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Full Length Research Paper

The Role of Salivary Composition in Caries Development: A Study of Adolescents

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The present study was undertaken to compare salivary flow rate, pH, calcium, buffering capacity and total protein between caries free and caries active adolescence. Un-stimulated whole saliva was collected from one hundred healthy adolescences with age range of 15 to 17 years who were divided to four groups: caries free female, caries free male, caries active female, caries free male. Then, flow rate of saliva was determined and samples were analyzed for pH, calcium, buffering capacity and total protein. The date was analyzed using student's t-test. The results showed that when all of these parameters were compared among caries free and caries active groups, buffering capacity of saliva decreased significantly in caries active group. Comparison of all of these parameters between girls and boys revealed the level of total protein and buffering capacity were significantly higher and pH was significantly lower in boys as compared to girls. Level of flow rate and composition of saliva were different between caries free and caries active adolescence. Moreover, buffering capacity decreased in caries active group. Notably difference in quantity and quality of saliva can contribute as an important causal factor in explaining sex difference in caries rate.

Key words: Dental caries, saliva, pH, buffers, calcium, salivary proteins.

INTRODUCTION

Dental caries is one of the most common, communicable and intractable infectious disease in human (Bowen et al., 2001; Amit and Robin Wendell, 2012). It remains the persistent and important oral health problem internationally, and particularly among developing countries (García-Godoy and Hicks, 2008; Tickle et al., 2011). It is also profoundly affected by many factors like oral hygiene and saliva (Preethi et al., 2010). Saliva is a biologic fluid in the oral cavity, composed of a mixture of secretary products from the major and minor salivary glands (Lima et al., 2010). Saliva plays key roles in lubrication, mastication, taste perception, prevention of oral infection and dental caries (Lima et al., 2010; Pink et al., 2009; Chiappin et al., 2007). By constantly bathing the teeth and oral mucosa with saliva functions as a cleansing solution, a lubricant, a buffer and ion reservoir of calcium and phosphate which are essential for re-mineralization of initial carious lesions (Preethi et al., 2010). Many studies discussed about salivary flow rate, pH, buffer capacity and total protein in relation to dental caries, but there are differences in obtained results between the studies in different regions. Hence, evaluation of these factors in saliva that may increase risk of dental caries is important. Therefore, the aim of this study was to evaluate the

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Table 1. Salivary parameters in caries active and caries free adolescence.

Group	Flow rate	pH	Calcium (mmol/L)	Total protein (mg/ml)	Buffering capacity
Caries active (n= 50)	0.730±0.878	7.624±0.354	1.115±0.807	8.878±6.104	41.224±16.99
Caries free (n=50)	0.692±0.255	7.737±0.356	0.891±0.504	7.873±6.777	54.540±54.125
P value	0.774	0.117	0.099	0.438	0.002*

*Significant.

Table 2. Salivary parameters according to sex.

Sex	Flow rate	pH	Calcium	Total protein	Buffering capacity
Female (n=50)	0.783±0.872	7.783±0.375	0.998±0.572	5.992±5.247	37.326±12.632
Male (n=50)	0.639±0.258	7.577±0.306	1.008±0.778	10.75±6.676	58.360±23.977
P value	0.264	0.004*	0.938	0.000*	0.000*

Data are presented as mean±SD; *Significant.

salivary flow rate, buffer capacity, pH, total protein and calcium in two caries free and caries active groups.

MATERIALS AND METHODS

Subjects

One hundred healthy high school students (50 females and 50 males) with age range of 15 to 17 years were randomly selected and participated in this study. Written informed consent was obtained from all subjects. Exclusion criteria were: having systemic disease, using medication, smoking, having periodontal diseases and poor oral hygiene. Subjects were divided to four groups as follows: caries free females (CF), caries active females (CA), caries free males, caries active males, each group consisted of 25 subjects.

Saliva sampling

Un-stimulated whole saliva specimens were collected in the morning, and it was asked from all selected students that brush their teeth and do not use any oral stimulation such as eating and drinking for 90 min prior to collection (Navazesh, 1993). Students were in sitting and anterior head protrusion position. Whole saliva samples were obtained by expectorating into polypropylene tubes within 5 min. The saliva samples were first weighed and reweighed again, then immediately were put on to ice and were stored at 4°C and transferred to the laboratory for up to 20 min and then were kept at -80°C until the analysis.

Clinical examination

All clinical examination was carried out by single examiner. Caries detection was based only on clinical caries observed with dental mirror and explorer and radiographic examination was not performed. CA group were selected within the subjects that had at least five clinical caries surface. CF group contained students that did not have any caries and filling and sign and symptom of sensitivity of teeth (Decayed/Missing/Filled Teeth (DMFT)=0). All selected groups had same age range.

pH and buffer capacity measurement

pH of saliva samples was determined using a pH meter (Hana Italy) To measure buffering capacity of saliva, after measuring pH, 1 ml of 0.1 N HCl was added to 1 ml of saliva and pH was recorded (Ericsson method) (Tulunoglu et al., 2006). Buffer capacity was calculated according to changes in pH.

Total protein assay

The protein content of saliva samples was measured using Bradford method (Nicholas, 2009).

Calcium assay

Saliva total calcium concentration was measured using spectrophotometric method (Pars Azmoon Kit, Iran).

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS; ver. 13). Statistical comparisons were performed using Student's t-test. The values are expressed as mean ± standard deviation (SD). A P value of <0.05 was considered statistically significant.

RESULTS

The results of the studied parameters are presented in Table 1. The obtained data showed that flow rate, calcium and total protein were slightly increased in caries active group as compared to caries free group, but pH of saliva decreased. Buffering capacity of saliva was decreased significantly in caries active group as compared to caries free (P=0.002). The difference in these parameters between two genders is presented in Table 2. The mean value of flow rate was slightly increased in girls as compared to boys, but the difference was not significant (P=0.264). The mean value of saliva

Table 3. Sex difference in salivary parameters in caries active and caries free groups.

Group	Sex	Flow rate	pH	Calcium (mmol/L)	Total protein (mg/ml)	Buffering capacity
Caries active	Female (n=25)	0.846±1.212	7.668±0.365	0.976±549	7.740±6.281	34.395±11.004
	Male (n= 25)	0.783±0.872	7.783±0.375	0.998±0.572	5.992±5.247	37.326±12.632
	P value	0.356	0.383	0.226	0.19	0.005*
Caries free	Female (n=25)	o.721±271	7.898±0.358	1.020±604	4.244±3.287	40.140±13.646
	Male (n=25)	0.664±0.241	7.575±0.276	0.762±0.354	11.502±7.451	68.940±23.786
	P value	0.438	0.001*	0.071	0.000*	0.000*

*Significant.

Table 4. Salivary parameters in caries active and caries free adolescent in girls and boys of group.

Sex	Group	Flow rate	pH	Calcium (mmol/L)	Total protein (mg/ml)	Buffering capacity
Girl	Caries active (n=25)	0.846±1.212	7.66±0.37	0.976±549	7.754±6.248	34.395±11.004
	Caries free (n= 25)	0.721±0.271	7.898±0.378	1.021±0.604	7.74±6.248	40.14±13.646
	P value	0.618	0.030*	0.79	0.017*	0.112
Boy	Caries active (n=25)	o.614±277	7.58±0.339	1.255±995	5.861±1.172	47.78±19.248
	Caries active (n=25)	0.664±0.241	7.575±0.276	0.762 ±0.354	7.551±1.490	68.94±23.786
	P value	0.497	0.953	0.024*	0.433	0.001*

*Significant.

pH was increased significantly in girls as compared to boys (P=0.004), but the mean level of buffering capacity was decreased significantly in girls when compared with those of boys (P=0.001). There was a slight but non-significant increase in calcium in boys. The mean value of total protein and buffering capacity were increased in boys and the difference as compared to those of the girls was statistically significant (p=0.000).

In caries active group, the mean level of flow rate and pH were increased in girls as compared to boys, but these difference was not significant (p=0.356, p=0.190, respectively). Furthermore, the mean level of calcium and pH were lower in girls in this group as compared to boys, but the difference was not statistically significant (P=0.226, P=0.383, respectively). The mean level of buffering capacity of saliva was decreased in female subjects in this group and the difference was statistically significant (P=0.005). In caries free group, the mean level of flow rate and calcium was higher in girls as compared to boys, but these differences were not significant (P=0.438, P=0.071, respectively). The mean level of pH was increased in girls, but total protein and buffering capacity of saliva decreased in this group as compared to boys and these differences were statistically significant (P=0.000) (Table 3). There was a higher but non-significant level of flow rate and total protein in caries active group as compared to caries free group (P=0.618 and 0.112, respectively). Comparing the mean of pH between caries active and caries free groups showed significantly lower level in caries active group (P=0.03). However, calcium and buffering capacity were decreased in caries active group as compared to caries free group; the difference was not statistically significant (P=0.790, P=0.112, respectively). The mean level of flow rate and total protein were decreased; however, non-significant in caries active group when compared with caries free group. The mean level of buffering capacity was lower in caries active group when compared with caries free group (P=0.001). In caries active group, the mean levels of pH and calcium were decreased as compared to caries free group, but the difference was not statistically significant (Table 4).

DISCUSSION

The obtained data in this study indicated no significant difference in salivary flow rate between two groups of caries active and carries free subjects. Lumikari and Loimaranta (2000) reported no correlation between salivary secretion and decay, which was parallel with the findings of this research. Also, Leone and Oppenheim (2001) showed that diseases such as Sjögren's syndrome as well as taking certain drugs can lead to hyposalivation, and lower salivary flow rate to the pathological levels dramatically elevates risk of caries.

Since all subjects included in this study were completely free from systemic or local disease and they took no particular medications which affect salivary secretion, the statistically non-significant difference between salivary flow rate in the two groups: caries active and caries free, in our study therefore can be justifiable. In relation to pH of saliva, this study showed that average pH of both groups was over 7, thus indicating no significant difference between individuals with active and free caries activity. Since dissolution of enamel minerals start to dissolve when the pH falls below critical pH (<5.5) (Lumikari and Loimaranta, 2000), thus the saliva pH in these two studied groups had not reached the critical limit to cause demineralization of inorganic substance of the tooth (Kleinberg et al., 1973).

Tulunoglu et al. (2006) reported no correlation between pH and caries activity in spite of age and gender. Also, as shown by Weiler et al. (1967), the acidic tendencies in resting plaques in children with rampant and moderate caries, even after eating nothing for hours indicates inherent tendencies toward caries in these children. Leone and Oppenheim (2001) research pointed out that pH reduction independent of buffering capacity of saliva is not a strong relationship to caries experience. In this research, salivary buffering capacity in individuals with active caries was lower than those of caries free group and this difference was in fact statistically significant. Similar to this finding, Pretthi et al. (2010) showed that the buffering capacity of the saliva was lower in a group including children with active caries as compared to caries free children, but the difference was not statistically significant.

The results of this study were different from the observations reported by Ericsson who showed that the buffering capacity of saliva was negatively correlated with caries (Pretthi et al., 2010). Leone and Oppenheim (2001) reported that eleven studies showed a correlation between low salivary buffering capacity and caries. Dreizen and Mann (1946) pointed out a strong correlation between buffering capacity and the chemical and bacteriological compound of the saliva with caries, and caries activity increases with lower salivary buffering capacity. Salivary buffering factors help keep salivary pH at a normal level as fast as possible. Although, these factors can elevate salivary pH; there is no conclusive evidence of their protective role against dental caries (Tenovuo, 1997).

In the present research, in caries active group, the mean calcium concentration was higher than those of caries free, but the difference was not statistically significant. Furthermore, Preethi et al. (2010) study showed (in contrast to our study) a lower mean of calcium concentration in individuals with caries active children as compared to caries free. Nevertheless, Leone and Oppenheim (2001) reported that the findings of seven studies indicated a moderate correlation between the low level of calcium and phosphate concentration in the saliva with caries susceptibility, whereas there wasno such correlation in these two studies. In this study, the total proteins level increased in individuals with caries activity as compared to caries free group; however, there was no significant difference between the two groups. Preethi et al. (2010) also found that the total proteins level in the saliva was increased in the group with active caries as compared to the caries free, which is in line with the findings of this research. Leone and Oppenheim (2001) reported that fourteen studies examined the correlation between caries and salivary proteins and found no correlation between them. Furthermore, Akyuz et al. (1995) reported that in diabetic children, protein level of saliva elevated, which leads to elevated saliva viscosity and reduced saliva quantities. Numerous studies pointed out a significant higher number of caries teeth in females as compared to males (Akyuz et al., 1995; Lukacs and Largaespada, 2006). In this study, the saliva flow rate was a little lower in girls than boys, although the difference was not statistically significant. Lukacs and Largaespada (2006) reported that women had a significantly lower mean saliva flow rate than men; for stimulated and un-stimulated parotid saliva. Perhaps female sexual hormones, specifically estrogen, have a significant role in the suppression of saliva flow (Lukacs and Largaespada, 2006; Temple, 2011). This study showed that the buffering capability of the saliva and also its total protein level was higher in the boys' group than in the girls' and the difference between the two genders was statistically significant, whereas in relation to pH, it is decreased in boys as compared to girls and the difference was statistically significant. Three factors can explain female's prevalence of caries as compared to males: (1) earlier eruption of teeth in girls, which causes longer exposure to cariogenic environment in the mouth,

(2) women consume more snacks and candy, and (3) pregnancy and hormonal influence (Lukacs, 2011). Also, the role of genetic effects, as well as the kind of nutrition greatly influences differences in caries prevalence between the two genders (Leone and Oppenheim, 2001).

Conclusion

Saliva is one of the most important factors in prevention of dental caries. Therefore, physical and chemical changes in saliva composition and particularly changes in its buffering capability play an important role on development and progression of caries.

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