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Comparing the Levels of Salivary CAVI in Children Receiving Fixed and Interceptive Orthodontic Treatment to Caries Matched Controls: An Exploratory Study

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Abstract

Aim: The aim of this study is to determine the influence of salivary CAVI on the dental caries status of children receiving fixed and interceptive orthodontic treatment and compare them to children who were not receiving orthodontic treatment.

Methods: The study participants comprised 60 patients aged 9–14 years allocated to one of three groups Control patients (with no fixed or interceptive treatment carried out), patients with interceptive orthodontic appliance and patients with fixed orthodontic appliance. All the participants were examined for DMFT/dmft, OHI and PH. Saliva was collected by sterile pasture pipette or by passive drool method. CAVI was estimated by using a commercially available Elisa kit.

Results: CAVI levels in the fixed orthodontic group exhibit the highest levels of the enzyme whereas in the control groups had the lowest levels. When the CAVI levels among groups were subjected to the Scheffe's post hoc test it was observed that while a significant difference existed between the CAVI levels and the other two groups ($p<0.05$) no significant differences were observed between the control group and the interceptive orthodontic group.

Conclusion: Children undergoing fixed orthodontic treatment have significantly higher CAVI expression than those undergoing interceptive orthodontic treatment or controls.

There is no significant difference in the CAVI levels of children undergoing interceptive orthodontic treatment and controls who were matched for oral hygiene and DMFT.

Keywords: Carbonic Anhydrase 6; Interceptive Orthodontic Treatment; Fixed orthodontic

Introduction

One of the most common dental disorders is malocclusion that may lead to increasing the risk of periodontal disease and dental caries orthodontic treatment often resolve malocclusion, or at least prevent further progression [1]. However, it may affect oral hygiene by influencing several factors including the saliva properties and microbial count [2]. These changes in saliva include decrease in pH, flow rate and buffering capacity. This may contribute to demineralization of enamel and increase the susceptibility to dental caries [3].

Another important factor in the dental caries dynamic process is the buffering capacity of saliva, which also plays an important role in maintaining the oral tissues homeostasis. Among its multiple functions, the clearance promoted by the salivary flow and the pH stability at acceptable levels stand out, mainly due to carbonate and phosphate buffers [4].

Among the defense systems of saliva, salivary carbonic anhydrase isoenzyme VI (CAVI) is the only known secreted isoenzyme of the carbonic anhydrase family, which has been detected in the saliva secreted by the serous acinar cells of mammalian parotid and submandibular glands. It catalyzes the reversible reaction of carbon dioxide in a reaction of CO_2+H_2O $H+HCO_3$. By catalyzing this reaction, CAVI is believed to provide a greater buffering capacity to saliva by penetrating dental biofilm and facilitating acid neutralization by salivary bicarbonate [5].

Given that orthodontic appliances create biofilm stagnation areas and complicate the oral hygiene, patients are susceptible to a higher biofilm accumulation, and mostly subjected to important biochemical and microbiological changes in saliva and biofilm. There is also debate in literature suggesting that the role played by fixed orthodontic appliances on the oral hygiene of children may differ from the role played by interceptive or removable appliances [3].

Thus, deliberating the behavior of CAVI in saliva of different types of orthodontic patient would be at great importance to investigate [6].

Methodology

The proposal was registered with the research center of Riyadh Elm University (FPGRP/43735003/228) and ethical approval was obtained from the Institutional Review Board (IRB) of Riyadh Elm University (RC/IRB/2018/1053).

A total of 60 patients, age 9 to 14 years, mixed dentition period, good general health attending the orthodontic clinics of Riyadh Elm University hospitals, were participated in the study and allocated to one of three groups; Group A, Group B, Group C. Represent respectively. Control patients (with no fixed or interceptive treatment carried out), patients with interceptive orthodontic appliance and patients with fixed orthodontic appliance.

Examination of oral health

Subjects were examined for caries using WHO (DMFT) Index Decay, Missing, and Filled Tooth. Oral hygiene level was assessed using the Oral Hygiene Index (Simplified) OHI(S) using a mirror and a WHO CPTIN type E probe.

OHI(S) was calculated as the mean score for the examined surfaces and was used as a measure for the average level of plaque accumulation. The salivary pH was calculated using test strips.

Saliva collection

Saliva of about 1 ml was collected using a sterile Pasteur pipette and transferred to a 1.5 ml Eppendorf tube from the participants. These sterile plastic collection tubes were transferred to the deep freezer of a – 600°C until they can be analyzed.

Assessment of CAVI activity

The assessment of CAVI activity was performed by the zymography method. It was performed on saliva since this isoenzyme can adhere to the acquired pellicle and promote the neutralization of excess acid by catalyzing the reaction of $H^+ + HCO_3^- \leftrightarrow CO_2 + H_2O$, which constitutes the most important buffer in the oral environment [7]. Salivary CAVI was measured using the commercially available CAVI ELISA kit.

This kit is based on sandwich enzyme-like immuno-sorbent assay technology. An antibody specific to CAVI is pre-coated onto a 96-well plate. The standards and samples are added to wells and incubated. Biotin conjugated anti-CAVI antibody. Next, Avidin conjugated to HRP is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain CAVI, biotin-conjugated antibody and enzyme-conjugated Avidin will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the CAVI amount bound on the plate. After that, CAVI O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of CAVI can be calculated.

Result

Descriptive statistics

The sample comprised of a total of 60 subjects (20 Fixed orthodontic, 20 interceptive orthodontic and 20 control) aged between 9 and 14 years. The mean age of the sample was 10.38 years (SD ± 2.1 years). There were no significant differences between genders (Table 1), Patients receiving fixed orthodontic treatment were significantly older than those receiving interceptive orthodontic treatment.

		Gender				Sig*
		Male age		Female age		
		Mean	Standard deviation	Mean	Standard deviation	
group	Controlab	10.56	1.72	10.62	1.5	0.346
	Interceptive Orthodonticsa	8.5	1.88	9.64	2.01	0.564
	Fixed orthodonticsb	11.32	1.2	12.31	2.1	0.455
*calculated using the independent t test						
a,b : Differences in superscript indicate significant difference in age (calculated using One-Way ANOVA and Scheffe's Post hoc test).						

Table 1: Age distribution of the sample.

		Mean	Std. deviation	Skewness		Kurtosis	
Group		Statistic	Statistic	Statistic	Std. error	Statistic	Std. error
Control	CAVI	26.245	5.17631	0.716	0.512	-0.065	0.992
Interceptive orthodontics	CAVI	17.675	1.61404	0.689	0.512	1.977	0.992
Fixed orthodontics	CAVI	13.005	2.46907	0.042	0.512	-0.993	0.992

Table 2: Descriptive statistics of the CAVI profile of the sample.

The descriptive statistics of the sample revealed no significant skew suggesting that the sample was not normally distributed and that parametric statistics should be used. The sample also revealed no significant Kurtosis (<3) suggesting the use of two-tailed tests (Table 2).

Oral hygiene of the different groups

		Mean	Std. deviation	F*	Sig
Saliva pH	Control	6.55	0.51042	0.427	0.657
	Interceptive orthodontics	6.7	0.80131		
	Fixed orthodontics	6.75	0.7864		
OHI	Control	2.54	0.63279	2.068	0.138
	Interceptive orthodontics	2.325	0.43392		
	Fixed orthodontics	2.66	0.50513		
* differences tested using the One-Way ANOVA					

Table 3: Comparison mean pH and OHI among groups.

The control group had the lowest salivary pH. While the fixed orthodontics group had the worst oral hygiene (Table 3). However, the One-Way ANOVA showed that the differences were not statistically significant.

Dental caries experience of the population

The dental caries in primary teeth was not recorded as the protocol of the university called for the extraction of all carious primary teeth prior to orthodontic treatment. When the Decayed (D), Missing (M) and Filled (F) permanent teeth were compared it was observed that there were no significant differences in the overall DMFT of any of the three groups (Table 4).

	Mean	Std. deviation	F*	Sig
Control	4.05	1.79991	0.673	0.123
Interceptive orthodontics	4.45	1.87715		
Fixed orthodontics	4.05	2.18789		
*differences tested using the One-Way ANOVA				

Table 4: Comparison of Mean DMFT across groups.

CAVI activity and orthodontic treatment

When the CAVI levels were compared in the saliva across groups it was observed that the control group had the lowest levels and the fixed orthodontics groups had the highest level. The differences among groups were statistically significant (Table 5).

	N	Mean	Std. deviation	F*	Sig
Fixed orthodontics	20	19.245	3.17631	46.221	<0.001**
Interceptive orthodontics	20	17.675	1.61404		
Control	20	13.005	2.46907		
* Calculated using the One-Way ANOVA					
** Differences significant at p<0.05					

Table 5: Comparison of CAVI levels among groups.

When the CAVI levels among groups were subjected to the Scheffe's post hoc test it was observed that while a significant difference existed between the CAVI levels and the other two groups ($p<0.05$) no significant differences were observed between the control group and the interceptive orthodontic group (Table 6).

Means for groups in homogeneous subsets are displayed.

When a regression model was formulated with CAVI levels as the dependent variable it was shown that the presence of orthodontic treatment and caries levels had a significant association with CAVI levels. The salivary pH and OHI scores had no significant association with the CAVI levels (Table 7).

CAVI			
Scheffea		Subset for alpha=0.05	
Group	N	1	2
Fixed orthodontics	20	19.245	
Interceptive orthodontics	20	17.675	
Control	20		13.005
Sig.		0.401	0.171
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size=20.000.			

Table 6: Post hoc comparisons among different groups.

Model	Unstandardized coefficients		Standardized coefficients beta		Sig.
	B	Std. error	Beta	t	
(Constant)	35.267	5.392		6.541	0
Orthodontic treatment	-6.815	0.557	-0.866	-12.244	.000*
DMFT	-0.397	0.196	-0.152	-2.028	.047*
Saliva pH	0.296	0.658	0.032	0.45	0.655
OHI	-0.553	0.87	-0.046	-0.635	0.528
*Association statistically significant					

Table 7: Regression model showing factors associated with CAVI levels. Dependent Variable: CAVI. Calculated using linear regression modelling.

Discussion

Dental caries is one of the most serious challenges of orthodontic treatment [8,9]. Dental caries is an irreversible microbial disease of the calcified tissues of teeth characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth which often leads to cavitation [10]. Caries is a complex and dynamic process where a multitude of factors influence and initiate the progression of the disease. It is well known that orthodontic treatment has the potential to cause damage to the hard and soft tissues. The presence of archwires complicates cleaning and makes access to plaque retaining areas difficult, especially when multiple loops, auxiliary archwires and different types of elastics are used [11]. The significantly higher expression of CAVI in the fixed orthodontic group confirms the hypothesis that the presence of arch wires is probably the most likely cause of dental caries in children undergoing orthodontic treatment.

Boersma et al. [3] showed positive correlation with caries prevalence was found for the bleeding and oral hygiene index scores of children undergoing orthodontic treatment (similar observations have been reported by various authors) [12,13]. The current study controlled for the overall oral hygiene among the different groups studied.

The presence of an increased inflammatory reaction in children undergoing orthodontic treatment has been previously documented [14]. The results of this study show that in fixed orthodontic treatment there is the greatest CAVI activity, a fact that is confirmatory of an increased response to inflammation in patients undergoing orthodontic treatment. Given that the difference was only significant in the fixed orthodontic group, it can be assumed that the continuous presence of wires in the patient's mouth presents a significantly higher inflammatory challenge, even when oral hygiene and dental caries are controlled for.

Salivary pH is an indicator of the buffering capacity of the saliva. Human saliva not only lubricates the oral tissues, making oral functions such as speaking, eating, and swallowing possible, but also protects teeth and oro-mucosal surfaces in different ways [15].

The result of our study showed that there was no significant difference in the pH among the different groups suggests the hypothesis that in cases of increased inflammatory challenges the salivary buffer systems are able to maintain the oral homeostasis [16].

Dental enamel is the hardest tissue in the human body, and the main challenge to it comes from acidic conditions in the oral cavity, which can cause dissolving of the mineral, i.e. dental caries or erosion [17-21]. However as shown by several studies, the buffering systems of the oral cavity are able to overcome the challenges of mild inflammation [22,23]. The buffering capacity (is the power to resist changes of pH when acid or alkali are added) of a complex solution like saliva will vary at different pH values because different systems of buffers are effective over different levels of the pH range [24].

The three major buffer systems responsible for buffer capacity of both unstimulated and stimulated saliva are the phosphate, the carbonic acid and the protien buffers [1,24]. Carbonic anhydrase is genetically expressed indicator for the functionality of the carbonic acid buffer.

Carbonic anhydrases are evolutionally old enzymes [25]. They are expressed in most tissues of the human body, participating in pH regulation, carbon dioxide and bicarbonate [26,27]. transport, as well as in the maintenance of water and electrolyte balance [28,29]. To date, seven isoenzymes have been identified in mammals, and all of them are expressed in the alimentary tract [30-34].

Of the isoenzymes expressed in the salivary glands, Cytoplasmic CAII is a high-activity isoenzyme which has been proposed to catalyze the production of salivary bicarbonate [35]. However, more recent research has shown that CAVI which is secreted into the saliva by the serous acinar cells of the parotid and submandibular glands is perhaps a better expressor of salivary buffering capacity [33,36,37]. The results of our study showed that the CAVI levels of children undergoing fixed orthodontics were elevated signficantly higher than those of the interceptive orthodontic and the control group. However there were no significant differences in pH. This, combined with the fact that the study design called for the matching of oral hygeine across groups lends credence to the argument that in cases where oral hygiene is maintained, the natural buffereing capacity of the saliva will help children compensate the inflammatory effects of arch wires [12,13].

The secretion of salivary CAVI is characterized [37,38] by a circadian periodicity, the concentration in saliva being very low during sleep and rapidly rising to the daytime levels after awakening [39-58]. This could help explain why fixed orthodontic patients, who had the presence of arch wires continuously, had significantly higher [59-94]. CAVI activity than those who had interceptive orthodontic appliances that were removed at night. This also explains the finding that there was no significant difference in the CAVI levels between control patients and those with interceptive orthodontic appliances [95-124].

Conclusion

Children undergoing fixed orthodontic treatment have significantly higher CAVI expression than those undergoing interceptive orthodontic treatment or controls.

There is no significant difference in the CAVI levels of children undergoing interceptive orthodontic treatment and controls who were matched for oral hygiene and DMFT.

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