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Buffering Capacity of Saliva in Patients with Active Dental Caries

[1]Mohammad Reza Malekipour, [2]Manoochehr Messripour and [3]Farzaneh Shirani
[1]Department of Operative Dentistry, Dental School,
[2]Department of Biochemistry, School of Medicine,
Islamic Azad University, Khorasgan Branch, Isfahan, Iran
[3]Department of Operative Dentistry, Dental School,
Isfahan University of Medical Sciences, Isfahan, Iran

Abstract: Saliva buffer act as an important factor to control the pH of the mouth environment. Because organic acids produced by the mouth microorganisms is associated with development of dental caries, the aim of this study was to compare the buffering capacity of saliva in active dental caries patients with caries free subjects. Saliva samples were collected without stimulation from 30 patients with more than 10 decayed teeth and 30 subjects with no dental caries. The pH of saliva was measured and the buffering capacity of each saliva sample was determined by either HCl (1:10 N) or NaOH (1:10 N) titration. The determination of pH values of patients with active caries and caries free subjects were 6.67±0.03 and 6.76±0.03, respectively which are not significantly different. However, the pattern of titration of the saliva in the patients was different from that of titration of the healthy subject. The differences were significant particularly after addition of 1-3 mL of either HCl or NaOH solution. The result suggested that the determination of the buffering capacity of the saliva may be used as an index for the development of dental caries.

Key words: Buffering capacity, saliva, active caries patients

INTRODUCTION

Saliva is one of the biological fluids containing several compounds by which the host can control mouth hemiparasites (Lagerlof, 1998). The saliva materials collaborate in order to prevent dental caries by mechanical washing, antimicrobial function, remineralization and buffering capacity of saliva. The buffer system is total of all conjugate acid-base pairs that help to regulate pH of any chemical environment and in particular that of extracellular and intracellular fluids of the body (Burton *et al*., 2001). The buffering system of saliva has an important role in preventing of major pH changes in the mouth environment. Several lines of evidence indicated produced acids may be neutralized by the buffering nature of saliva (Alamoudi *et al*., 2004; Cogulu *et al*., 2006). As in plasma, bicarbonate-carbonic acid and phosphate have the most important buffer value for the regulation of saliva pH (Stephen, 1997). Most of the hydrogen ions that produced in mouth will react with saliva bicarbonate to form H_2CO_3. Bicarbonate system can quickly neutralize strong acids in pH≤6 and is more effective than phosphate (Stephen, 1997). A number of observations have indicated that buffering capacity of saliva increases, as the amount of saliva secretion increases. These studies indicated that the lack of buffering capacity in saliva is an important factor in tooth caries (Sullivan, 1990). It is very well known that with consumption of sugar, the pH of dental plaque decreases to 5 quickly, since

Corresponding Author: M. Messripour, Department of Biochemistry, School of Medicine, Islamic Azad University, Khorasgan Branch, P.O. Box 81595-158, Isfahan, Iran
Tel: 0098 311 5354059 Fax: 0098 311 5211265

lactic acid and other organic acids are produced by the mouth microorganisms and then it will return to the neutral level by bicarbonate and phosphate buffer system of saliva (Stephen, 1997). Because buffering capacity of saliva is the most important mechanism in neutralizing acids in the mouth and identifying this capacity in different people (Larsen *et al*., 1999; Beel *et al*., 1991), the present study was undertaken to compare buffering capacity of saliva in dental caries patients with caries free subjects.

MATERIALS AND METHODS

The buffering capacity of saliva was measured in saliva from patients with active dental caries and volunteer healthy subjects without any tooth decay or filled teeth (control). The study was carried out from September 2004, to March 2005, in the Dental School of Islamic Azad University of Khorasgan, Isfahan, Iran. The patients and control subjects were examined and after making sure that they are not smokers and do not suffering from systemic diseases or health problems they were allowed to enter the study. The subjects did not have any medications during the last two weeks, 15 female and 15 male contributed to each group (age were matched 43-55 years old). Saliva samples were collected from the subjects before breakfast and before there usual morning teeth brushing and mouth rinsing. About 5 mL of saliva samples were collected in a tube containing 1 mL of mineral oil to prevent loss of carbon dioxide. The sampling was carried on without stimulation during approximately 10 min between 9-11 am while they were sitting in the armed chairs of the dental clinic. Just after sample collection, the pH and the buffering capacity of the saliva sample was measured by a pH meter (Horiba). The buffering capacity of each saliva sample was determined by the titration method. Two milliliter of saliva sample was diluted with 2 mL double glass distilled water and the pH of the solution was measured by addition of each increment drops of HCl (1:10 N) or NaOH (1:10 N).

The mean pH values were plotted versus the amounts (mL) of HCl or NaOH solution to represent titration curve. The calculated standard deviations (SD) of the plots were between 0.01-0.06. Statistical comparisons were made and the deviations from the null hypothesis were calculated using unpaired Student's t-test.

RESULTS

The determination of pH values of patients with active caries (n = 30) and caries free subjects (n = 30) were 6.67±0.03 and 6.76±0.03, respectively which are not significantly different. Because the pH values and titration patterns of the saliva samples of female and male the control subjects and also female and male patients were not significantly different the data were not analyses for each sexes separately. Figure 1 shows the changes in pH that resulted from the addition of varying amount of strong acid (1:10N HCl) or base (1:10N NaOH) to double glass distilled water and saliva samples collected from the active caries patients and caries free subjects. The pH falls as increasing amounts of strong acid are added to the saliva samples, but not nearly as much as it would fall if the strong acid were added to water. Similarly, the pH rises as increasing amounts of strong base are added to the samples, but not as much as it would rise if the strong base were added to water. It is clear that the slope of the water curves is almost vertical by addition of 1 mL of either HCl or NaOH solutions to water, indicating radical changes of pH from about 2 to 12, but smaller changes are seen in case of both group of samples. However, the pattern of HCl and NaOH titration of the patient samples is different to that of the healthy subjects. The differences were significant particularly after addition of 1, 2 and 3 mL of either HCl or NaOH solution. The isoelectric points (IP) of buffering systems of the saliva samples of the patients and healthy subjects as calculated by *p*ka and *p*kb of the titration curves are given in Table 1. The IP of the patient samples is lower than IP of the samples from healthy subjects. The mean IPs was similar to the mean of pH of the saliva samples.

Table 1: Comparison of buffering capacity of saliva samples from active caries patients with caries free subjects

Experiment	*p*ka	*p*kb	Isoelectric point
Caries free subjects	1.37±0.02	12.12±0.04	6.73±0.03
Active caries patients	1.20±0.04*	12.00±0.05	6.60±0.03

The values of *p*ka and *p*kb were estimated from the titration curves of each sample and isoelectric point (IP) of the sample was calculated. Results are mean±SD of 30 separate experiments. * The values is different significantly as compared with corresponding value of caries free subjects (unpaired Student's t-test)

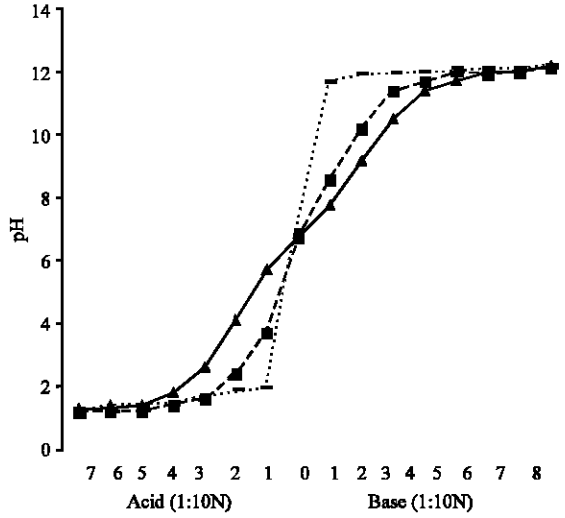

Fig. 1: Saliva titration curves of active caries patients and healthy subjects. Double glass distilled water (-■-) and saliva samples from the active caries patients (···■···) and caries free subjects (—▲—) were tittered by addition of HCl (1:10N) and NaOH (1:10N) solutions. Each point represents a mean of 30 separate experiments with SD of 0.01-0.06. Differences of the values of the patient curve at plots of 1, 2 and 3 mL of acid and 1, 2, 3 mL of base are statistically significant as compared with corresponding values of caries free subjects (unpaired Student's t-test)

DISCUSSION

The major finding of the present study is that the pattern of acid/base titration of saliva samples from patients with active caries is different from the pattern of caries free subjects. As can be seen in the Fig. 1 at pH values near *p*ka and *p*kb the differences are particularly significant. Values of *p*ka and *p*kb are known as the cationic and anionic zones that having buffering power (Lehninger, 1982). Therefore, it is evident from the titration results of the two groups that buffering capacities are significantly different. It is likely that the pattern of the samples from healthy subjects tends to keep the pH near the natural status, but this tendency seems to be less effective in the samples from the patients. Although saliva pH values of the two groups are approximately similar, results indicated that the buffering capacity of the patients is weaker than that of normal healthy subjects. This is consistent with the suggestion that buffering effect can not be judged only by determination of saliva pH (Larsen *et al*., 1999). Moreover, the results of the present study are in accord with the previous studies that reported high level of saliva secretion has a cariostatic effect as it accelerates buffering effect and therefore less caries were observed as compared with those who secrete low level of saliva (Larsen *et al*., 1999; Beel *et al*., 1991; Alausva and Kvjala, 1990).

It is concluded that salivary buffering capacity may be taken as a measure to predicate the future caries condition.

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