

# Root resorption and anti-dentine antibody level in serum and saliva of well-controlled type I diabetic patients undergoing orthodontic treatment

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## ABSTRACT

**Background:** Diabetic mellitus type I is a metabolic disorder of diverse etiological factors, characterized by hyperglycemia resulting from an absolute deficiency of insulin affected childhood and adolescent. Some of these patients seek an orthodontic care. The orthodontist who is treating these medically compromised patients should have a working knowledge of the multitude of medically complex problems. This information will support and enable for delivery of high standards of dental care in general and orthodontic care in particular. The aim of this study was to analyze serum IgG levels and salivary secretory IgA (sIgA) levels in human dentine extract (HDE) before (T0) and 6 months after (T6) orthodontic treatment and to correlate anti-HDE autoantibodies to root resorption in well-controlled type I diabetic patients.

**Materials and methods:** Sixty individuals, who were attending to Al-Mustansiriya National Diabetes Center from April to October, 2012 and classified as well-controlled type I diabetic patients (HbA1c <8.5), were participating in this study. The mean age of the whole samples was (15±1 SD) years, thirty three of them (18 males and 15 females) were not wearing orthodontic appliance and were selected as the controls, while twenty seven of them (12 males and 15 females) were wearing orthodontic appliance. Periapical radiographs of the upper central incisors, unstimulated saliva and serum samples were obtained of all patients before (T0) and 6 months after (T6) orthodontic treatment. Anti-dentine antibody (Ab) levels were determined by mean of enzyme linked immune sorbant assay (ELISA) technique. At T6, root resorption was classified as grade 0 (no resorption), grade 1 (slight resorption), and grade 2 (moderate to severe resorption). Chi square test and T-test were used to assess the association between qualitative and quantitative results respectively, while paired t-test was used to analyze the results before (T0) and 6 months after (T6) orthodontic treatment. Differences were considered significant at P<0.05.

**Results:** There was statistical significant difference in the level of (anti-dentine Ab) in saliva between the two study groups at T0 and T6, its level was higher in the wearing group comparing with non wearing group, while it didn't differ in serum. In the wearing group, the level of anti-dentine antibody in serum and saliva significantly decreased at T6 comparing with its level at T0. High level of the (anti-dentin Ab) shown in serum and saliva in grade 1 root resorption (R1) comparing with grade 0 root resorption (R0) at T0 and also at T6.

**Conclusions:** The results of this study indicate that the anti-dentin antibody plays an important role in the detection of root resorption during orthodontic treatment in well-controlled diabetic patients type I and its level is different according to the grade of root resorption in both saliva and serum.

**Keywords:** Diabetes mellitus type I, Anti-dentine antibody, Root resorption, Orthodontic treatment. (J Bagh Coll Dentistry 2013; 25(3):134-141).

## INTRODUCTION

Insulin Dependent Diabetic Mellitus (IDDM) is the most common chronic metabolic autoimmune disease <sup>(1)</sup>. It is characterized by hyperglycemia due to the infiltration of lymphocytes in the pancreas causing destruction of insulin producing beta-cells <sup>(2,3)</sup>. The incidence of (IDDM) is reported to be increasing by 3-5% per year, and the number of people with diabetes is estimated to reach 380 million by 2025<sup>(4)</sup>. The major cause of (IDDM) is attributed to changes in their habitual lifestyle represents a major clinical and public health problem <sup>(5,6)</sup>. Some of these diabetic patients for a reason or another seek an orthodontic treatment which is not restricted only for healthy patients <sup>(7)</sup>.

The orthodontic treatment is a discipline in dentistry, like many other disciplines in this field; it can have adverse effects associated with the execution of treatment. These effects can be related to the patient or practitioner. Some of these effects are not fully understood, such as root resorption, and others are associated with orthodontic treatment without supporting evidence <sup>(8)</sup>. Consideration of risk factors prior to treatment is important, because dental resorptions constitute a challenge to dentistry due to their organic complexity. The concern and curiosity on this subject are not recent. The oldest report about resorptions of the dental structures was described by Michael Blum in 1530, probably the first book about the topic. However, the scientific study of root resorptions is considered recent, embracing nearly two decades <sup>(9)</sup>.

Numerous potential factors, related to both the individual patient and to treatment, have been suggested as risk factors for root resorption, but

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direct causal factors have not been identified<sup>(10)</sup>. The degree of resorption can be very variable, highlighting the importance of individual susceptibility over and above other risk factors<sup>(11)</sup>. It was hypothesized that susceptibility to root resorption may be associated with autoimmune responses against dentine matrix proteins, based on evidence that anti-dentine antibodies could be detected in experimental root lesions in mice and in traumatized patients with root resorption<sup>(12-14)</sup>. Autoimmune responses can influence the resorption of calcified tissues through interactions among immune and clast cells or through the production of cytokines and other mediators that modulate local inflammatory responses<sup>(15,16)</sup>. Orthodontic forces induce an inflammatory cell infiltration on periodontal tissues that produce signals and cytokines for differentiation and activation of clast cells<sup>(13,17-21)</sup>. The chronic inflammatory process may aid the presentation of autoantigens to the immune system and the breakdown of immunological tolerance<sup>(22,23)</sup>. Migration of immunocompetent cells to the periodontal ligament, such as lymphocytes, plasma cells, and antigen-presenting cells (macrophages and dendritic cells), has been reported during orthodontic movement<sup>(24,25)</sup>. In patients with pathological root resorption, the presence of antibodies against dentine antigens, increased serum IgG, and low levels of IgM suggests that an autoimmune reaction is present<sup>(14)</sup>.

Secretory IgA (sIgA) is the main line of defence of the oral cavity and upper respiratory tract surfaces and is secreted in large amounts into saliva by the salivary glands<sup>(22,23,26,27)</sup>. sIgA represents the local response of adaptive immune systems to environmental antigens found in the digestive and upper respiratory tract<sup>(26,28,29)</sup>. Alterations of the salivary levels of sIgA autoantibodies may represent a local imbalance of the immune response in the oral cavity<sup>(30)</sup>. Autoantibodies (sIgA) can be detected in saliva samples of patients with autoimmune diseases<sup>(31-34)</sup>. Currently, information is available concerning the presence of autoantibodies in the saliva of patients with orthodontic root resorption. Salivary antibodies may be a more suitable approach to study oral pathological disorders since they represent the local immune response, are a non-invasive method, and can be easily sampled<sup>(35)</sup>.

## MATERIALS AND METHODS

### The sample:

The sample of this study had been selected from patients who were attending to Al-

Mustansiriya National Diabetes Center from April, 2012 till October, 2012. Before starting the study an approval was obtained and the objective of the study was explained to the parents of each participant. The sample consist of sixty well-controlled type I diabetic patients with mean age (15±1 SD) years, of certain criteria, thirty three of them (18 males and 15 females) were not undergoing orthodontic treatment and were selected as the controls, while twenty seven of them (12 males and 15 females) who had class I malocclusion (crowding in the upper anterior teeth need to be treated without premolar extraction) were undergoing orthodontic treatment by using straight wire fixed orthodontic appliances with 0.018×0.025 inch bracket slots. The degree of upper central incisor root resorption, Anti-HDE IgG and Anti-HDE sIgA levels were analyzed in the saliva and serum of all patients before (T0) and 6 months after (T6) orthodontic treatment.

### The inclusion criteria:

Certain points were considered in the selection of the sample.

- 1- The participants were classified as well-controlled type I diabetic patients (HbA1c <8.5) with no history of other autoimmune diseases or chronic inflammatory diseases.
- 2- None of the patients or controls reported previous trauma of permanent dentition.
- 3- The participants didn't use steroidal and non-steroidal anti-inflammatory drugs for at least one month before sampling.
- 4- No history of previous orthodontic treatment.
- 5- The patients didn't show clinical or radiographic signs of periodontal diseases, periapical lesions, or root resorption before starting this study.
- 6- Patients with active caries or oral mucosa lesions were excluded.
- 7- Presence of only permanent dentition.

### Blood sample:

After an overnight fast, (5 ml) of blood samples were collected from well-controlled type I diabetic patients of both groups by venipuncture between 8.30 a.m. and 10.30 a.m., allowed to clot, then these blood samples centrifuged at 30 rpm for 10 minutes to obtain serum samples to assess FBS (fasting blood sugar level) and HbA1c (glycosylated hemoglobin) and then stored at -20°C until using for detection of Anti-HDE IgG level.

### Saliva sample:

Unstimulated whole saliva (2ml) were collected from patients of both groups by expectoration into sterilized vials after they rinsed

their mouth twice with water to avoid the effect of the circadian cycle of cortisol on secreted sIgA into saliva because the amount of secreted sIgA into saliva is decreased during early morning by cortisol variation<sup>(36)</sup>, samples were obtained between 10.00 a.m. and 4.00 p.m., then saliva sample were centrifuged at 12000 rpm for 10 minutes and the supernatants were stored at -20°C until use.

#### **Radiographs:**

Periapical radiographs were obtained for all patients at T0 and T6. The radiographs (70 kV, 10 mA, exposure time 0.7 seconds) of the upper central incisors were taken using the long cone paralleling technique. The most resorbed incisor was considered for analysis. The degree of root resorption was classified using the criteria described by Malmgren et al.<sup>(37)</sup>. Tooth length was measured from the incisal edge to the apex. Root and crown length was measured from the incisal edge to the apex using cemento-enamel junction as the limit. Image distortion was determined by comparing the image length to the real length of a radiopaque object placed on the film. Image distortion between T0 and T6 radiographs was determined by comparing crown length. The maximum acceptable distortion was 5%. Root resorption was graded from 0 to 2, where 0=no discernible root resorption; 1=slight root resorption (less than 2mm); 2 moderate to severe resorption (more than or equal to 2 mm).

#### **Laboratory investigation:**

Serum anti-dentin Ab was measured by using ELISA (Enzyme Linked Immune Sorbant Assay) technique by procedure of the test as follows:

##### 1-Antigen preparation:

The human dentin extract (HDE) which containing the organic materials of the dentin extract, was used as antigen. HDE was obtained through a modification of the technique described by Bradford<sup>(38)</sup>, using third molars donated by patients in which extractions were indicated. The dentin was drilled out using a high speed bit. The precipitate was placed in a demineralizing solution diluted 1:1 (guanidine-HCl 5 M, 10 percent enzyme-linked immunosorbant assay (EDTA), 5 μM phenylmetilsulfonylflouride, pH 5.0) for 14 days at 4°C and then centrifuged at 12000 rpm for 20 minutes (HERE Z 323K/Germany). The supernatant was dialyzed overnight against phosphate-buffered saline (PBS; pH 7.2) at 4°C. Protein concentration (ranging from 300 to 400 μg/ml) was determined. HDE was stored at -20°C until use. This procedure was done in Institute of Molecular biotechnology and Genetic

engineering for postgraduate study at Baghdad University.

##### 2- Determination of protein concentration:

Protein concentration was carried out using the method of Bradford(1976) and standard curve bovine serum albumin was done using different concentration, each one was pipetted in duplicated sterilized test tubes, then protein concentrations were measured using the method of Bradford(1976) and then read the optical density(O.D). The absorbency was plotted against the corresponding concentration of bovine serum albumin.

##### 3- ELISA for detection of serum anti-HDE IgG:

- a- Standards concentration was constituted to 100ng/ml. (5,10,15,20,25)ng/ml.
- b- HDE (100 μg/ml) in carbonate-bicarbonate buffer (Na<sub>2</sub>CO<sub>3</sub> 1.59g, NaHCO<sub>3</sub> 2.93g, distilled water qsp1000ml, pH 9.6) was used to coat 96-well immunoplates for 1 hour at 37°C and then stored overnight at 4°C.
- c- The plates were washed four times with PBS containing 0.05 percent Tween 20 (PBS-T) blocked with PBS-T-5 percent skimmed milk for 1 hour at room temperature.
- d- After washing, The serum sample (1/10 in PBS), were incubated at 37 °C for 1 hour, then washed four times, incubated with goat anti-human IgG labeled with peroxidase (Sigma-Aldrich, St Louis, USA) diluted 1:4000 at 37°C for 1 hour.
- e- The substrate solution (100 μl), after washing, was added (5 mg orthophenylenediamine, 10ml of 0.1M citrate buffer, pH 4.5, and 5 μl H<sub>2</sub>O<sub>2</sub>).
- f- The reaction was stopped after 15 minutes with 50 μl H<sub>2</sub>SO<sub>4</sub> 4N and the absorbance was read in ELISA reader at 492nm (Biotek, USA) at Immunology Unit/ Alkaramh Teaching Hospital.

##### 4- ELISA for detection of salivary anti-HDE IgA:

By using (ELISA Test) started by sensitizing and blocking the immunoplates as described before, followed by washing, then 100 μl from undiluted saliva samples were added and incubated at 37°C for 2 hours, then rewashed, and 100 μl of mouse monoclonal IgG to human secretory chain (Sigma-Aldrich, St Louis, USA) were added for each well and incubated at 37°C for 2 hour after preparation of the dilution 1/4000. After washing the plate wells, 100 μl from goat anti-mouse IgG labeled with peroxidase (Sigma-Aldrich, St Louis, USA) were added for each well and incubated for 1 hour at 37°C, then stopped the reaction and the absorbance was read.

### Statistical analysis

The data were entered and analyzed on SPSS version-20, and were summarized using frequency and proportions. Chi-square test, t-test and paired t-test were used for assessing significance of association. P-value of equal or less than 0.05 was used as the level of significance<sup>(39,40)</sup>.

## RESULTS

The results of this study show that there was no significant difference between the two study groups according to gender and age which were classified as: wearing orthodontic appliance group includes 44.4% male and 55.6% female and non-wearing orthodontic appliance (control) group which includes 54.5% male and 45.5% female as seen in Table 1.

The results also showed that 33.3% of wearing group develops grade R1 of root resorption with significant statistical differences by chi-square test (p value = 0.05). Table 2.

Regarding the level of anti-dentine Ab in serum, it was reported that there was no significant difference between wearing and non-wearing (control) groups at T0 and T6 seen in Table 3, while there was statistical significant difference in anti-dentine Ab level in saliva between the two study groups, where the mean level of antibody was higher in the wearing group comparing with non-wearing group at T0 and T6 (p value=0.059, 0.02 respectively) as seen in Table 4.

In the wearing orthodontic appliance group, the concentration of anti-HDE in serum and saliva showed statistical significant differences between T0 and after 6 months (T6) of wearing orthodontic appliance (p value=0.02) as shown in Table 5, where the level of anti-HDE IgG was at T6 (20.222) which is less than that at T0 (22.422) and the level of anti-HDE sIgA was at T6 (21.181) which also less than that at T0 (23.356).

It can be also seen high statistical significant differences in the concentration of anti-dentine Ab in serum between R0 and R1 at T0 and T6, in which the result revealed, increased the serum level of antibody in (R1) in wearing group as seen in Table 6.

The results also demonstrated high significant difference regarding the level of anti-dentine Ab in saliva in wearing group between (R0) and (R1) at zero time, and also at T6 (after 6 months of wearing the orthodontic appliance) which is also more in (R1) than in (R0) at two different times with (P value=0.002) seen in Table 7.

## DISCUSSION

External root resorption is a phenomenon that has been associated with orthodontic treatment. There are numerous potential factors, related to both the individual patient and to treatment, have been suggested as risk factors for root resorption; therefore, it is a multifactorial occurrence and can be detected early during orthodontic treatment, after 6 months of force application<sup>(41-45)</sup>. The susceptibility to root resorption may be associated with autoimmune responses against dentine matrix protein, based on evidence that anti-dentine antibodies could be detected in experimental root lesions in mice and in traumatized patients with root resorption<sup>(12-14)</sup>.

The presence of autoantibodies may not cause root resorption, where as autoimmune aggression occurs when the tissue antigen are accessible to specific receptors of the immune system and there are costimulatory stimuli<sup>(46)</sup>. During orthodontic treatment, the compression areas and hyaline necrosis in the periodontium may damage the cementum layer and expose the dentine matrix<sup>(13)</sup>. The resulting inflammation caused by damaged periodontal tissue can result in recruitment of antigen-presenting cells<sup>(47)</sup> and can also induce the expression of costimulatory molecules that favor lymphocyte activation<sup>(25)</sup> which are the primary cells of the immunologic system, and developed one of the most sophisticated defense mechanisms in the biological system<sup>(48)</sup>; therefore, the levels of anti-dentine antibody in saliva and serum can be detected and they were higher in patients who were wearing orthodontic appliances comparing with non-wearing group.

The anti-dentine antibodies can be also detected in the patients who were not undergoing orthodontic treatment because the organic matrix of dentine shares common components with bone matrix protein, especially type I collagen, as well as non-collagenous proteins and serum components<sup>(49)</sup>. There is an evidence demonstrated that some proteins considered exclusively expressed by odontoblasts, such as cleavage products of dentine sialophosphoprotein, are now known to be expressed in periodontium and bone<sup>(50,51)</sup>. The mean concentration of some of these dentine protein (dentine matrix protein - 1, dentine sialoprotein, and dentine phosphoprotein) is higher in the dentine matrix<sup>(50,52)</sup>. For this reason, the emergence of anti-dentine antibodies could be caused by several factors, such as periodontal or root damage or oral inflammatory processes, thus the level of anti-dentine antibody is high in saliva and serum of

well-controlled type I diabetic patients who were wearing orthodontic appliances and suffering from root resorption grade 1 (R1) at T6 comparing with patients who were also wearing orthodontic appliances but didn't suffer from root resorption (R0).

The resorption of mineralized tissues by clast cells is influenced by cytokines and co-stimulatory molecules produced by lymphocytes<sup>(15,16)</sup>. The present results suggest that a local and systemic immunomodulation of specific B lymphocytes to dentine may occur during orthodontic treatment. The modulation of T and B lymphocyte responses has been observed in other inflammatory diseases where clasts play a pivotal role, and this phenomenon induces bone destruction<sup>(15,16)</sup>.

Some inflammatory cytokines of the innate immune response, which induced by orthodontic force, may affect the production and delivery of sIgA on the mucosal surface such as Tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) and interleukine-1 (IL-1)<sup>(19,46,53,54)</sup>. Both can stimulate sIgA transposition throughout the epithelial barriers and stimulate clast differentiation and activation<sup>(15,55)</sup>, thus the level of anti-HDE sIgA was increased in the wearing group comparing with non-wearing at T6 (after 6 months orthodontic treatment). However, patients with significant degree of root resorption cannot maintain increased levels of sIgA during the application of orthodontic force suggesting that local anti-HDE antibody production was not exclusively supported by unspecific inflammatory responses<sup>54</sup>, since it excluded from the beginning such as periodontal diseases, caries and trauma; therefore, the level of anti-HDE sIgA was decreasing at T6 comparing with its level at T0 in the wearing group who suffering from root resorption grade 1 (R1). These findings agreed with Solange et al.<sup>(35)</sup> in healthy patients undergoing orthodontic treatment with root resorption grade 2 (R2).

The level of anti-HDE IgG was also found decrease at T6 comparing with the same wearing group at T0 who suffering from root resorption grade 1(R1), suggesting that the formation of immunocomplexes may be responsible for such a difference, and this is agreed with the experiments were done in mice by Wheeler and Stroup<sup>(12)</sup>, and in traumatized patients with root resorption by Hidalgo et al.<sup>(14)</sup>. These findings also agreed with Solange et al.<sup>(35)</sup> in healthy patients undergoing orthodontic treatment with root resorption grade 2 (R2).

The level of anti-HDE antibody in serum and saliva of well-controlled type I diabetic patients was higher in patients from the beginning of

orthodontic treatment who later showed root resorption after 6 months of treatment. These findings suggest that analysis of serum IgG anti-HDE and saliva sIgA anti-HDE before starting orthodontic treatment are preferable to give an idea about the susceptibility of these patients to develop root resorption after wearing orthodontic appliance in the future.

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**Table 1. Distribution of study groups according to gender and age**

Variables	Non-Wearing (Control)		Wearing (Study group)		Comparison
	No.	%	No.	%	P-value
Male	18	54.50%	12	44.40%	NS
Female	15	45.50%	15	55.60%	
Total	33	100%	27	100%	
Age (year)	15±1SD		15±1SD		NS

SD=standard deviation, NS=Non significant

**Table 2. Distribution of study groups according to root resorption.**

Groups	No. and %	Grades		Total	Comparison	
		R0	R1		Chi square	P-value
Wearing	No.	18	9	27	12.94	HS
	% within wearing/ Non-wearing	66.7	33.3	100		
	% of Total	30	15	45		
Non-Wearing	No.	33	0	33		
	% within wearing/ Non-wearing	100	0	100		
	% of Total	55	0	55		
Total	No.	51	9	60		
	% within wearing/ Non-wearing	85	15	100		
	% of Total	85	15	100		

**Table 3. Mean of anti-dentine antibody level of study groups in serum at two different time.**

Conc. of anti-HDE Ab	Wearing/ Not wearing	No.	Mean	S.D.	t-test	p- value
T0	wearing	27	22.42	8.25	1.7	0.09
	Non- wearing	33	19.23	5.69		
T6	Wearing	27	20.22	5.34	0.822	0.4
	Non- wearing	33	19.22	4.08		

**Table 4. Mean of antibody in saliva for wearing and non-wearing at two different time.**

Conc. of anti-HDE Ab	wearing/not wearing	No.	Mean	S.D.	t-test	p- value
T0	Wearing	27	23.35	7.91	1.929	0.059
	Not Wearing	33	19.82	6.27		
T6	Wearing	27	21.18	4.99	2.261	*0.02
	Not Wearing	33	18.50	3.96		

\*HS: Highly significant difference

**Table 5. Concentration of anti-dentine antibody in serum and saliva of wearing group at different times.**

anti-dentine antibody		No.	Mean	S.D.	t-test	p- value
Conc. in serum	T0	27	22.422	8.2509	2.420	* 0.02
	T6	27	20.222	5.3448		
Conc. in saliva	T0	27	23.356	7.9135	2.338	* 0.02
	T6	27	21.181	4.9973		

**Table 6. Mean of anti-dentine antibody level in serum of two grades at two different times in wearing group**

Conc. of anti-HDE in serum	Root resorption grade	No.	Mean	S.D.	t-test	p- value
<b>T0</b>	<b>R 0</b>	18	17.989	4.8475	-6.119	HS
	<b>R 1</b>	9	31.289	6.2170		
<b>T6</b>	<b>R 0</b>	18	18.378	4.1098	-2.866	0.008
	<b>R 1</b>	9	23.911	5.8298		

**Table 7. Mean of antibody level in saliva of two grades of root resorption at two different times in wearing group.**

Conc . of anti-HDE Ab level in saliva	Root resorption grade	No.	Mean	S.D.	t-test	p- value
<b>T0</b>	<b>R 0</b>	18	18.772	4.8149	-7.578	HS
	<b>R 1</b>	9	32.522	3.5309		
<b>T6</b>	<b>R 0</b>	18	19.222	3.7217	-3.424	0.002
	<b>R 1</b>	9	25.100	5.0811		