Comparison of Salivary Ion Activity Product for Hydroxyapatite (I_{PHA}), Alkaline Phosphatase and Buffering Capacity of Adults According to Age and Caries Severity

Vahedi M.[a], Davoodi P.[b], Goodarzi MT.[c], Rezaei-Soufi L.[d], Jazaeri M.[b], Mortazavi H.[e], Moghimbeigi E.[f]

[a] Dept. of Oral Medicine, Dental Research Center, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, IRAN
[b] Dept of Oral Medicine, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, IRAN
[c] Dept. of Biochemistry and Nutrition, Research Center for Molecular Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IRAN
[d] Dept. of Operative Dentistry and Dental Research Center, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, IRAN
[e] Dept. of Oral Medicine, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, IRAN
[f] Dept. of Biostatistics and Epidemiology, School of Public Health and Center for Health Research, Hamadan University of Medical Sciences, Hamadan, IRAN

ABSTRACT

Statement of Problem: Tooth caries is influenced by different biochemical characteristics of saliva. As hydroxyapatite is the main component of enamel, salivary ion activity product for hydroxyapatite (I_{PHA}) as well as alkaline phosphatase may be attributed to dental caries.

Purpose: The aim of the present study was to compare salivary buffering capacity, alkaline phosphatase and I_{PHA} of adults according to the dental caries and age.

Materials and Method: One hundred and twenty 19 to 44 years old male individuals were divided into four groups according to the dental caries rate and age: group 1: 19-35 years old low dental caries (DMFT <5); group 2: 19-35 years old high dental caries (DMFT 5<); group 3: 35-44 years old low dental caries (DMFT <11) and 35-44 years old high dental caries (DMFT 11<). Five millilitre of unstimulated saliva was collected, and then buffering capacity, the level of alkaline phosphatase activity and I_{PHA} was determined for each sample. Data was analyzed by soft ware SPSS using two-way ANOVA, Friedman and Mann-Whitney tests.

Results: Mean and standard deviation of buffering capacity of group 1 to 4 was 2.66±0.54, 2.64±0.56, 2.70±0.70 and 2.26±0.82, respectively. The difference was not significance (p= 0. 305). Mean and standard deviation of alkaline phosphatase activity of group 1 to 4 was 5.82±2.91, 5.30±1.52, 4.77±1.82 and 4.55±1.61, respectively. There was no significant difference (p= 0.692). Mean and standard deviation of I_{PHA} of group 1 to 4 was 29.39±0.61, 29.51±0.76, 29.14±0.56 and 29.75±0.75, respectively. The difference was significant (p= 0.049).

Conclusion: Based on the results of the present study, buffering capacity and the level of alkaline phosphatase couldn't affect dental caries, independently. However, the higher value of I_{PHA} may be attributed to the higher dental caries rate. Ageing decreases alkaline phosphatase activity.

Corresponding Author: Rezaei-Soufi L., Dept. of Operative Dentistry, School of Dentistry, Hamedan University of Medical Sciences, Shahid Fahmideh Blvd, Hamedan, Iran   Tel: +98-09123750364   Fax: +98-0811-8283647
Email: loghmansofi@umsha.ac.ir

Introduction

Tooth caries, a common chronic infectious disease of the oral cavity, is considered as a complex phenomenon and is involved with different important factors including saliva characteristics, tooth surface morphology, oral hygiene. Different biochemical characteristics of

saliva such as salivary flow rate, buffering capacity, inorganic component, as well as proteins may affect the development of dental caries [1-2].

The normal structure of enamel is mainly consisted of hydroxyapatite, which contains a high degree of calcium and phosphate. It is expected that saliva might be effective on enamel maturation and remineralisation [3-4], with respect to its high level of calcium and phosphate [5-6]. However, spontaneous precipitation of these ions from saliva to tooth structure cannot occur [2]. Studies show that there are factors which may influence the function of calcium and phosphate on tooth remineralisation. Alkaline phosphatase, a salivary protein, may increase the concentration of salivary phosphate and the balance of demineralisation to remineralisation process of enamel. It seems that the function of this protein relatively depends on the salivary pH and buffering capacity [7].

To show the degree of saturation of the solutions, many authors used the ion activity product for hydroxyapatite (I_{PHA}) calculated by employing the equation $I_{PHA} = \{Ca^{2+}\}^{5} \{PO4^{3-}\}^{3} \{OH^{-}\}$ [8-10]. Different factors such as pH, buffering capacity, and temperature have shown, in an in-vitro experiment, to be effective on the I_{PHA} [11]; however, it seems that there is a relationship between the values of I_{PHA} and de- and remineralisation of the enamel [12].

To the best of our knowledge, no clinical study has been performed yet to evaluate the relationship between the I_{PHA} of saliva and tooth caries experience. Therefore it seems necessary to conduct a study to compare the I_{PHA}; reflecting salivary calcium and phosphate concentration [12], alkaline phosphatase; which may balance enamel remineralisation [4], as well as buffering capacity; that may affect alkaline phosphatase function and the quantity of ion activity product for hydroxyapatite [7,12], regarding the caries risk and age.

The aim of the present study was to compare salivary I_{PHA}, alkaline phosphatase, as well as buffering capacity of saliva in adults concerning the tooth caries prevalence.

Materials and Methods
Study population
In this observational cross sectional study [13], 120 men with age range of 19 to 44 years who were seeking for routine dental care in oral medicine department of Hamadan dental school, Hamadan, Iran, were participated. Respecting the previous studies [12, 14], those who had positive history of illnesses or treatments which could cause alteration in salivary rate and composition including diabetes, rickets, deformans osteitis, periodontal disease, history of radiotherapy or chemotherapy, dehydration, using antibiotic in recent two weeks, and mouth breath, were excluded from the study. The only inclusion criterion was regular teeth brush, for at least once in 24 hours. Informed consent was obtained from each individual, before any data collection and examination of the oral health status.

Dental examination
Data was collected by interview and clinical examination. An examiner was trained and informed with WHO instructions on tooth caries diagnosis. Prior to examination, teeth were isolated by cotton rolls. With patients sitting on the dental unit, a WHO periodontal probe and a No.4 flat mouth mirrors were used for each dental examination. Teeth lost or restored due to trauma, orthodontic treatment or aesthetic reasons were not considered as missed or filled teeth. Decayed (D), missed (M) and filled teeth were detected and DMFT were calculated.

According to the mean DMFT values of Iranian population of different ages reported in the previous studies [15-16], participants were divided into low and high risk groups; those with DMFT value higher than the mean DMFT of aged-matched population considered as high risk group and vice versa. Regarding the DMFT value and the age, participants were divided into four groups as follows; group 1 was composed of 30 low risk male individuals with age range of 19-35 years (DMFT<5), group 2 (was included 30 high risk men with age range of 19-35 years (DMFT>5), group 3 consisted of 30 low risk male individuals with 35 to 44 years old (DMFT<11) and finally 30 high risk men with age range of 35-44 years old (DMFT>11) were placed in to group 4.

Saliva collection and salivary buffering capacity
To reduce circadian effect, saliva collection was done between 9 and 11 am. Five millilitres of unstimulated saliva was collected in centrifuge tubes (HAILUN, Jiangsu, China) after 2 hours with subjects being prevented of eating, drinking or brushing. Collected samples were delivered to the biochemistry lab. in 2 hours

[17-18]. Shortly after delivering, 0.5 ml of each sample was used to measure the pH value within 30 seconds after the placement of pH-sensitive electrode (HANNA instruments®, Inc. Michigan, USA). Buffering capacity was determined by modified Ericsson method [19]. Regarding the salivary pH, after adding 1.5ml of 5 mmol/L hydrochloric acid into the 0.5 ml saliva; samples were ranked to have: (1) low (pH<4.5); (2) medium (4.5<pH<5.5); and (3) high buffering capacity (pH 5.5) [20-21].

Measurement of total salivary calcium and phosphate concentration

In order to measure ion activity product for hydroxyapatite (I_{PHA}); salivary calcium and phosphate concentrations were needed to be measured. For measuring calcium concentration, colorimetric method using Cresolphthalin complex, provided by Pars Azmoon diagnostic kit (Pars Azmoon Co, Karaj, Iran), were performed. In this method calcium compounds to Cresolphthalein complexone and produces purple solution in which the colour intensity is proportional to the calcium concentration. To examine the colour intensity, the light absorption of the solution was measured by spectrophotometer (JENWAY, Staffordshire, UK) in 570 nanometre wavelength, and finally calcium concentration was calculated in mg/dL [20].

Measuring salivary phosphate concentration was basically similar to that of calcium; however, two different solutions containing ammonium molibidate and sulphuric acid used to produce coloured solution. Light absorption of the solutions was measured by spectrophotometer in 340 nanometre wavelength and phosphate concentration was calculated in mg/dL [22].

Measurement of alkaline phosphatase concentration

The level of alkaline phosphatase activity (unit/L) was measured by kinetic method. In this method alkaline phosphatase converts 4- nitrophenyl phosphate into 4-nitrophenol and produces a yellow solution. The light absorption which matches up to the activity of alkaline phosphatase was assessed by spectrophotometer in 405 nanometre (nm) wavelength [4, 20].

Calculation of ion activity product for hydroxyapatite (I_{PHA})

As it is clear that the pH + pOH are 14 [23], after measurement of the pH of the samples, pOH was used to calculate the concentration of {OH^+}. I_{PHA} was calculated by placement of the concentration of calcium^{2+}, phosphate^{3-} and OH$^-$ in the following equation: I_{HA}= {Ca^{2+}}^{5}{$PO4^{3-}$}^{3}{OH^-}, in which any value in the curly brackets shows the ion activity in saliva. In the present study, like the previous one [12], the negative logarithms of I_{PHA} (pI_{PHA}) have been used for convenience in order to determine the enamel solubility. All calculations were done by current Microsoft Office Excel software.

Statistical analysis

Data was analyzed by SPSS version 13.00 software. The mean salivary alkaline phosphatase and pI_{PHA} were compared among four experimental groups using Two-way ANOVA. In order to compare salivary buffering capacity of the groups, Friedman and Mann-Whitney tests were used. *P*-value of <0.05 was considered statistically significant in all analysis.

Results

The mean age of the individuals participated in the present study was 32.95±8.09. The mean numbers of decayed (D), missed (M) and filled (F) teeth, as well as DMFT for each experimental group are shown in Table 1. Salivary parameters including the average of salivary flow rate, pH, calcium and phosphate concentration of the four groups are illustrated in Table 2, separately. The average of pI_{PHA} for group 1 to 4 was 29.39±0.61, 29.51±0.76, 29.14±0.56 and 29.75±0.75, respectively.

Table 1 The mean and standard deviation of decayed (D), missed (M) and filled (F) teeth, DMFT of experimental groups

Experimental group	Mean D ± SD	Mean M ± SD	Mean F ± SD	Mean DMFT ± SD
1	1.24±1.10	0.93±0.50	1.96±1.62	3.70±1.51
2	2.76±2.47	1.71±1.5	4.46±2.68	8.70±2.34
3	1.80±1.42	2.52±2.50	3.60±2.11	7.90±1.90
4	4.50±4.07	6.06±4.71	5.06±4.08	15.60±2.96

Table 2 The mean and standard deviation of salivary flow rate (FR), pH, calcium(Ca) and phosphate (Ph) concentration of experimental groups

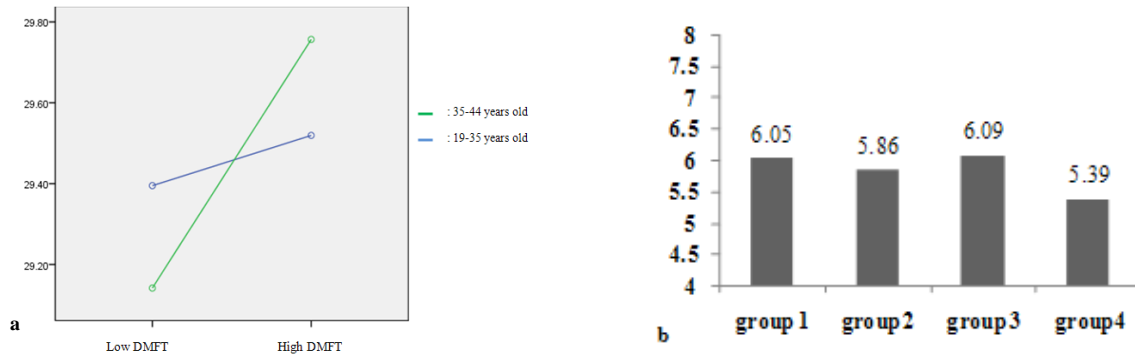| Experimental group	Mean FR ± SD	Mean pH ± SD	Mean Ca ± SD	Mean Ph ± SD
1	0.84±0.29	6.62±0.70	4.86±1.02	9.76±2.03
2	0.81±0.38	6.59±0.68	4.73±0.68	10.16±2.99
3	0.64±0.29	6.90±0.40	5.00±1.01	10.53±2.09
4	0.62±0.37	6.49±0.60	4.56±0.79	10.66±2.06

Figure 1a I$_{PHA}$ changes according to dental caries rate in different age groups **b** The mean of secondary pH for different experimental groups

The results of Two-way ANOVA showed that the difference between the experimental groups were significant ($p= 0.049$). Although, pI$_{PHA}$ was significantly different according to the DMFT ($p= 0.003$), there was no significant difference between pI$_{PHA}$ of participants with different ages. The changes of I$_{PHA}$ according to the DMFT values of different age groups are shown in Figure 1.

The mean level of alkaline phosphatase activity of group one to four was 5.82 ± 2.91, 5.30 ± 1.52, 4.77 ± 1.82 and 4.55 ± 1.61, respectively. Two way ANOVA analysis showed that regarding the age, the difference was significant ($p= 0.018$), however; neither the difference among four experimental groups ($p= 0.692$) nor between high and low dental caries risk groups ($p= 0.318$) was significant.

Figure 2 illustrates the average of secondary pH of saliva of four experimental groups. According to the mean salivary buffering capacity which was 2.66 ± 0.56 for group 1, 2.64 ± 0.56 for group 2, 2.70 ± 0.70 for group 3 and 2.66 ± 0.82 for group 4 all groups had medium buffering capacity. The results of Friedman and Mann-Whitney test indicated that the difference between four experimental groups ($p= 0.305$) as well as between high and low risk groups ($p= 0.058$) was not significant.

Discussion

Saliva not only has important protecting effects on oral tissue but also is a lubricant which facilitates oral function including talking, chewing and so on. Recent studies showed that saliva has different effects on dental caries regarding to its organic components, such as alkaline phosphatase, and its inorganic materials, including calcium, phosphate and so on. [21-22].

In the present study, similar to the previous study [24], total or rest saliva was used because of its long time contact with the teeth. Total saliva, compared to the limited secretion of stimulated saliva; is imparted in our mouth for approximately 14 hours a day [25]. As there is hormonal and physiological difference between two genders, we selected our subjects to be only male individuals in order to reduce inter and intra group differences. Regarding the possible effects of aging on salivary secretion and composition, the population participated in the present study were placed into two groups according to their age [26].

The balance between demineralisation and remineralisation of teeth surface depends on the salivary calcium and phosphate saturation [27]. Due to the controversial results of the previous studies which were conducted to evaluate the relationship between salivary calcium and phosphate concentration and tooth caries [28-30], in the present study; pI$_{PHA}$ was measured considering its ability to reflect calcium and phosphate saturation together and also its relationship with buffering capacity [11]. According to the results of the present study, the amount of I$_{PHA}$ is significantly different between low and high dental caries rate groups in all ages, but the difference is more prominent in older adults (Figure 1). Earlier in-vitro experiments reported different values for pI$_{PHA,}$ ranging from 51.8 to 58.8 depended on the different condition, including temperature [9]. To the best of our knowledge, no clinical study was performed to evaluate the relation between pI$_{PHA}$ and tooth caries; however, Auichi et al. [11] reported that the mean value of pI$_{PHA}$ was 40 and was related to the salivary buffering capacity. In the present study the mean pI$_{PHA}$ was 29.45. Compared to previous study [9], the

results of the present study showed a lower amount of pI_{PHA}. This difference may be contributed to the different variants such as gender of the participants whom the salivary samples were collected from, as well as initial pH of saliva.

The mean level of alkaline phosphatase activity in the present study was 5.11 unit/L and similar to the finding of Shahrabi et al [4] and Afshar et al [31] showed no significant difference between low and high dental-caries risk groups. Previous studies on salivary alkaline phosphatase activity showed different results. Contrary to our findings, Gandhy et al [12] reported that higher level of alkaline phosphatase activity is related to rampant caries. Based on our searching, no study compared the salivary alkaline phosphatase activity in different ages; however the results of this study showed that the older is patient, the lower is alkaline phosphatase activity.

Three different buffering capacity including bicarbonates, phosphates and proteins are responsible for salivary buffering [32]. Different factors such as hormones, metabolic conditions and general health could affect this capacity. So in the current study all participants were men with no systemic diseases in order to decrease the influence of the factors that could alter the results. In this study Ericson method [2] was used to evaluate salivary buffering capacity. According to this method, salivary pH after adding acid was considered as an appraisal factor for buffering capacity [2].

Results of the present study were similar to the findings of Monezgo et al. [33] and Gabris et al. [34]. It showed that there is no significant difference in buffering capacity of saliva according to dental caries risk. On the contrary, Lundgren et al. [35] observed that lower buffering capacity increased root caries prevalence. This differing result could be due to dissimilar methods of the two studies. Of these inconsistencies, structural difference between the root and crown, unstimulated or stimulated saliva could be declared. Age as well as gender was another difference of methods between these two studies [36].

Opposite to the results of the current study, Ruiz et al. [37] explained that buffering capacity could be deliberated as a predictive factor for dental caries prevalence. Different gender of the participants could be the cause of different results delivered by these two studies

respecting the Heintze [36] study which reported sex hormones could affect the buffering capacity and it was higher in male subjects.

The results of the present study similar to the results of Palomares et al. [25], Kitasako et al. [38] and Farsi et al. [39], showed that there is no relationship between age and salivary buffering capacity.

Based on the current findings, salivary buffering capacity and alkaline phosphatase had no significant relationship with dental caries rate in male adults. Although salivary alkaline phosphatase activity reduces with age, no significant change occurs in buffering capacity. According to the results of the present study, since there is a significant relation between pI_{PHA} and dental caries, it could be considered as a predictor of dental caries; however performing further research in a wider population, regarding the age, sex and race is suggested.

Conclusion

Based on the results of the present study, although buffering capacity and the level of alkaline phosphatase is not related to the severity of dental caries, I_{PHA} index in individuals with higher teeth caries prevalence is higher than those with lower caries prevalence. Ageing decreases the level of alkaline phosphatase activity.

Acknowledgment

References

[1] Roberson TM, Heymann HO, Swift E J. Art and science of operative dentistry. 5th ed., St Louis: Mosby Co; 2006. p. 67.

[2] Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000; 14: 40-47.

[3] Pinkham J, Casmassimo P, Mc Tigue D, Fields H, Nowak A. Pediatric Dentistry Infancy through Adolescence .4th ed., China: Elsevier Inc; 2005. p. 199-204.

[4] Shahrabi M, Nikfarjam J, Alikhani A, Akhoundi N,

Ashtiani M, Seraj B. A comparison of salivary calcium, phosphate, and alkaline phosphatase in children with severe, moderate caries, and caries free in Tehran's kindergartens. J Indian Soc Pedod Prev Dent 2008; 26: 74-77.

[5] Hay DI, Schluckebier SK, Moreno EC. Equilibrium dialysis and ultrafiltration studies of calcium and phosphate binding by human salivary proteins. Implications for salivary supersaturation with respect to calcium phosphate salts. Calcif Tissue Int 1982; 34: 531-538.

[6] Lagerlöf F. Effects of flow rate and pH on calcium phosphate saturation in human parotid saliva. Caries Res 1983; 17: 403-411.

[7] Harada M, Udagawa N, Fukasawa K, Hiraoka BY, Mogi M. Inorganic pyrophosphatase activity of purified bovine pulp alkaline phosphatase at physiological pH. J Dent Res 1986; 65: 125-127.

[8] Shellis RP, Wilson RM. Apparent solubility distributions of hydroxyapatite and enamel apatite. J Colloid Interface Sci 2004; 278: 325-332.

[9] Margolis HC, Zhang YP, Lee CY, Kent RL Jr, Moreno EC. Kinetics of enamel demineralization in vitro. J Dent Res 1999; 78: 1326-1335.

[10] Patel MV, Fox JL, Higuchi WI. Effect of acid type on kinetics and mechanism of dental enamel demineralization. J Dent Res 1987; 66: 1425-1430.

[11] Aiuchi H, Kitasako Y, Fukuda Y, Nakashima S, Burrow MF, Tagami J. Relationship between quantitative assessents of salivary buffering capacity and ion activity product for hydroxyapatite in relation to cariogenic potential. Aust Dent J 2008; 53: 167-171.

[12] Gandhy M, Damle SG. Relation of salivary inorganic phosphorus and alkaline phosphatase to the dental caries status in children. J Indian Soc Pedod Prev Dent 2003; 21: 135-138.

[13] Mann CJ. Observational research methods. Research design II: cohort, cross sectional, and case-control studies. Emerg Med J 2003; 20: 54-60.

[14] von Bültzingslöwen I, Sollecito TP, Fox PC, Daniels T, Jonsson R, Lockhart PB, et al. Salivary dysfunction associated with systemic diseases: systematic review and clinical management recommendations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103: 1-15.

[15] Hessari H, Vehkalahti MM, Eghbal MJ, Murtomaa HT. Oral health among 35- to 44-year-old Iranians. Med Princ Pract 2007; 16: 280-285.

[16] Hessari H, Vehkalahti MM, Eghbal MJ, Samadzadeh H,

Murtomaa HT. Oral health and treatment needs among 18-year-old Iranians. Med Princ Pract 2008; 17: 302-307.

[17] Larsen MJ, Jensen AF, Madsen DM, Pearce EI. Individual variations of pH, buffer capacity, and concentrations of calcium and phosphate in unstimulated whole saliva. Arch Oral Biol 1999; 44: 111-117.

[18] Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci 1993; 694: 72-77.

[19] Moritsuka M, Kitasako Y, Burrow MF, Ikeda M, Tagami J. The pH change after HCl titration into resting and stimulated saliva for a buffering capacity test. Aust Dent J 2006; 51: 170-174.

[20] Thomas L. Clinical laboratory diagnosis. 1st ed., Frankfort: th-books verlagsgeselleschaft; 1998. p. 136-146.

[21] Wiktorsson AM, Martinsson T, Zimmerman M. Salivary levels of lactobacilli, buffer capacity and salivary flow rate related to caries activity among adults in communities with optimal and low water fluoride concentrations. Swed Dent J 1992; 16: 231-237.

[22] Powell LV, Leroux BG, Persson RE, Kiyak HA. Factors associated with caries incidence in an elderly population. Community Dent Oral Epidemiol 1998; 26: 170-176.

[23] Mortimer RG. Physical chemistry. 3th ed., Massachusetts: Academic press; 2008. p. 82-94.

[24] Varma S, Banerjee A, Bartlett D. An in vivo investigation of associations between saliva properties, caries prevalence and potential lesion activity in an adult UK population. J Dent 2008; 36: 294-299.

[25] Fenoll-Palomares C, Muñoz Montagud JV, Sanchiz V, Herreros B, Hernández V, Mínguez M, Benages A. Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers. Rev Esp Enferm Dig 2004; 96: 773-783.

[26] Tanida T, Ueta E, Tobiume A, Hamada T, Rao F, Osaki T. Influence of aging on candidal growth and adhesion regulatory agents in saliva. J Oral Pathol Med 2001; 30: 328-335.

[27] Farsi N. Dental caries in relation to salivary factors in Saudi population groups. J Contemp Dent Pract 2008; 9: 16-23.

[28] Karshan M. Factors in Saliva Correlated with Dental Caries. J Dent Res 1939; 18: 395-407.

[29] Ashley FP, Wilson RF. The relationship between calcium and phosphorus concentrations of human saliva and dental plaque. Arch Oral Biol 1978; 23: 69-73.

[30] Elizarova VM, Petrovich IuA. Ionized calcium in the

saliva of children with multiple caries. Stomatologiia Mosk 1997; 76: 6-8.

[31] Afshar H, Seraj B, Shafizadeh N. The relationship between rampant caries and salivary situation of 4-5 years old children living in Tehran. J Islamic Dent Assoc Iran 1380; 13: 18-25.

[32] Miura H, Isogai E, Hirose K, Wakizaka H, Ueda I, Ito N. Application of a sucrose indicator strip to evaluate salivary sucrose clearance. J Dent 1991; 19: 189-191.

[33] Mazengo MC, Tenovuo J, Hausen H. Dental caries in relation to diet, saliva and cariogenic microorganisms in Tanzanians of selected age groups. Community Dent Oral Epidemiol 1996; 24: 169-174.

[34] Gábris K, Nagy G, Madléna M, Dénes Z, Márton S, Keszthelyi G, et al. Associations between microbiological and salivary caries activity tests and caries experience in Hungarian adolescents. Caries Res 1999; 33: 191-195.

[35] Lundgren M, Emilson CG, Osterberg T. Root caries and some related factors in 88-year-old carriers and non-carriers of Streptococcus sobrinus in saliva. Caries Res 1998; 32: 93-99.

[36] Heintze U, Birkhed D, Björn H. Secretion rate and buffe-reffect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J 1983; 7: 227-238.

[37] Ruiz Miravet A, Montiel Company JM, Almerich Silla JM. Evaluation of caries risk in a young adult population. Med Oral Patol Oral Cir Bucal 2007; 12: 412-418.

[38] Kitasako Y, Ikeda M, Burrow MF, Tagami J. Oral health status in relation to stimulated saliva buffering capacity among Japanese adults above or below 35 years of age. J Med Dent Sci 2006; 53: 175-180.

[39] Farsi N, Al Amoudi N, Farsi J, Bokhary S, Sonbul H. Periodontal health and its relationship with salivary factors among different age groups in a Saudi population. Oral Health Prev Dent 2008; 6: 147-154.