual mouth breathers and nasal breathers

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ABSTRACT

Background: The study was conducted to analyze some standard parameters of the unstimulated whole saliva of habitual mouth-breathers and a control group of nasal breathers Also, the study aimed to determine whether there is any difference in these variables when compared amongst mouth-breathers and the control group. **Setting and Design**: Saliva samples were collected from 100 children aged 10 - 16 years; 50 were habitual mouth-breathers and 50 were nose-breathers. **Method:** The unstimulated salivary specimen was collected. After that salivary flow rate and pH were determined. The samples were then immediately analyzed for determination of free, bound and total sialic acid levels. **Results:** Statistically significant result was observed in the flow rate, pH, bound and total sialic acid levels of unstimulated whole saliva saliva, in habitual mouth breathers and nasal breathers. However, the levels of free sialic acid of the unstimulated saliva were not statistically significantly in the mouth breather compared to the control group. **Conclusion:** pH and flow rate were found to be decreased in habitual mouth breathers which indicates an acidic oral flora and dryness respectively, compared to nasal breathers. Since higher levels of bound and total sialic acid suggests an increase in the number of bacteria in saliva, our findings indicate that mouth-breathers retain more bacteria in oral tissues.

KEYWORDS: Habitual Mouth Breathers, Flow Rate, Saliva; Sialic Acid

INTRODUCTION

Saliva is a odorless, tasteless, slightly acidic, viscous fluid consisting of secretions from major and minor salivary glands. It is a biologic fluid, utilized for diagnosis of disease other than analysis of cellular and chemical constituents of blood, with some distinct advantages.[1] Saliva is easily available, noninvasive diagnostic medium and is a cost effective approach for the screening of large population.[1]

Saliva can be collected as stimulated or unstimulated saliva. Stimulated saliva is collected by masticatory action or by gustatory stimulation. Stimulation affects the pH of saliva. Unstimulated saliva is collected without exogenous, gustatory stimulation or masticatory or mechanical action.[1] It possesses tremendous multi-functionality that is; it is involved in diverse range of functions such as buffering action, lubrication, protection, maintenance of tooth, mucous membrane integrity, antimicrobial action, taste and digestion. Important salivary constituents are antimicrobial factors, inorganic ions, factors affecting mineralization, buffering factors like bicarbonates, urea and phosphates and sialic acid. These substances in conjunction with others provide first line of defense against microbial invasion.[2]

The human mouth is quite frequently exposed to components whose pH differs from normal salivary Ph (6.5-7.5). These components may cause erosion of teeth or damage to mucosal surfaces. Salivary buffers, however, try to bring the pH back to normal range as fast as possible. In unstimulated saliva, the major buffering agent is inorganic phosphate and in stimulated saliva carbonic acid/bicarbonate system.[2]

The buffering capacity of saliva plays an important role in the maintenance of salivary pH, and in dental remineralization. It correlates with salivary flow rate, as any factor which decreases salivary flow rate tends to decrease its bufferng capacity and hence, increases the risk of caries development.[3] Diminution of salivary flow rate is also associated with oral dryness in mouth breathers.[4]

The state of inhaling and exhaling through the mouth is referred to as mouth breathing. At rest or while doing light exercise healthy individual breathes through the

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nose and during vigorous aerobic exercise, in order to supply sufficient oxygen a healthy individual breathes simultaneously through both the nose and mouth During excessive mouth breathing air bypasses the nasal canal and paranasal sinuses, and dries out the mouth[4] this air is not filtered and warmed and can prove problematic. Salivary secretions like salivary glycoproteins are important for oral health and they play an important role in properties and functions of saliva.[5] Sialic acids are terminal sugar components of the oligosaccharide chains of glycoproteins and glycolipids.[6] In human beings it is present in body fluids (blood plasma, breast milk, gallbladder excretions, synovial fluid, sweat, gastric juices and urine) and tissues (erythrocytes, leucocytes, platelets, salivary glands, throat, stomach, cervix, colon, cartilage etc).[7] In humans so far the only sialic acid to be found from over a dozen different sources has been N Acetyl Neuraminic acid.[8] Sialic acid also acts as a marker of chronic inflammatory response. In children with poor oral health status sialic acid is found to be present at higher levels.[9]

Due to the dynamic nature of saliva, there are variations in properties and composition of saliva both quantitatively and qualitatively due to various physiological and pathological factors. Also there is very little literature available about physicochemical properties and mouth breathing. Hence this study was undertaken to find and analyze some standard parameters (Salivary pH, Salivary Flow Rate, Sialic acid) of the unstimulated saliva of habitual mouth breathers and control group (Nasal breathers) and to determine if these variables differ in their values in mouth breathers and the control group, since these parameters of saliva can influence the oral health status of an individual.

MATERIALS AND METHODS

Subjects: One hundred subjects aged 10 - 16 years were selected from the patients attending the outpatient department at Department of Paedodontics and Preventive Dentistry (Manav Rachna Dental College, Faridabad, India.) The criteria for inclusion in this study demanded that the patients should not be taking any medication, should be free of systemic disease, and should not have open cavities in the teeth and/or unhealthy gums. To diagnose the habitual mouth-breathing, the subjects were submitted to specific physical examination and observation by a Pediatrician. This research was approved by the Ethical committee of the respective institute and informed consent was obtained from the parent or guardian of the subjects.

Those children who fulfilled the inclusion criteria were divided into two groups Group A (Control Group: Nasal breathers) and Group B (Experimental Group: Habitual Mouth Breathers.) While 50 patients were nasal-breathers, 50 patients were habitual mouth-breathers.

Saliva Collection: Unstimulated whole saliva sample was collected in the morning to make allowances for circadian rhythms and each patient was asked not to eat or drink for 1 hour prior to saliva collection. The patient was asked to sit on the dental chair with his/her head bend forward so as to collect unstimulated saliva (Fig 1). Subsequently, after an initial swallow saliva was allowed to accumulate on the floor of the mouth and the subject was instructed to spit into a sterilized plastic container.

Fig 1: Salivary Sample Collection

Salivary flow rate: Immediately after collection, saliva volume was measured and the salivary flow rate (SFR) was calculated in ml/min, by dividing the total volume by the time of collection. Calibrated disposable sterile test tubes (Fig 2) were used for measuring the amount of saliva in milliliter (Fig 3) , as the test tubes were calibrated from 1 to 15 millilitres, with a unit of 1 millilitre each.[10]

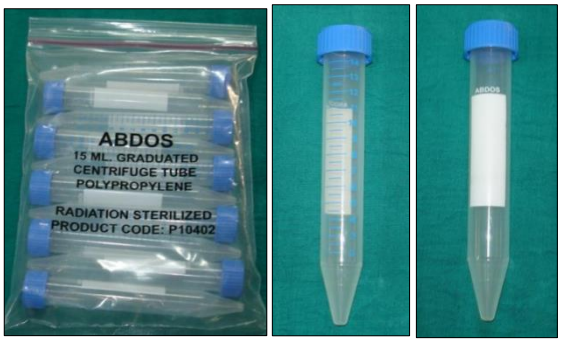

Fig 2: Sterilized Calibrated Disposable Test Tubes for measuring Salivary Flow Rate

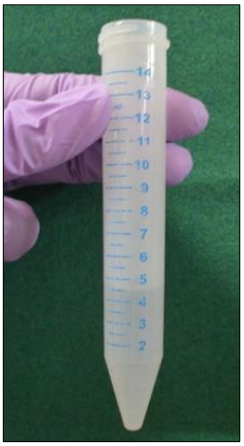

Fig 3: Measuring Amount of Saliva Collected (ml) for Salivary Flow Rate

Salivary Ph: After evaluating the flow rate, the pH of the sample was measured with the digital pH meter (HANNA Instruments) (Figure 4, 5).Thereafter, the containers were sealed and the samples were delivered to the Department of Biotechnology, MRIU Faridabad in an insulated ice box for immediate evaluation of bound sialic acid, unbound sialic acid and total sialic acid.

Fig 4: Digital pH meter, Figure 5: Measuring pH with Digital pH Meter

Salivary Sialic Acid: The levels of sialic acid (free, bound and total) were determined using the method of Yao et al 1987 works on the principle that in presence of acidic medium, sialic acid reacts with the acid ninhydrin reagent which yields a stable color which is measured calorimetrically, with an absorption maximum at 470nm in a colorimeter (Figure 6). NAcetylneuraminic acid (Sigma-Aldrich Corporation, USA) was used as standard.

Fig 6: Colorimeter

Preparation of standard curve: A standard of N-acetyl neuraminic acid was prepared having a concentration of 0.1mg/ml. This stock was diluted appropriately using distilled water to obtain varying concentration of N-acetyl neuraminic acid i.e. S1 - 20 µg, S2 - 40 µg, S3-60 µg, S4-80 µg and S5 -100 µg were taken in the test tubes. The volume was made up to 1 ml with distilled water. To this 1 ml of glacial acetic acid [Merk] and 1 ml of ninhydrin reagent was added and kept in boiling water bath for 10 minutes. The ninhydrin reagent contained 250mg of ninhydrin (Merk) in a mixture of 6ml of acetic acid and 4ml of concentrated HCl (Merk), with repeated mixing at room temperature for 20-30 min.

Procedure for analysis of sialic acid: Saliva was first centrifuged at 3000 rpm for 15 minutes. The pellet was discarded and sialic acid was estimated in supernatant. To the supernatant, 9 ml ethanol was added to separate the bound sialic acid from free sialic acid. They were mixed properly and checked for the formation of precipitates. The precipitates obtained were separated from the solvent. The precipitates contained bound sialic acid while the solvent contained free sialic acid. 1ml each, of the solvent and the precipitate were taken in separate test tubes and to them 1ml glacial acetic acid and 1ml freshly prepared ninhydrin reagent were added. The test tubes containing this reaction mixture were kept in boiling water bath for 10 minutes and the optical density was measured at 470nm. Distilled water was used as blank. The concentration of free and bound sialic acid was calculated on the basis of optical density using the standard curve prepared above.The total sialic acid was obtained by determining the sum of the levels of free sialic acid and bound sialic acid.

Statistical Package for the Social Sciences software program, version 16.0 (SPSS Ver. 19.0, Inc., Chicago, IL,USA) was used to conduct the statistical analyses. Continuous variables were presented by means, SD and compared using unpaired Student's t distribution test.

RESULTS

The mean and standard deviation showed for nasal breather and mouth breather group concerning the pH, can be verified in Table 1. The result was found to be statistically significant.

	Group	N	Mean	Std. Deviation	t-value	p-value
pH	Nasal Breather	50	6.85	0.36	3.852	<0.001**
	Mouth Breather	50	6.53	0.45		

Table 1. pH

The results in Tables 2, show that there was a statistically significant difference in the flow rate of unstimulated whole saliva of mouth and nasal-breathers.

	Group	N	Mean	Std. Dev	t-val	p-value
Flow Rate [ml/min]	Nasal Breather	50	0.53	0.16	3.764	<0.001**
	Mouth Breather	50	0.41	0.15		

Table 2: Flow Rate

Table 3 shows the results for the free, bound and total sialic acid levels in unstimulated saliva, the statistically significant difference found was that mouth-breathers had greater levels of bound and total sialic acid in their unstimulated saliva than the control group. No statistically significant difference was observed in free

sialic acid scores of habitual mouth- and nasal breathers.

Following inferences were drawn from this study:

- The pH values of nasal breathers is more than the pH values of mouth breathers.
- The flow rate value of nasal breathers is more than flow rate value of mouth breathers.
- The free sialic acid was higher in habitual mouth breathers than nasal breathers, although the t test is not statistically significant.
- The bound sialic acid value of Nasal Breathers is less than the bound sialic acid value of mouth breathers.
- The total sialic acid value of Nasal Breathers is less than the total sialic acid value of mouth breathers.
- The salivary pH and salivary flow rate decreased in habitual mouth breathers whereas free sialic acid, bound sialic acid and total sialic acid was found to be increased in mouth breathers.

	Group	N	Mean	Std. Deviation	t-value	p-value
Free Sialic Acid [µg/ml]	Nasal Breather	50	3.58	1.44	0.858	0.393
	Mouth Breather	50	3.87	1.87		
Bound Sialic Acid [µg/ml]	Nasal Breather	50	3.61	2.30	2.168	0.033 *
	Mouth Breather	50	4.64	2.44		
Total Sialic Acid [µg/ml]	Nasal Breather	50	7.19	3.25	1.916	0.058
	Mouth Breather	50	8.50	3.61		

Table 3 Free Sialic Acid, Bound Sialic Acid and Total Sialic Acid

DISCUSSION

Saliva is a complex fluid composed of a wide variety of organic and inorganic constituents that collectively act to modulate the oral environment.[11] Collection of saliva is easy, non painful to patient and is less infectious to the health care provider.[12]

The unstimulated saliva was collected for the purpose of investigation. The decision to collect unstimulated saliva in this study was chosen as unstimulated saliva yields valuable information and usually correlates to the clinical condition more accurately than stimulated saliva as supported in a study by Mori F et al.[13]

In the present study physicochemical properties of mouth breathers were compared to the normal healthy individuals i.e. nasal breathers. The studies on salivary composition of subjects with mouth breathing are limited. So mouth breathers were taken as an experimental group to find if mouth breathing could alter some of the salivary variables and cause damage to the oral cavity. The result of this study suggests that the adolescents with mouth breathing, present alteration in the salivary parameters, that can increase the risk of oral disease in them.

In the present study physicochemical properties of saliva i.e. pH, flow rate, free sialic acid, bound sialic acid and total sialic acid were determined for mouth breathers and nasal breathers, since these parameters of saliva can influence the oral health.

As reported by Tram TH et al[14], sialic acid contributes to viscosity of saliva. Enzymatic cleavage of sialic acid residue by neuraminidase reduces viscosity of saliva and subsequently causes precipitation of some salivary components.[15] Also flow rate of mouth breathers have been reported to be less in comparison to nasal breathers due to evaporation of water from the saliva of constant mouth breathers that can reach 0.24mL/min[16], which often leads to oral dryness. Thus a possible explanation to viscous saliva in mouth breathers could be increased sialic acid content in saliva of mouth breathers. Thus flow rate and sialic acids were determined in the present study.

The sialic acids are a family of 9-carbon carboxylated

sugars usually found as terminal monosaccharides of animal oligosaccharides. The most common is N-acetyl-neuraminic acid (2-keto-5-acetamido-3,5-dideoxy-D-glycero-D-galactononulopyranos-1-onic acid) (Neu5Ac), which is believed to be the biosynthetic precursor for all other members of the family.[17] Alterations in the level of sialic acid in the saliva have been reported with elevated amount found in diabetics, cancer cases, pregnancy, Down syndrome[18] and in plaque and calculus formation. Interestingly elevated levels of sialic acid in human milk have also been related to higher IQ in children.[14] Thus this study was undertaken to know the effect of mouth breathing on the salivary sialic acid and to compare its clinical significance with normal healthy subjects i.e. nasal breathers.

In this study the unstimulated whole saliva sample was collected in the morning as many investigations have shown that the salivary flow rate fluctuates with the circadian cycle.[19] It has been suggested that the unstimulated flow rate may be at its maximum in the mid-afternoon.[20] Moreover, variation of unstimulated whole saliva flow rate over different time-spans and at different times of the year yield changes in flow rate[19]. To avoid the circadian effect, obtaining saliva at the same time of day is essential.

In the present study pH was determined by a digital pH meter. The pH reading of the digital pH meter was standardized by using buffer capsules of pH 7.0 ± 0.05. For this each buffer capsule was first dissolved in 10 ml of CO_2 free water and then made till 100 ml and pH was adjusted in the pH meter at 7.0 for standardization. The mean ± SD of salivary pH for the nasal breathers i.e. normal healthy individuals [control group] was 6.85 ± 0.36 and for mouth breathers was 6.53 ± 0.45. In our present study the salivary pH was decreased in mouth breathing children compared to nasal breathing children which was highly statistically significant.

Similar results in accordance with our control group were seen in the studies conducted by Preethi BP et al[12], Shaila M et al[21], S Kuriakose et al[22], Moreira AR et al [23] and Baliga S et al.[24]

Contrary studies were reported by Fiyaz M et al[25], Englander H.R et al[26], Dogra S et al[27] who showed varied levels of pH in relation to the results in control group of our study.

Literature search revealed no similar studies comparing the pH of mouth breathers. Hence our study gives a value to the pH of mouth breathers for the age group of 10 – 16 years. The mean ± SD value was found out to be 6.53 ± 0.45.

The salivary flow measurement is frequently used in the evaluation of oral and systemic diseases. Measurement of salivary flow rate is used as a screening method to identify people with low salivary flow which could often, but not always be related to mouth breathing. In general, higher the flow rate, faster the clearance and higher the buffer capacity, lesser is the microbial attacks[28]. Normal unstimulated salivary flow rate ranges from 0.3 to 0.5 mL/min, and flow rates between 0.10 and 0.001 mL/min are considered hyposalivation.[29]

In the present study unstimulated salivary flow rate was determined by caliberated disposable sterile test tubes for measuring the amount of saliva in milliliters. The test tubes were calibrated with a unit of 1 ml each ranging from 1 to 15 ml.[29]. In this study, saliva was collected without stimulation. Collection was made by expectoration, as the methods involving suction or use of cotton wool cause a certain degree of stimulation in the oral cavity, which could have prejudiced measurement. Also this method is more reliable than weighing the saliva in gm/ml and then converting it to mL/min.[29]

The mean ± SD salivary flow rate for the nasal breathers i.e. normal healthy individuals [control group] was 0.53 ± 0.16 mL/min and for mouth breathers was 0.41 ± 0.15 mL/min. In our present study the salivary flow rate was decreased in mouth breathing children compared to nasal breathing healthy children and was highly statistically significant.

In the study by Weiler RME[4] she found the flow rate to be 0.41 ± 0.35 mL/min in nasal breathers and flow rate of 0.36 ± 0.24 mL/min in mouth breathers which coincide with the results of our study. Similar results were seen in studies conducted by Shaila M et al[21], Fiyaz et al [25] and Foglio-Bonda P.L. et al.[30]

Results contrary to our results were shown in studies reported by Santos MT et al[31], Narhi TO[32], Avsar A et al[28], Hidas A et al[33], Alves C et al [29] which were both higher and lower than the results obtained in our study.

Sialic acid is an important structural component of glycoproteins, playing a part in enhancing bacterial agglutination[34], as well as participating in formation of the acquired pellicle and dental plaque.[4]

In the present study three forms of sialic acid i.e free sialic acid, bound sialic acid and total sialic acid were determined calorimetrically by a method using acidic nynhydrin reaction given by Yao et al (1987).[35] This is a comparatively newer method than the previously used Thiobarbituric acid method by D. Aminoff – 1961[36], Warren L. (1959)[37]; Direct Ehrlich method (1952) and Resorcinol method by G.W Jourdian 1971[38] used for sialic acid determination.

In our present study the salivary free sialic acid value in mouth breathers and nasal breathers is not statistically significant. Salivary bound sialic acid value for nasal breathers is less than that of mouth breathers which was statistically significant. Salivary total sialic acid value for nasal breathers is less than that of mouth breathers which was statistically significant.

Contrary studies were reported by Tram TH et al[14], Yarat A et al[9] and Williams JE[39], who showed varied levels of sialic acid in relation to the results in control group of our study.

Extensive literature search revealed no similar studies comparing sialic acid in mouth breathers. Hence our study gives a value to the sialic acid content of mouth breathers for the age group of 10 – 16 years. The mean ± SD value of free sialic acid, bound sialic acid and total sialic acid for mouth breathers was found out to be 3.87 ± 1.87 µg/ml, 4.64 ± 2.44 µg/ml and 8.50 ± 3.61 µg/ml respectively.

The physicochemical properties of saliva like pH, salivary flow rate, free sialic acid, bound sialic acid and total sialic acid play a major role in different patterns of breathing. Still there are very few studies in support or contrast to this result.

Hence more clinical and laboratory studies are needed to determine the exact relationship between these physicochemical properties of saliva and mouth breathing.

CONCLUSION

The present study was carried out to estimate and compare the pH, flow rate and free sialic acid, bound sialic acid and total sialic acid in nasal breathers and habitual mouth breathers. The enzyme systems responsible for the release and breakdown of sialic acid from salivary mucin is bacterial in origin, is mainly present in saliva and correspond in action to the enzyme neuraminidase and aldolase. The amount of sialic acid in the saliva can be a useful index of the severity of oral disease.

Following inferences were drawn from this study:

- The pH values of nasal breathers is more than the pH values of mouth breathers.
- The flow rate value of nasal breathers is more than flow rate value of mouth breathers.
- The free sialic acid was higher in habitual mouth breathers than nasal breathers, although the T test is not statistically significant.
- The bound sialic acid value of Nasal Breathers is less than the bound sialic acid value of Mouth Breathers.
- The total sialic acid value of Nasal Breathers is less than the total sialic acid value of Mouth Breathers.

- The salivary pH and salivary flow rate decreased in habitual mouth breathers whereas free sialic acid, bound sialic acid and total sialic acid was found to be increased in mouth breathers.

The physicochemical properties of saliva like pH, salivary flow rate, free sialic acid, bound sialic acid and total sialic acid play a major role in different patterns of breathing. Still there are very few studies in support or contrast to this result. Hence more clinical and laboratory studies are needed to determine the exact relationship between these physicochemical properties of saliva and mouth breathing.

REFERENCES

1. Kaufman E, Lamster IB. The diagnostic applications of saliva-- a review. Crit Rev Oral Biol Med 2002;13(2):197-212.
2. Prabhakar AR, Gulati A, Mehta D, Sugandhan S. Diagnostic applications of saliva in dentistry. Int J Clinic Ped Dent 2009;2(3):7-13.
3. Sanchiz V, Herreros B, Hernández V, Mínguez M. Unstimulated salivary flow rate , pH and buffer capacity of saliva. Rev Esp Enferm Dig 2004;96:773-83.
4. Weiler RME, Fisberg M, Barroso AS, Nicolau J, Simi R, Siqueira WL. A study of the influence of mouth-breathing in some parameters of unstimulated and stimulated whole saliva of adolescents. Int J Pediatr Otorhinolaryngol 2006;70(5):799-805.
5. Sanjay PR, Hallikeri K, Shivashankara AR. Evaluation of salivary sialic acid, total protein, and total sugar in oral cancer: a preliminary report. Indian J Dent Res 2008;19(4):288-91.6. Varki A, Schauer R. Chapter 14 Sialic Acids. 1957;
6. Nigam PK, Narain VS, Kumar A. Sialic acid in cardiovascular diseases. Indian J Clin Biochem 2006 ;21(1):54-61.
7. Leach SA. Release and breakdown of sialic acid from human salivary mucin and its role in the formation of dental plaque. Nature 1963;199(4892):486-87.
8. Oztürk LK, Furuncuoğlu H, Atala MH, Uluköylü O, Akyüz S, Yarat A. Association between dental-oral health in young adults and salivary glutathione, lipid peroxidation and sialic acid levels and carbonic anhydrase activity. Braz J Med Biol Res 2008;41(11):956-9.
9. Navazesh M, Kumar SKS. Measuring salivary flow: Challenges and opportunities. JADA 2008;139(suppl 2):35S-40S
10. Edgar WM. Saliva: its secretion, composition and functions. Br Dent J 1992;172:305-12.
11. Preethi BP, Pyati A, Dodawad R. Evaluation of flow rate , pH , buffering capacity , calcium , total protein and total antioxidant levels of saliva in caries free and caries active children - An in vivo study. Biomed Res 2010;21(3):289-94.
12. Mori F, Hiraishi N, Otsuki M, Tagami J. Effect of mastication on flow and properties of saliva. Asian Pac J Dent 2012;12:1-5.
13. Tram TH, Brand Miller JC, McNeil Y, McVeagh P. Sialic acid content of infant saliva: comparison of breast fed with formula fed infants. Arch Dis Child 1997;77(4):315-8.
14. Briscoe JM, Pruitt KM, Caldwell RC. The effect of neuraminidase on the properties of salivary proteins. J Dent Res 1971;51(3):819-24.
15. B. Guggenheim, Cardiology Today, S. Karger, Basel, 1984, 70 pp.
16. Varki A. Diversity in the sialic acids. Glycobiology 1992;2(1):25-40.
17. Yarat A, Akyuz S, Koc L, Erdem H, Emekli N. Salivary sialic acid , protein , salivary flow rate , pH , buffering capacity and caries indices in subjects with Down's syndrome. J Dent 1999;27:115-18.
18. Kavanagh DA, Svehla G. Variation of salivary calcium, phosphate and buffering capacity in adolescents. Arch Oral Biol 1998;43(12):1023-7.
19. Dawes C. Circadian rhythms in human salivary flow rate and composition. J Physiol.1972;220(3):529-45.
20. Shaila M, Pai GP, Shetty P. Salivary protein concentration , flow rate, buffer capacity and pH estimation : A comparative study among young and elderly subjects , both normal and with gingivitis and periodontitis. J Indian Soc Periodontol 2013;17(1):42-6.
21. Kuriakose S, Sundaresan C, Mathai V, Khosla E, Gaffoor F. A comparative study of salivary buffering capacity, fl ow rate, resting pH, and salivary Immunoglobulin A in children with rampant caries and caries-resistant children. J Indian Soc Pedod Prev Dent 2013;31:69-73.
22. Inoue H, Ono K, Masuda W, Morimoto Y, Tanaka T, Yokota M, Inenaga K. Gender difference in unstimulated whole saliva flow rate and salivary gland sizes. Arch Oral Biol 2006;51(12):1055-60.
23. Baliga S, Muglikar S, Kale R. Salivary pH : A diagnostic biomarker. J Indian Soc Periodontol 2013;17:461-5.
24. Fiyaz M, Ramesh A, Ramalingam K, Thomas B, Shetty S, Prakash P. Association of salivary calcium, phosphate, pH and flow rate on oral health: A study on 90 subjects. J Indian Soc Periodontol 2013;17:454-60.
25. Englander HR, Mau LM, Hoerman KC, Chauncey HH. Dental caries activity, and the pH, titratable alkalinity, and rate of flow of human parotid saliva. J Dent Res 1958;37(5):906-11.
26. Dogra S, Bhayya D, Arora R, Singh D, Thakur D. Evaluation of physio-chemical properties of saliva and comparison of its relation with dental caries. J Indian Soc Pedod Prev Dent 2013;31(4):221-4.
27. Avşar A, Darka O, Bodrumlu EH, Bek Y. Evaluation of the relationship between passive smoking and salivary electrolytes, protein, secretory IgA, sialic acid and amylase in young children. Arch Oral Biol 2009;54(5):457-63.
28. Alves C, Brandão M, Andion J, Menezes R. Use of graduated syringes for measuring salivary flow rate: a pilot study. Braz Dent J 2010;21(5):401-4.
29. Foglio-Bonda PL, Migliario M, Rocchetti V, Pattarino F, Bonda FA. Daily and annually variation of unstimulated whole saliva flow rate and pH and their relation with body profile in healthy young adults. Eur Rev Med Pharmacol Sci 2013;17(18):2538-45.
30. Rodrigues Santos MTB, Siqueira WL, Nicolau J. Amylase and peroxidase activities and sialic acid concentration in saliva of adolescents with cerebral palsy. Quintessence Int 2007;38(6):467-72.
31. Närhi TO, Tenovuo J, Ainamo a, Vilja P. Antimicrobial factors, sialic acid, and protein concentration in whole saliva of the elderly. Scand J Dent Res 1994;102(2):120-5.
32. Hidas A, Noy AF, Birman N, Shapita J, Matot I, Steinberg D, MoskovitzM. Oral health status, salivary flow rate and salivary quality in children,
adolescents and young adults with ADHD. Arch Oral Biol 2011;56(10):1137-41.
33. Dezan CC, Nicolau J, Souza DN, Walter LRF. Flow rate, amylase activity, and protein and sialic acid concentrations of saliva from children aged 18, 30 and 42

months attending a baby clinic. Arch Oral Biol 2002;47(6):423-7.
34. Yao K, Ubuka T. Determination of sialic acids by acidic ninhydrin reaction. Acta Med Okayama 1987;41(6):237-41.
35. Aminoff D. Methods for the quantitative estimation of N-acetylneuraminic acid and their application to hydrolysates of sialomucoids. Biochem. J 1961;81:384-92.
36. Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem 1959;234(8):1971-5.
37. Jourdian GW, Dean L, Roseman S. The sialic acids. XI. A periodate-resorcinol method for the quantitative estimation of free sialic acids and their glycosides. J Biol Chem 1971;246(2):430-5.
38. Williams JE. Relationship of sialic acid to dental caries. J Dent Res 1966;46(3):514-21.

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