

Correlation between pH, buffering capacity, calcium and dental caries in schoolchildren

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Abstract

The aim of this study was to determine salivary pH, buffering capacity and calcium levels in caries-free and caries-active children.

We examined 80 children of both genders, 15 years of age. Subjects were divided into four groups as follows: caries-free females, caries-active females, caries-free males, caries-active males; each group consisted of 20 subjects. The unstimulated saliva sample was collected by the spitting method and then pH, buffering capacity and calcium in saliva was measured.

The results showed that mean level of buffering capacity of saliva was decreased significantly in the caries-active group as compared to caries-free group. The obtained data showed that the mean levels of pH and calcium were decreased in the caries-active group as compared to the caries-free group, but the difference was not statistically significant.

The saliva with its constituents plays an important role in maintaining oral and especially dental health. Salivary pH values were found to be higher in the caries-free group. In our study, there was no significant correlation of pH values and caries activity with gender. Buffer capacity values were significantly lower in the caries-active group than in the caries-free group. There were significant differences when the groups were compared in the caries-active group where buffer capacity values were higher in boys than in girls.

Calcium content of saliva was higher in the caries-free group.

The results obtained in this study related to the values of the pH, buffering capacity and calcium in saliva, may serve as parameters for determining the caries risk patients, and accordingly to plan and carry appropriate caries preventive measures.

Keywords: saliva, dental caries, pH, buffer capacity, calcium

Introduction

Saliva has an important role in protecting the oral health. The whole saliva represents a mixture of the secretions of the major (submandibular, sublingual, parotid) and minor (accessory) salivary glands, together with the gingi-

val fluid. There are many biological factors in saliva that protect enamel, dentin and cementum from caries development and facilitate the remineralization. The ability of saliva to affect caries development is dependent upon the quantity and composition of the secretions (Preethi et al., 2010).

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Saliva and dental caries

The buffer capacity of saliva has a direct and important impact on the time-interval required for the establishment of normal acid of the saliva. The role of buffer maintenance of acido-basis balance, or oral homeostasis, is one of the most important natural protective functions of saliva (Mandel, 1990).

Saliva possesses antimicrobial components and a buffering agent that act to protect and maintain oral tissues. Saliva plays a fundamental role in maintaining the physical-chemical integrity of tooth enamel by modulating remineralization and demineralization. The main factors controlling the stability of enamel hydroxyapatite are the active concentrations free of calcium, phosphate, and fluoride in solution and the salivary pH (Featherstone, 2008; Fiyaz et al., 2013; Shaw et al., 1983). The high concentrations of calcium and phosphate in saliva guarantee ionic exchanges directed towards the tooth surfaces that begin with tooth eruption resulting in post-eruptive maturation. Remineralization of a carious tooth before cavitation occurs is possible, mainly due to the availability of calcium and phosphate ions in saliva (Jazaeri et al., 2015; Li et al., 2014). Depending on the pH, salivary calcium can be ionized or linked. Ionized calcium is important for establishing equilibrium between the calcium phosphates of enamel and its adjacent liquid. Non-ionized calcium can be linked to inorganic ions (inorganic phosphate, bicarbonate, fluoride), to small organic ions (citrate), and to macromolecules (statherin, histidine-rich peptides, and proline-rich proteins). In addition to moderating microbial factors and encouraging preventive dietary behaviors, a core goal in caries prevention is promoting the natural protective mechanisms of saliva. In healthy teeth, the loss of minerals is balanced by the reparative mechanisms of saliva (Afshar et al., 2001; Dawes and Watanabe, 1987; de Almeida, 2008).

The aim of this study was to determine salivary pH, buffering capacity and calcium levels in caries-free and caries-active children.

Material and method

We included 80 children of both genders, 15 years of age, randomly selected for participation in this study. Exclusion criteria were: existence of systemic disease, use of medications, smoking, periodontal diseases and poor oral hygiene. Subjects were divided into four groups as follows: caries-free females (CF), caries-active females (CA), caries-free males, caries-active males, each group consisted of 20 subjects.

Clinical examination

Caries detection was based only on clinical caries observed with dental mirror and explorer. Caries-active group was selected from the subjects that had at least five clinical

cal caries surface. Caries-free group consisted of students who had no caries and filling or any signs and symptoms of the sensitivity of teeth (Decayed/Missing/Filled Teeth (DMFT) = 0).

The examined parameters were followed in the same examinees in a sample of saliva taken in the morning before consuming any food or implementation of oral hygiene.

pH and buffer capacity measurement

The saliva samples were frozen at -80 °C, 30 minutes after sampling. Before analyzing, the samples were equilibrated to room temperature. The pH of each salivary sample was determined using a digital pH meter (Piccolo plus, ATC pH meter, Hanna Instruments, Italy).

Buffer capacity was calculated according to changes in pH. The buffer capacity was determined by the method of Ericsson (1959) modified for smaller volumes. This method involved the addition of 0.5 mL of saliva to 1.5 mL of 5 mmol/L HCl. The mixture was vigorously shaken and allowed to stand for 10 minutes before the final pH was measured.

Calcium measurement

Saliva total calcium concentration was measured using spectrophotometric method by the Arsenazo-III method. The absorbency absorption of the reagent was measured bichromatically at 650 and 700 nm immediately before and 21 s after saliva sample addition. The difference in absorption before and after sample addition was directly proportional to the amount of calcium in the sample (Tulunoglu et al., 2006).

Statistical analysis

Statistical analysis of salivary parameters in caries-active and caries-free children as well as among girls and boys was performed using Student t-test ($p < 0.05$).

Results and discussion

Dental caries is a complex and dynamic process where a multitude of factors influence and initiate the progression of the disease. One of the most important factors which influence the development of dental caries is saliva. The physicochemical properties of saliva like pH, buffering capacity, salivary flow rate, the concentration of various components like proteins, calcium play a major role in the development of caries.

The saliva circulating in the mouth at any given time is termed as the whole saliva and comprises a mixture of secretions from the major, minor salivary glands and traces from the gingival crevicular fluid. Since this fluid constantly bathes the teeth and oral mucosa, it acts as a cleans-

ing solution, a lubricant, buffer, and ion reservoir of calcium and phosphate, which is essential for remineralization of the initial carious lesion (Animireddy et al., 2014).

Dawes (1987) described the terms “unstimulated” saliva, when no exogenous or pharmacological stimulation is used and “stimulated” saliva, when secretion is promoted by mechanical or gustatory stimuli or by pharmacological agents. In our study unstimulated saliva sample was collected as Stookey et al. (2008) reported that stimulating the flow of saliva can alter its composition. Kaufman and Lamster (2002) reported that salivary stimulation affected the quantity of saliva, concentration of some constituents and pH of the fluid.

In our study we examined the whole saliva. The unstimulated saliva sample was collected by the spitting method that appeared to be the most reproducible one. In the oral cavity, as an initial part of the digestive system, the substances are imported through food and affect the concentration level of hydrogen ions (pH) in saliva. Due to a salivary buffer system, this level is maintained within certain narrow limits (from pH 6.8 to 7.2) (Leone and Oppenheim, 2001).

Distribution of participants groups is shown in Table 1.

Table 2 shows the mean level of buffering capacity, pH, and calcium in both groups. Buffering capacity of saliva was decreased in the caries-active group as compared to caries-free. The obtained data showed that the mean levels of pH were decreased in the caries-active group as compared to the caries-free group. The difference was statistically significant. The study showed that pH and buffering capacity had a correlation with caries activity. Therefore, it can be said that other factors like micro flora, diet and retention of food might have a dominant role in the buffering capacity to initiate caries, which is a multifactorial disease. Similar results were obtained in a study conducted by Tulunoglu et al. (2006), where no correlation between pH values and caries activity regardless of the age and gender. They were dependent upon individual and environmental variations.

As presented by Ahmadi-Motamayel et al. (2013), salivary buffer capacity has an inverse relationship with human caries incidence. In our study, buffer capacity values were significantly lower in the caries-active group than in the caries-free group. There were significant differences only in the caries-active group where buffer capacity values were higher in boys than in girls.

Other studies support our finding as they have report-

Table 1. Distribution of participants in groups

Group	Boys	Girls	Total
Caries-active	20	20	40
Caries-free	20	20	40
Total	40	40	80

Table 2. Salivary parameters in caries-active and caries-free children

Group	Buffering capacity	pH	Calcium (mmol/L)
Caries-active	42.423	7.385	1.528
Caries-free	46.553	7.506	1.441
t value	-7.258	-6.678	0.395
p value	1.342E-10*	1.68E-09*	0.346

* significant

Table 3. Salivary parameters according to gender

Gender	Buffering capacity	pH	Calcium (mmol/L)
Girls	44.227	7.450	1.409
Boys	45.162	7.453	1.552
t value	-1.283	-0.108	-0.665
p value	0.101	0.457	0.253

* significant

ed that larger quantities and faster rates of acid production in caries active individuals than that in caries-free individuals. There is strong evidence showing that salivary buffering capacity protects the tooth from dental caries and low buffering capacity is usually associated with caries development because of its impaired neutralization of plaque acids and reduced remineralization of early enamel lesions. This association between low caries levels and high salivary buffering capacity has been demonstrated and individuals with a high salivary buffer capacity are often caries-resistant (El-kwatehy et al., 2016).

The mean pH level and buffering capacity of saliva were increased in boys comparison to girls and these differences were not statistically significant. The mean level of buffering capacity of saliva was decreased in girls in the caries-active group and the difference was not statistically significant. In this group, the mean level of pH and calcium was increased in boys as compared to girls, but this difference was not significant. In a caries-free group, the mean level of pH was increased in girls, but the buffering capacity of saliva decreased in this group as compared to boys. These differences were not statistically significant (Table 3).

In our study calcium content of saliva was higher in the caries-free group. The decrease in caries in children with high calcium concentration in saliva is attributed to the process of remineralization of the incipient caries lesions. The saliva which is supersaturated with calcium and phosphate acts as a reservoir for these essential ions. In such a conducive environment the process of remineralization overrides demineralization. The study of Tulunoglu et al. (2006) also reported increased calcium level in the caries-free group. Conflicting results have been obtained from investigations of calcium and phosphate contents of saliva and their relationship to dental caries. Ashley et al. (1991) found that the salivary calcium increased with decreasing caries activity.

This study demonstrates that the mean salivary calcium level in children with severe caries was higher in the caries-free group, but this difference was not significant. Our results are in agreement with the literature data (Cornejo et al., 2008; Gandhi and Damle, 2002; Jolly and Shetty, 2014; Masamura et al., 1995; Marray et al., 1983).

Conclusion

The saliva with its constituents plays an important role in maintaining oral and especially dental health.

Salivary pH values were found to be higher in the caries-free group. In our study, there was no significant correlation of pH values and caries activity with gender.

Buffer capacity values were significantly lower in the caries-active group than in the caries-free group. There were significant differences regarding buffer capacity values, which were higher in boys than in girls in the caries-active group.

The obtained results for the calcium content have shown that calcium content of saliva was higher in the caries-free group and did not differ significantly from previously published studies.

References

- Afshar, H., Seraj, B., ShapZadeh, N., 2001. The relationship between rampant caries and salivary situation of 4-5 year old children living in Tehran. *J. Islamic Dent. Assoc.* 13, 18-35.
- Ahmadi-Motamayel, F., Goodarzi, M.T., Hendi, S.S., Abdolsamadi, H., Rafielan, N., 2013. Evaluation of salivary flow rate, pH, buffering capacity, calcium and total protein levels in caries free and caries active adolescence. *Academic Journal* 5(4), 35-39.
- Animireddy, D., Reddy Bekkem, V.T., Vallala, P., Kotha, S.B., Ankireddy, S., Mohammad, N., 2014. Evaluation of pH, buffering capacity, viscosity and flow rate levels of saliva in caries-free, minimal caries and nursing caries children: An *in vivo* study. *Contemp. Clin. Dent.* 5(3), 324-328.
- Ashley, F.P., Coward, P.Y., Jalil, R.A., Wilson, R.F., 1991. Relationship between calcium and inorganic phosphorus concentrations of both resting and stimulated saliva and dental plaque in children and young adults. *Arch. Oral. Biol.* 36, 431-434.
- Cornejo, L.S., Brunotto, M., Hilas, E., 2008. Salivary factors associated to the prevalence and increase of dental caries in rural schoolchildren. *Rev. Saude. Publica.* 42, 19-25.
- Dawes, C., 1987. Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *J. Dent. Res.* 66, 648-653.
- Dawes, C., Watanabe, S., 1987. The effect of taste adaptation on salivary flow rate and salivary sugar clearance. *J. Dent. Res.* 66(3), 740-744.
- de Almeida, P.D., Grégio, A.M., Machado, M.A., de Lima, A.A., Azevedo, L.R., 2008. Saliva composition and functions: a comprehensive review. *J. Contemp. Dent. Pract.* 9(3), 72-80.
- El-kwatehy, W.M., Youssef, A.R., 2016. Salivary Biomarkers in Caries Affected and Caries Free Children. *Int. J. Dentistry Oral Sci.* 3(10), 348-352.
- Ericsson, Y., 1959. Clinical investigations of the salivary buffering action. *Acta Odontologica Scandinavica* 17, 131-165.
- Featherstone, J.D., 2008. Dental caries: a dynamic disease process. *Aust. Dent. J.* 53(3), 286-291.
- Fiyaz, M., Ramesh A., Ramalingam, K., Thomas, B., Shetty, S., Prakash, P., 2013. Association of salivary calcium, phosphate, pH and flow rate on oral health: A study on 90 subjects. *J. Indian Soc. Periodont.* 17(4), 454-460.
- Gandhy, M., Damle, G., 2002. Relation of salivary inorganic phosphorous and alkaline phosphatase to the dental caries status in children. *J. Indian Soc. Pedod. Prev. Dent.* 21, 135-138.
- Jazaeri, M., Malekzadeh, H., Abdolsamadi, H., Rezei-Soufi, L., Samami, M., 2015. Relationship between salivary alkaline phosphatase enzyme activity and the concentrations of salivary calcium and phosphate ions. *Cell. J. Spring.* 17(1), 159-162.
- Jolly, L., Shetty, A., 2014. Calcium and inorganic phosphorous levels in stimulated and unstimulated saliva in early childhood caries - A comparative study. *Journal of Academy of Dental Education* 1(2), 5-11.

- Kaufman, E., Lamster, I.B., 2002. The diagnostic applications of saliva - A review. Crit. Rev. Oral. Biol. Med. 13(2), 197-212.
- Leone, C.W., Oppenheim, F.G., 2001. Physical and chemical aspects of saliva as indicators of risk for dental caries in human. J. Dent. Educ. 65(10), 1054-1064.
- Li, X., Wang, J., Joiner, A., Chang, J., 2014. The remineralisation of enamel: a review of the literature. J. Dent. 42 (Suppl 1), 12-20.
- Mandel, I.D., 1990. The diagnostic uses of saliva. J. Oral. Pathol. Med. 19(3), 119-125.
- Masumura, K., Inaba, R., Iwata, H., 1995. Salivary calcium and total protein in relation to dental caries. Nippon Zasshi 50, 882-892.
- Preethi, B.P., Reshma, D., Anand, P., 2010. 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 vitro study. Indian J. Clin. Biochem. 25(4), 425-428.
- Shaw, L., Marray, J.J., Burchell, C.K., Best, J.S., 1983. Calcium and phosphorous content of plaque and saliva in relation to dental caries. Caries Res. 17, 543-548.
- Stookey, G.K., 2008. The effect of saliva on dental caries. J. Am. Dent. Assoc. 139, 272-285.
- Tulunoglu, O., Demirtas, S., Tulunoglu, I., 2006. Total antioxidant levels of saliva in children related to caries, age, and gender. Int. J. Paediatr. Dent. 16, 186-191.

Резиме

Корелација помеѓу рН вредноста, пуферскиот капацитет, калциумот и забниот кариес кај деца на училишна возраст

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Клучни зборови: плунка, забен кариес, рН, пуферски капацитет, калциум

Целта на оваа студија беше да се утврди рН на плунката, пуферскиот капацитет и нивото на калциум кај деца без и со присутен забен кариес.

Испитувањето опфати 80 деца од двата пола на 15-годишна возраст. Испитаниците беа поделени во четири групи и тоа: женски деца без кариес, женски деца со кариес, машки деца без кариес и машки деца со кариес. Секоја група се состоеше од 20 испитаници.

Во нашата студија беше собиран примерок од нестимулирана плунка со помош на методот на плукање, а потоа се одредуваше рН, пуферскиот капацитет и нивото на калциумот во плунката.

Резултатите од студијата покажаа дека средното ниво на пуферскиот капацитет на плунката беше значајно намалено кај кариес-активната група на испитаници, во споредба со групата без кариес. Добиените податоци покажаа дека средните вредности на рН и калциум беа намалени во групата со присутен кариес, во споредба со групата без кариес, но разликата не беше статистички значајна.

Плунката со своите конституенти има важна улога во одржувањето на оралното и посебно на денталното здравје. Резултатите покажаа дека рН вредноста на плунката беше повисока во групата без кариес. Не беше забележана значајна корелација помеѓу вредностите на рН и полот кај испитаниците со кариес. Вредностите на пуферскиот капацитетот беа значајно пониски во групата со кариес отколку во групата без кариес. Вредностите на пуферскиот капацитет беа повисоки кај момчињата отколку кај девојчињата во кариес-активната група и беа статистички значајни.

Нивото на калциумот во плунката се покажа дека е поголемо во групата без кариес.

Резултатите од ова истражување, односно вредностите на рН, пуферскиот капацитет и калциумот во плунката кај пациентите од детска возраст може да послужат како параметри за утврдување на ризикот од кариес, и планирање и спроведување на соодветни кариес-превентивни мерки.

