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EVALUATION OF THE ASSOCIATION OF SURFACE HARDNESS OF ENAMEL WITH SALIVA COMPONENTS IN ADOLESCENCE WITH AND WITHOUT TOOTH CARIES

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ABSTRACT

Background and Aims: Tooth caries is a common disease of mankind in all ages and is considered as a multi factorial disease. One of the factors contributing to tooth caries is enamel surface hardness (ESH) and saliva composition. The objective of the current study was to evaluate the association of ESH with the saliva components in adolescence with and without caries.

Methods: ESH and saliva components (IgA, Fluoride, calcium and phosphate concentration) were evaluated in premolars of adolescence whom underwent premolar extraction for orthodontic reasons.

Result: ESH was 298.2 ± 23.11 and 312.47 ± 22.2 for the caries and caries-free groups respectively (p=0.05). IgA concentration was 16.40 ± 3.13 mg/dl and 10.43 ± 1.49 mg/dl for the

first and second groups respectively (p=0.001). ESH had a negative association with IgA concentration (p=0.008) and a positive association with Fluoride concentration (p=0.05). **Discussion and conclusion:** ESH was lower in the caries group as compared to the caries-free one. IgA concentration was higher in caries group. ESH was inversely correlated with IgA and positively correlated with Fluoride concentration. The results of the current study confirm the role of Fluoride concentration in saliva and the immune response in caries development in adolescents.

Keywords: Enamel surface hardness, tooth caries, Calcium, Phosphorous, Fluoride, IgA, Saliva

[I] INTRODUCTION

Tooth enamel is the coating layer of teeth protecting them from microorganisms present in the oral cavity (1). It is the hardest tissue present in human body, acellular and is impermeable so that fluids could penetrate the most superficial layers (2). Due to the lack of cells, no regeneration occurs following injury to the tooth and replacement of lost enamel does not occur in the oral cavity (3). Therefore, enamel is an extraordinary tissue due to the lack of potential to regenerate and its high level of hardness which protects teeth against injuries applied to the teeth (4). Saliva components play an important role in the balance of enamel composition and the progress of tooth caries (5). It is previously demonstrated that fluoride concentration plays an important role in either prevention or progression of tooth caries and the body immune response is an essential contributor to the progression of tooth caries (6).

Enamel surface hardness (ESH) is a very important factor in determination of the speed of caries progression(7) and it is the rationale of fluoride therapy and fissure sealant therapy with fluoride releasing agents (8). Previous studies have demonstrated the role of fluoride in halting the progress of tooth caries and imply the necessity for fluoride therapy for subjects at risk of caries (9). There are also reports on the effect of saliva mineral concentration on ESH. So that subjects with a low concentration of minerals are at a higher risk of developing tooth caries (10, 11).

According to the previous studies and the lack of a direct correlation among ESH and saliva mineral components, this case-control study was performed to evaluate the possible

association among ESH, saliva mineral concentration and IgA concentration..

[II] MATERIALS AND METHODS

This cross-sectional case-control study was performed on 39 subjects aging 12-18 years old. Subjects referring to Kerman orthodontists' offices that needed premolar extraction due to orthodontic reasons were enrolled in the study. They were included if they had no systemic disease, no prior fluoride therapy or specific drugs that might alter the composition of saliva. Informed consent was obtained from the parents to use the extracted teeth in the current study. The study was approved by Kerman university of medical sciences ethics committee.

Subjects were grouped into caries and non-caries groups based on their DMFT score; DMFT< 5 were classified as non-caries and DMFT> were considered as caries group.

Teeth were extracted and kept in normal saline. Saliva specimen were collected using the following method: subjects were instructed to hold their head forward and bent for two minutes and the secreted saliva was collected in a glass vial and then stored in -70˚C.

To evaluate the concentration of calcium, atomic absorbtion spectroscopy method was recruited (Varian, Australia)(12).

To measure the level of fluoride and phosphate ions, Ion-chromatography method was recruited. Briefly, 30µL of saliva specimen was filtered using 0.4µm filters and then analyzed using the Ion-chromatograph device (MEtvohm, Switzerland).

IgA was measured using ELISA method as described in other studies (Elyza Reader, Biotek, USA) (13).

To assess the surface hardness, Vickers hardness test was recruited. Briefly, teeth were sectioned longitudinally and then put in the device. Surface hardness was measure in Vickers score (100N force, 10 s duration)(14).

Data were analyzed using SPSS v.16 (IBM, USA). $P<0.05$ was considered statistically significant.

[III] RESULTS

The current study was performed on 39 subjects and a total of 40 teeth. Demographic characteristics of the subjects and the average DMFT score is presented in Table 1.

The average ESH was 312.47 ± 22.20 and 298.2 ± 23.11 for the caries-free and caries groups respectively ($p<0.05$). Fluoride concentration was 0.289 ± 0.063 and 0.254 ± 0.557 mg/dl for the first and second group respectively ($p>0.05$). The concentration of calcium was 40.98 ± 7.57 mg/l and 40.49 ± 8.05 mg/l for the two groups respectively.

Concentration of phosphate in the two groups was 423.57 ± 49.97 mg/l and 420.57 ± 64.4 mg/l ($p>0.05$ for both variables) . The IgA concentration for the first and second group was as follows: 10.43 ± 1.49 mg/dl and 16.4 ± 3.68 mg/dl ($p<0.05$) (Table 2).

EHS was inversely correlated with IgA concentration ($p=0.008$, $R= -0.415$). There was a positive correlation between EHS and Fluoride concentration ($p=0.05$, $R= 0.302$). There was no significant correlation among EHS and either calcium or phosphate concentration (Table 3).

[IV] DISCUSSION

Results of the current study revealed that there is a significant difference in enamel surface hardness (ESH) and fluoride and IgA concentration in teeth and saliva of subjects with or without tooth caries. There was an inverse correlation observed between ESH and saliva IgA concentration. A positive correlation was observed between ESH and saliva fluoride concentration.

Studies performed on the ESH reveal a wide range of values for this important property of enamel. Differences arise due to different case selection, different methodology to obtain ESH and evaluate that. In a previous study by Craig et al. (15), EHS was measured from 292 to 390. In another study performed by Gaspersic et al. (16), EHS at the occlusal surface was in 359.5 – 424.3 range. These differences are due to the different methodologies recruited in the studies and different teeth evaluated (8, 16). EHS has a wide range of variability among individuals and teeth in the same individual and it is important to evaluate it in one location of the teeth studied to obtain a homogenous value (17). EHS average was 305.15 in the current study. This value was obtained from the occlusal surface of upper premolars in different individuals and this point must be considered while interpreting the results.

Saliva has an important role in maintaining the chemical, microbiological and physical balance of the oral cavity (18, 19). Immunoglobulins present in the saliva such as IgA play an important role to protect the oral cavity against microbiological insults (20). IgA protects oral cavity through binding with the microorganisms and inhibition of their adherence to the mucosa (20, 21). Several studies have been performed to associate the level of IgA with caries rate (22-24), but different and sometimes controversial results have been obtained. This inconsistency might be due to different reasons. IgA level is influenced by several factors including the rate of saliva secretion, age, hormonal changes and genetics. The methodology used to obtain the results are

also diverse, which confounds the problem (25, 26). In the current study, ELISA method was recruited to evaluate the level of IgA. The level of IgA was lower in caries-free group as compared to the caries group. It might be implied that IgA has a protective role in the saliva, therefore, it is higher in the environment which is threatened by cariogenic microorganism. This finding is similar to the Poureslami et al. (2011) study. Their study was conducted on subjects younger than 3 years old, which is the major difference with the current study (27). In other studies by AlAmoudi et al. (2007) and defarias et al. (2003) (28, 29), the same findings were observed so that the level of IgA was higher in children with early childhood caries as compared to the subjects with no caries.

One of the other components present in the oral cavity which has an important role in remineralization process is Fluoride. Entrance of the Fluoride into the tooth surface is dependent upon several factors including the plaque age, carbohydrates types, pH, saliva buffering capacity (30, 31). The increased concentration of Fluoride in saliva leads to the increased distribution of fluoride into the dental plaque and therefore prevents from tooth caries (28). In the current study, concentration of Fluoride was higher in non-caries group saliva samples, but the difference with caries group was not statistically significant. Further studies are required to evaluate this issue in larger samples, though the results were consistent with Jawed et al. (2006) (32).

ESH was inversely correlated with IgA concentration. This finding might be justified through the fact that the immune system of subjects with lower caries has a decreased activation through bacterial process, which is not an odd finding. There was also a significant association between ESH and fluoride concentration. Since fluoride has an important role in the remineralization process (33) and it contributes to the structure of enamel, higher fluoride concentration in the saliva means a higher level of fluoride in the tooth structure and thus, increased enamel hardness.

[V] CONCLUSION

Results of the current study revealed that enamel surface hardness is higher in non-caries subjects and IgA concentration is higher in the saliva of subjects with tooth caries. An inverse correlation between ESH and IgA concentration was observed. There was also a positive correlation between ESH and fluoride concentration. Further studies are suggested to evaluate the possible effect

of fluoride therapy on caries incidence in a longidutenal study.

Conflict of interest:

The authors declare no conflict of interest. This study was funded as a thesis for granting pediatric dentistry degree by Kerman University of medical sciences.

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Table 1: demographic characteristics of the study subjects. DMFT= decay, missing, filling of tooth, S.D. = standard deviation

Study group	DMFT (mean ± S.D.)	Age (Mean ± S.D.)
Non-caries	2.34 ± 1.04	14.68 ± 2.02
Caries	5.65 ± 1.6	13.75 ± 1.74

Table 2: results of the Vickers hardness test (VHN) and Fluoride, IgA, Calcium and phosphate measured in saliva specimen of non-caries and caries groups. S.D. = standard deviation

Variable	Non-caries	Caries	p-value
VHN	312.47 ± 22.20	298.2 ± 23.11	0.05
Fluoride (mg/l)	0.289 ± 0.063	0.254 ± 0.557	0.32
IgA (mg/dl)	10.43 ± 1.49	16.4 ± 3.13	0.001
Calcium (mg/l)	40.58 ± 7.24	40.97 ± 8.05	0.87
Phosphate (mg/l)	423.57 ± 49.970	420.57 ± 64.4	0.80

Table 3: correlation among different variables studied in the current experiment. Pearson's correlation score and p-value is presented for each correlation.

	Correlation with the:	Pearson's correlation score	p-value
Enamel surface hardness	Fluoride (mg/ml)	0.302	0.05
	IgA (mg/dl)	-0.415	0.008
	Calcium (mg/l)	-0.07	0.98
	Phosphate (mg/l)	0.0041	0.98