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Relationship of Dental Caries Experience in Paediatric subjectswithinorganic constituents of saliva: A quantitative study.

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ABSTRACT: Background: Saliva is undoubtedly one of the most important factor in regulating oral health, with flow rate and composition changing throughout development and during disease. Saliva can affect incidence of dental caries in four general ways, firstly as a mechanical cleansing, secondly by reducing enamel solubility by means of calcium, phosphate and fluoride, thirdly by buffering and neutralizing the acids produced by cariogenic organisms and finally by anti-bacterialactivity. Thus, the present study aimed to assess the levels of salivary immunoglobulin A (IgA), immunoglobulin G (IgG), proteins, calcium, inorganic phosphorous and alkaline phosphatase levels in caries free and caries active children.

Material and methods: Forty school children in the age group of 12-15 years with full complement of permanent dentition except third molars were included by stratified random sampling method. They were divided into two groups of 20 each based on DMFS score, Group I – Caries free (DMFS score=0) and Group II – Caries active (DMFSscore ≥ 10). Unstimulated midmorning saliva samples were collected and analyzedcolorimetrically and by radial immunodiffusion method for constituents of saliva under study.

Results: The mean salivary IgA levels in children in Group-I (caries free children) was 10.63±2.85 mg/dl whichwas statistically higher as compared to caries active children in Group-II (8.50 ± 1.43 mg/dl). The mean salivary proteinlevel in children of Group-II was statistically higher at 3.28 ± 0.12 mg/dl as compared to Group-I (2.89 ± 0.11 mg/dl).

Conclusion: An inverse relationship was noticed between the salivary IgA levels and dental caries experience and higher salivary protein levels were associated with high caries experience whereas no significant difference was observed in levels of calcium, inorganic phosphorous, alkaline phosphatase and IgG in saliva samples of children with and without dental caries.

Keywords: Alkaline phosphatase; Dental caries; Immunoglobulin A (IgA); Immunoglobulin G (IgG); Saliva

I. INTRODUCTION

Oral health is an integral part of the general health of an individual.Dental caries is acomplex multifactorial disease caused by the interplaybetween a susceptible host, fermentable substrate, microflora and saliva.Saliva is essential for maintaining the oral equilibrium; and the effectsof saliva and its constituents on the oral micro-organisms influence thedevelopment of dental caries. Salivary components (immunoglobulins, salivary protein, salivary calcium, and inorganic phosphorous andalkaline phosphatase levels), its flow rate, viscosity, buffering capacity,pH etc plays a major role in initiation, and progression of dental caries[1].

Mutans streptococci (MS), gram-positive micro-organism,are implicated as the primary causative agent in the formation ofdental caries in humans. Early colonization and growth of mutansstreptococci, changes local conditions, e. g. pH, thereby enabling moreorganisms to further colonize the oral biofilm, forming dental

plaquewhich results in demineralization of tooth structure and consequentlydental caries ensues [2]. The infective nature of dental caries suggeststhat the host immunity regulates caries activity [3,4]. The specificimmune defense against mutans streptococci is provided through theCommon Mucosal Immune System (CMIS). Immunoglobulin A (IgA)is predominantly released by common mucosal immune system inhuman body secretions including saliva. Naturally occurring salivaryIgA antibodies against different streptococcal antigens are present insaliva and constitute major defensive actions against dental caries [2,5].The gingival crevicular mechanism involves the humoral andcellular components of the systemic immune system. The gingivalcrevicular fluid, serve as a source of secretory immunoglobulin G(IgG) as well as some monomeric IgA contributing towards hostdefense against dental caries. IgG are capable of opsonizing bacteria forphagocytosis and thus, intervene in the colonization and pathogenicactivity of cariogenic microorganisms.

Saliva also contains various inorganic and organic constituentsapart from immunoglobulin. A major part of organic component isformed by salivary proteins which play an important role in modulatingmicrobial colonization and formation of enamel pellicle. Salivaryproteins bind with calcium and phosphate ions and help to maintainthese in a supersaturated state, in respect to enamel and maintain toothintegrity via the common ion effect [6,7].

Alkaline phosphatase, an enzyme present in saliva, is active at pH9-10 and is important for the process of remineralization. A variationin the level of alkaline phosphatase affects the ionic concentration ofphosphate and calcium, which in turn can alter the equilibrium ofdemineralization and remineralization. The interplay of the variouscomponents of saliva and their protective role against dental carieshas been of much interest. Thus, the present study further investigated and estimated the levels of salivary immunoglobulin A (IgA),immunoglobulin G (IgG), proteins, calcium, inorganic phosphorousand alkaline phosphatase levels in association with presence andabsence of dental caries among children.

II. MATERIALS AND METHODS

The quantitative determination of constituents in saliva and itsrelationship with dental caries experience among forty school childrenselected by stratified random sampling were assessed in this study.

Ethical approval was obtained from the ethics committee and permissionwas also taken from the school authorities. A written explanatory notewas sent to the parents regarding the objectives of the study and writtenconsent was received from them. A total of 150 children between 12-15years of age, were initially screened from a Municipal school in Moradabad. Prior to commencement of oralexamination relevant medical and dental history was elicited fromthe parents. The fluoride level in drinking water of different parts ofMoradabadwas estimated using selective fluoride ion electrode (Orion)and was found to be within the range of 0.15-0.48 ppm.

Inclusion criteria

- Children in the age group of 12-15 years
- Good general health
- No history of intake of antibiotics or any preventive treatment for
past 6 months
- Regular attendance in school
- Permanent residents of Moradabad

Exclusion criteria

- Medically compromised children or children with physical
limitations
- Children undergoing orthodontic treatment
- Children with moderate – severe gingivitis or any significant softtissue pathology

The children were examined in their classrooms under natural daylight, comfortably seated on ordinary chairs. A thorough oral and softtissue examination was done with mouth mirror and CPI probe. Thedental caries status was assessed and recorded as per WHO criteria(1997) [8]. The examination revealed 100 caries-active and 50 caries-free children. Based on the inclusion and exclusion criteria of the study,82 children were selected, 49 caries-active and 33 caries-free children ofwhich 48 were boys and 34 were girls.

The selected children were further divided into 2 groups – GroupI – Caries free children (DMFS score=0) which included 30 boys and 19girls and Group II – Caries active children (DMFS score $\geq$ 10) with 18boys and 15 girls. Within each group, the boys and girls were allottedseparate sequential numbers. Through randomized draw of lots doneby one of the child, a sample size of 10 boys and 10 girls were obtained(total 20 children) for each group, in the study.

Saliva collection

The unstimulated mid-morning whole saliva samples werecollected. The saliva samples

were taken one and half hour after schoolhad commenced so that sufficient time had elapsed after breakfast. Thechildren were then asked to allow saliva to drool from the oral cavityinto the sterile, labelled disposable containers, to determine the salivaryflow rate expressed as ml/min. A total of 5 ml saliva was collected fromeach child and transported immediately in a thermostat container forfurther analysis.

Estimation of immunoglobulin A (IgA) and immunoglobulinG (IgG) in saliva

Immunoglobulin A and Immunoglobulin G levels in saliva wereestimated by Single Radial Immunodiffusion method described byMancini et al. [9] It is based on the principle that a quantitative relationexists between the amount of antigen placed in well of agar antibody plate and the resulting ring of precipitation.

Two ml of each salivary sample was centrifuged at 4000 rpm for20 min, to remove the particulates [10]. 5 µl of the supernatant wasthen placed in each well of the immunodiffusion plate (DiffuplateTMBioscientifica S. A, batch no. 1017-IgA and 1013-IgG) using amicropipette (CE Biosystems) with disposable tips. After 30 minutes,5 µl of the supernatant was added to each well; and the plates wereincubated at room temperature for 48 hours. Antigen-antibodyprecipitate formation was observed in agar in the form of concentricring around the antigen well, and measured with a Tripartigen ruler(DiffuplateTM Bioscientifica S. A) after 48 hours.

Estimation of salivary proteins

The level of proteins in saliva was estimated by the proceduredescribed by Lowry et al. [11] The saliva samples were treated withalkaline copper sulphate (CuSO4) and FolinCiocalteau reagent. Thecolor change was noted colorimetrically (A. E ERMA INC) at 660 nm.

Estimation of salivary calcium

Salivary calcium was measured using Trinder´s method [12]. Thesample was treated with calcium reagent and the precipitate was mixedwith EDTA and treated with ferric nitrate. The reddish brown colorcomplex was measured colorimetrically (AE ERMA INC) at 470 nm.
The intensity and color is directly proportional to the calcium contentof saliva.

Estimation of salivary inorganic phosphorous

The level of salivary inorganic phosphorus was measured by Fiskeand Subarrow method [13]. The inorganic phosphorous in a protein freefiltrate reacts with molybdic acid to form a hexavalent phosphomolybdicacid which is further reduced to 1,2,4-aminonaphthol sulphonic acidto give blue colored complex, and the intensities were read at 660 nmusing a colorimeter (AE ERMA INC).

Estimation of salivary alkaline phosphatase

The level of alkaline phosphatase was measured by the King-Armstrong method using disodium phenyl phosphate as a substrate[14]. The reddish brown color was read colorimetrically (AE ERMAINC) at 530 nm.

Statistical Analysis

The collected data was tabulated and statistically analyzed by:
A) Unpaired t-test
B) Chi-square test

III. RESULTS

A total of forty children, 20 in group I (DMFS=0), and 20 in groupII (DMFS ≥ 10) were selected by stratified random sampling fromMunicipal school of the Moradabad.

The mean salivary IgA levels of children in Group-I was 10.63 ±2.85 mg/dl, which was significantly higher as compared to children inGroup-II with 8.50 ± 1.43 mg/dl (t-value = 2.600, p-value = 0.015). Themean salivary IgG levels of children in Group-I was 1.04 ± 0.31 mg/dlas compared to Group-II with 0.87 ± 0.14 mg/dl, which was statistically insignificant with t-value = 1.793, p-value = 0.085 (Table 1).

The mean salivary protein levels in children in Group-I was 2.89± 0.11 mg/ml while in Group-II, it was significantly higher i.e., 3.28 ±0.12 mg/dl with t-value = -10.766, p-value = 0.015 (Table 1).The levels of salivary calcium in Group-I was 6.81 ± 0.72 mg/dl,while in Group-II, it was 6.39 ± 0.59 mg/dl (t-value = 1.966, p-value =0.057). Inorganic phosphorous levels for children in Group-I was 17.45± 1.34 mg/dl, and levels for Group-II children was 16.74 ± 1.02 mg/dl(t-value = 1.896, p-value = 0.066). The levels of alkaline phosphatasein Group-I was 2.41 ± 0.47 KA units, and Group-II showed 2.70 ±0.46 KA units (t-value = -1.965, p-value = 0.057). These results werestatistically insignificant (Table 1).

The intra-group comparison of salivary IgG levels in girls (GroupI) was significantly higher than boys (t-value = -2.327, p-value = 0.038)but was not statistical significant for IgA (t-value = 1.802, p-value= 0.093) and protein levels

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(t-value = 1.802, p-value = 0.293). Thedifference between boys and girls in Group I with respect to salivarycalcium (t-value = -0.708, p-value = 0.488), inorganic phosphorous(t-value = 0.989, p-value = 0.336) and alkaline phosphatase (t-value= 0.811, p-value = 0.335) was statistically not significant (Table 2).

In Group II, the mean salivary IgA (t-value = 1.866, p-value = 0.085),IgG (t-value = 2.191, p-value = 0.051), protein levels (t-value = 0.000,p-value = 1.000), salivary calcium (t-value = -0.846, p-value = 0.409),inorganic phosphorous (t-value = 0.592, p-value = 0.561) and alkaline phosphatase (t-value = -0.198, p-value = 0.845) was not statisticalsignificant between boys and girls (Table 2).

IV. DISCUSSION

Oral cavity is a distinctive ecosystem, which performs a wide rangeof functions, harbours a plethora of microorganisms and is unique inaccommodating exposed mineralized tissues. The saliva bathes thisecosystem and possesses a large number of components, plays a major role in the etiopathogenesis of dental caries [15,16].

The present study included school children in the age group of12-15 years, as both cell mediated and humoral immune system areknown to be fully functional at this age group [17]. The unstimulatedmidmorning whole saliva samples were collected at least two hoursafter breakfast as this period has been reported to have less diurnalvariations in the flow rate and composition of saliva. A total of 5 ml ofsaliva was collected from each child and transported immediately forthe estimation of salivary constituents under study.

Prolonged storageof saliva samples should be avoided as it leads to variable loss of proteinincluding immunoglobulins [10,11].

In the present study, the mean salivary IgA level in children inGroup-I {Caries free (DMFS=0)} was significantly higher than Group-II {Caries active (DMFS $\geq$ 10)}, suggesting a possible protective roleof IgA in prevention of dental caries. Lehner et al. [10] reported that subjects with caries had decreased IgA concentrations, as compared tothose with no detectable caries and proposed it could be due to thedeficient transport mechanism, stimulation of immune system via pulp,deficient local immunoglobulin synthesis and molecular size of IgA.

Challacombe in a study also reported a significant inverse relationshipbetween the IgA secretion rate and dental caries and stated that theraised IgA and IgG levels in serum reflect past caries experience [15].

Rose et al. [18] compared the IgA levels of whole saliva and parotidsaliva of caries susceptible and caries-resistant children aged 7-11years using enzyme linked immunosorbant assay and concluded thatwhole saliva and not parotid saliva in caries resistant children had a significantly higher IgA levels as compared to caries prone group.

However, Camling et al. [19] have reported a negative correlationbetween the degree of caries activity and salivary IgA concentration.Bhatia et al. [20] concluded that higher levels of salivary IgA exist incaries susceptible group, which could be due to either a cumulativeantigenic or recent antigenic stimulation, with higher caries experienceas compared to caries resistant group. Some authors also reportedthat the saliva of caries-free subjects includes significant IgA antibodyagainst antigen I/II of Streptococcus mutans, indicating a protective mechanism [21,22]. However, microorganisms may protect themselvesfrom host immune attack by forming biofilms and decreasingexpression of antigen I/II. The finding of Cogulu et al. [23] tends tosupport the hypothesis that higher levels of salivary IgA may provide protection against dental caries.

The mean salivary IgG levels in the present study were higherin Group I as compared to Group II; however, the difference wasstatistically insignificant. Lehner et al [10] and Everhart et al. [24]reported that salivary IgG does not seem to play any role in preventionof dental caries.

Variables		Group	
		I	II
lgA (mg/dl)	**Mean**	10.63	**8.50**
	SD	2.85	**1.43**
lgG (mg/dl)	**Mean**	1.04	**0.87**
	SD	0.31	**0.14**
Protein (mg/ml)	**Mean**	2.89	**3.28**
	SD	0.11	**0.12**
Calcium (mg/dl)	**Mean**	6.81	**6.369**
	SD	0.72	**0.59**
Inorganic Phosphorous (mg/dl)	**Mean**	17.45	**16.74**
	SD	1.34	**1.02**

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Alkaline Phosphatase (KA units)	**Mean**	2.41	**2.70**
	SD	**0.47**	**0.46**

Table 1: Comparison of immunoglobulin A (lgA) and immunoglobulin G (lgG), salivary protein, salivary calcium, inorganic phosphorous and alkaline phosphatise levels between Group I and Group II.

Variables		Group I		Group II	
		Boys	**Girls**	**Boys**	Girls
lgA (mg/dl)	**Mean**	11.82	9.43	9.18	**7.91**
	SD	3.43	1.53	0.83	**1.63**
lgG (mg/dl)	**Mean**	0.87	1.20	0.94	**0.79**
	SD	0.20	0.31	0.15	**0.09**
Protein (mg/ml)	**Mean**	2.91	2.86	3.28	**3.28**
	SD	0.11	0.11	0.14	**0.10**
Calcium (mg/dl)	**Mean**	6.69	6.92	6.28	**6.51**
	SD	0.80	0.66	0.66	**0.53**
Inorganic Phosphorous (mg/dl)	**Mean**	17.74	17.15	16.87	**16.60**
	SD	1.18	1.47	0.86	**1.20**
Alkaline Phosphatase (KA units)	**Mean**	2.49	2.32	2.68	**2.72**
	SD	**0.45**	**0.50**	**0.53**	**0.41**

Table 2: Intra-group comparison of immunoglobulin A (lgA), immunoglobulin G (lgG), salivary protein, salivary calcium, inorganic phosphorous and alkaline phosphatase levels in Group I and Group II according to gender.

Bagherian et al. [25] reported that the high concentration ofsalivary immunoglobulin in children with early childhood cariesmay be associated with an increased antigenic load, leading to highproduction of antibodies. According to a study by Kirtaniya et al. [26],the increase in serum IgG, IgA and total antibody titer showed a directcorrelation with the increase in number of carious lesions.

The various organic and inorganic constituents of saliva help inmaintaining the integrity of teeth and other oral structures. Salivaryproteins possess antimicrobial, lubricative and digestive propertiesand play an important role in modulating the microbial colonizationof teeth and soft tissues [6]. The salivary protein levels were estimatedby the method using Folin phenol reagent. The Folin-Ciocalteaureagent contains phosphomolybdic acid. The reduction of Mo6+ andMo4+ of phosphomolybdic acid by tyrosine and tryptophan presentin the salivary proteins gives a blue colored complex which is readcolorimetrically at 660 nm [11].

The mean salivary protein level in the present study in cariesactive group i.e. Group-II was higher as compared to Group-I whichis in accordance with Tulungolu et al. [27] and Kargul et al. [28]who observed an increase in the salivary protein concentration withincreased caries activity.

The role of saliva in remineralization of enamel and calculusformation is dependent upon the saturation of saliva with respect tocalcium and phosphorous. The salivary alkaline phosphatase is one ofthe factors governing the calcium and phosphorous levels in saliva [29].

The mean salivary calcium and inorganic phosphorous levels in cariesfree group (Group-I) were higher as compared to caries active group(Group-II) and this difference was statistically not significant whichare in accordance with the result of Gandhy and Damle [29], Marray and Shaw [30], Afshar et al. [31], Cornejo et al. [32], Masamurak etal. [33]. These studies investigated the relationship between alkalinephosphatase and inorganic phosphorous with decayed, filled surfaceand observed that the level of alkaline phosphatase and inorganic phosphorous in rampant caries children was higher than the caries freechildren.

The level of alkaline phosphatase for caries active (Group-II) washigher as compared to caries free (Group-I) in the present study.Kumar et al. [34], Pandey et al. [35], Bai et al. [36], Vijayaprasad et al.[37] and Mahjoub et al. [38] have also reported a positive correlationbetween salivary alkaline phosphatase activities with dental caries.

In the present study, mean of inorganic phosphorous and alkalinephosphatase in caries free group was higher than the other groups, butthis difference was not significant after statistical analysis.

V. CONCLUSION

Saliva's buffering capability, ability to wash the tooth surface,antibacterial activities, and to control demineralization, and perhapsother

mechanisms all contribute to its essential role in preventing caries.

The present study shows decreased levels of salivary immunoglobulinA and high concentration of salivary protein in children with increasedcaries experience which is indicative of the protective role of salivaryconstituents in caries free children.

REFERENCES

[1]. Clarkson BH (1999) Introduction to cariology. Dent Clin North Am 43: 569-578.

[2]. Gregory RL, Filler SJ (1987) Protective secretory immunoglobulin A antibodiesin humans following oral immunization with Streptococcus mutans. InfectImmun 55: 2409-2415.

[3]. Bolton RW, Hlava GL (1982) Evaluation of salivary IgA antibodies to cariogenicmicroorganisms in children. Correlation with dental caries activity. J Dent Res61: 1225-1228.

[4]. Loesche WJ (1986) Role of Streptococcus mutans in human dental decay.Microbiol Rev 50: 353-380.

[5]. Arnold RR, Mestecky J, McGhee JR (1976) Naturally occurring secretoryimmunoglobulin A antibodies to Streptococcus mutans in human colostrum andsaliva. Infect Immun 14: 355-362.

[6]. Edgar WM (1990) Saliva and dental health. Clinical implications of saliva:report of a consensus meeting. Br Dent J 169: 96-98.

[7]. Mandel ID (1989) Impact of saliva on dental caries. CompendSuppl S476-S481.

[8]. W.H.O. (1997) Oral health surveys: Basic methods (4thedn).

[9]. Mancini G, Carbonara AO, Heremans JF (1965) Immunochemical quantitationof antigens by single radial immunodiffusion. Immunochemistry 2: 235-254.

[10]. Lehner T, Clarry ED, Cardwell JE (1967) Immunoglobulins in saliva and serumin dental caries. Lancet 1: 1294-1296.

[11]. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurementwith the Folin phenol reagent. J BiolChem 193: 265-275.

[12]. Wootton ID (1974) Electrolytes. In: Microanalysis in medical biochemistry.(5thedn), Edinburgh, Churchill Livingstone.

[13]. Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorous.J BiolChem 66: 375.

[14]. Varley H (1996) Practical clinical biochemistry. (6thedn), CBE publishers.

[15]. Challacombe SJ (1976) Immunoglobulins in parotid saliva and serum in relationto dental caries in man. Caries Res 10: 165-177.

[16]. Edgar WM, Higham SM, Manning RH (1994) Saliva stimulation and cariesprevention. Adv Dent Res 8: 239-245.

[17]. Lehner T (1980) Future possibilities for the prevention of caries and periodontaldisease. Br Dent J 149: 318-325.

[18]. Rose PT, Gregory RL, Gfell LE, Hughes CV (1994) IgA antibodies toStreptococcus mutans in caries-resistant and -susceptible children. PediatrDent 16: 272-275.

[19]. Camling E, Gahnberg L, Krasse B (1987) The relationship between IgAantibodies to Streptococcus mutans antigens in human saliva and breast milkand the numbers of indigenous oral Streptococcus mutans. Arch Oral Biol 32:21-25.

[20]. Bhatia S, Chawla HS, Tewari A, Ganguly NK (1986) Naturally occurring s-IgAsaliva of adults and children--correlation with dental caries activity. J Indian SocPedodPrev Dent 4: 1-7.

[21]. Sanui T, Gregory RL (2009) Analysis of Streptococcus mutans biofilm proteinsrecognized by salivary immunoglobulin A. Oral Microbiol Immunol 24: 361-368.

[22]. Gregory RL, Kindle JC, Hobbs LC, Filler SJ, Malmstrom HS (1990) Functionof anti-Streptococcus mutans antibodies: inhibition of virulence factors andenzyme neutralization. Oral Microbiol Immunol 5: 181-188.

[23]. Cogulu D, Sabah E, Kutukculer N, Ozkinay F (2006) Evaluation of therelationship between caries indices and salivary secretory IgA, salivary pH,buffering capacity and flow rate in children with Down's syndrome. Arch OralBiol 51: 23-28.

[24]. Everhart DL, Grigsby WR, Carter WH Jr (1973) Human dental caries experiencerelated to age, sex, race, and certein salivary properties. J Dent Res 52: 242-247.

[25]. Bagherian A, Jafarzadeh A, Rezaeian M, Ahmadi S, Rezaity MT (2008)Comparison of the salivary immunoglobulin concentration levels betweenchildren with early childhood caries and caries-free children. Iran J Immunol5: 217-221.

[26]. Kirtaniya BC, Chawla HS, Tiwari A, Ganguly NK, Sachdev V (2010) Naturalprevalence of antibody titers to glucosyltransferase of Streptococcus

mutansin serum in high and low caries active children. J Indian SocPedodPrev Dent28: 91-94.

[27]. Tulunoglu O, Demirtas S, Tulunoglu I (2006) Total antioxidant levels of salivain children related to caries, age, and gender. Int J Paediatr Dent 16: 186-191.

[28]. Kargül B, Yarat A, Tanboğa I, Emekli N (1994) Salivary protein and someinorganic element levels in healthy children and their relationship to caries. JMarmara Univ Dent Fac 2: 434-440.

[29]. Gandhy M, Damle SG (2003) Relation of salivary inorganic phosphorus andalkaline phosphatase to the dental caries status in children. J Indian SocPedodPrev Dent 21: 135-138.

[30]. Shaw L, Murray JJ, Burchell CK, Best JS (1983) Calcium and phosphoruscontent of plaque and saliva in relation to dental caries. Caries Res 17: 543-548.

[31]. Afshar H, Seraj B, Shafizadeh N (2001) The relationship between rampantcaries and salivary situation of 4-5 year old children living in Tehran. J IslamicDent Assoc Iran 13: 18-35.

[32]. Cornejo LS, Brunotto M, Hilas E (2008) [Salivary factors associated to theprevalence and increase of dental caries in rural schoolchildren]. Rev SaudePublica 42: 19-25.

[33]. Masamura K, Inaba R, Iwata H (1995) [Salivary calcium and total protein inrelation to dental caries]. Nihon EiseigakuZasshi 50: 886-892.

[34]. Kumar J, Kumar SS, Pradhan RK (2011) Studies on excessive daytimesleepiness (EDS) and alkaline phosphatase activity in smoker and alcoholicperson. World Journal of Science and Technology 1: 33-38.

[35]. Pandey RK, Tripathi A, Chandra S, Pandey A (1990) Relation of salivaryphosphorus and alkaline phosphatase to the incidence of dental caries inchildren. J Pedod 14: 144-146.

[36]. Bai J, Zhou Q, Bao ZY, Li XX, Qin M (2007) [Comparison of salivary proteinsbetween children with early childhood caries and children without caries].Zhonghua Kou Qiang Yi XueZaZhi 42: 21-23.

[37]. Vijayaprasad KE, Ravichandra KS, Vasa AA, Suzan S (2010) Relation ofsalivary calcium, phosphorus and alkaline phosphatase with the incidence ofdental caries in children. J Indian SocPedodPrev Dent 28: 156-161.

[38]. Mahjoub S, Ghasempour M, Mohammadi A (2007) Salivary alkalinephosphatase activity and inorganic phosphorus concentration in children withdifferent dental caries. J Babol Uni Medical Sciences 9: 23-28.