

The correlation between root resorption and some immune parameters in well- controlled type I diabetic patients during orthodontic treatment

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Key words.

Diabetes mellitus type I; Immune parameter; Root resorption ; Orthodontic treatment.

Abstract

Background:Type 1 diabetes mellitus is the result of a breakdown in immune regulation that leads to expansion of auto reactive CD₄and CD₈T- cells, autoantibody-producing B lymphocytes and activation of the innate immune system, so disturbances of immune system may be the cause and/or associated with diabetes mellitus. Some of these diabetic patients seek an orthodontic care. The orthodontist must remain vigilant as they may be the only health care professional seen by otherwise fit, young patients on a regular basis, and it is also important to implement risk control procedures during and after orthodontic treatment.

Aims: The aim of this study was to analyze serum levels of immunoglobulins (IgG, IgA , and IgM) ,and complement components (C3, and C4) after 6 months (T6) of orthodontic treatment in well-controlled type I diabetic patients wearing orthodontic appliance comparing with non-wearing group and to correlate these immune parameters with grade of root resorption which sometimes happen during orthodontic treatment .

Materials and methods: Sixty well- controlled type I diabetic patients (HbA_{1c} <8.5), were participating in this study. Thirty cases of them(16 male and 14 females) were wearing fixed orthodontic appliance, and the other thirty cases didn't wear orthodontic appliances and considered as the controls (12 male and 18 females).The mean age of study groups was(15± 1SD) years. Periapical radiographs of the upper central incisors were obtained of all patients before(T0) and 6 months after(T6) orthodontic treatment. At (T6), root resorption was classified as grade 0 (no resorption) ,grade 1 (slight resorption), and grade 2 (moderate to severe resorption). Serum level of IgG , IgA, IgM ,C3, and C4 were analyzed after 6 months orthodontic treatment were determined using single radial immunodiffusion method (SRID) to the two study groups. 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 after 6 months (T6) orthodontic treatment, differences were considered significant at P<0.05.

Results: There was statistical significant difference in the serum level of IgG , IgA, IgM ,C3, and C4 after 6 months orthodontic

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treatment between the two study groups, there were an increase in the serum level of (IgG, and IgA), and a significant decrease in the serum level of (IgM, C3, and C4) in the wearing group comparing with non-wearing group; moreover, a high mean of serum level of IgG and IgA in root resorption grade 1 (R1) than in grade 0 (R0), while low mean of serum level of IgM, C3, and C4 were reported in (R1) grade than (R0) grade.

Conclusions: There were significant differences in the mean of serum levels of IgG, IgA, IgM, C3, and C4 between wearing orthodontic appliance and non-wearing groups of well controlled type I diabetic patients and also between root resorption grade 1 comparing with root resorption grade 0 in the wearing group.

Introduction

Type 1 diabetes mellitus is an autoimmune disease that involves the progressive destruction of the insulin-producing beta cells in the islets of Langerhans. It is a complex process that results from the loss of tolerance to insulin and other beta-cell-specific antigens. Various genetic and environmental factors have been studied so far, but precise causation has yet to be established. Numerous studies in rodents and human subjects have been performed in order to elucidate the role of B and T cells, which determine the risk of development and progression of diabetes. These studies have demonstrated that while this disease is fundamentally a T-cell-mediated autoimmune response, the development of it results from complex interactions between the adaptive and innate immune systems, with numerous cell types thought to contribute to pathogenesis. Like any complex disease, the variation in severity and incidence of this disease can be attributed to a combination of genetic and environmental factors^{1,2}. Some of these diabetic patients seek an orthodontic treatment which is not restricted for healthy patients.³

Orthodontic treatment, like many other interventions, has inherent risks and complications; but the advantages it offers should outweigh any possible damage. One of these adverse effects is root resorption⁴, which is a common iatrogenic consequence in the field of orthodontics. With this in mind, orthodontists should take all known measures to reduce its occurrence^{5,6}.

The root resorption may be described as mild, moderate and severe. Usually it is mild and clinically insignificant; however it can occur in large amounts, i.e. loss of over one third of the root length, in some patients⁷. Close radiographic examination of orthodontically treated individuals show some loss of root length in nearly every patient^{8,9}. The incisors are the most susceptible while the molars seem to be the least affected¹⁰. These orthodontically-induced root resorption areas after 7 weeks of treatment, verified histologically, are not visible in periapical radiographs. Thus, using film-based radiography, the diagnosis is uncertain during the first months of treatment. After 5-6 months a reliable radiographic diagnosis of apical root resorption can be performed¹¹.

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^{12,13,14}.

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 stimuli¹⁵. During orthodontic treatment, the compression areas and hyaline necrosis in the periodontium may damage the cementum layer and expose the dentine matrix¹³. The resulting inflammation caused by damaged periodontal tissue can result in

recruitment of antigen –presenting cells¹⁶ and can also induce the expression of costimulatory molecules that favor lymphocyte activation ¹⁷which are the primary cells of the immunologic system, and developed one of the most sophisticated defense mechanisms in the biological system¹⁸; The resorption of mineralized tissues by clast cells is influenced by cytokines and co-stimulatory molecules produced by lymphocytes^{19,20}.The immune modulation of T and B lymphocyte responses to dentine has been observed in other inflammatory diseases where clasts play a pivotal role, and this phenomenon induces bone destruction^{19,20}.

T lymphocytes appear in the form of two subpopulations of opposite regulative function: T-helper and T-killer of cytotoxic cells. T-helper lymphocytes stimulate the transformation of B lymphocytes into plasma cells. The plasma cells are in turn responsible for the formation of antibody which is a type of glycoprotein molecule, also called immunoglobulin (Ig) that binds to antigen, often with a high degree of specificity and affinity. Every individual has millions of different antibodies (IgA, IgG, IgM, IgD, and IgE), each with a unique antigen-binding site. Secreted antibodies perform various effectors' functions, including neutralizing antigen, promoting leukocyte – dependent destruction of microbes ,and activating complement.²¹

The complement system , which activated by antigen- antibody complexes, is a complex series of some 30 proteins, two-thirds of which circulate in the plasma while the remainder are present on cell and tissue surfaces. Complement components are rapidly synthesized and released into the circulation following trauma or inflammation, so its functions includes control of inflammatory reactions and chemotaxis, clearance of immune complexes, cellular activation and antimicrobial defense. ^{21, 22}

The previous studies on immunoglobulin levels in diabetic patients have revealed higher levels of IgG, IgA ,and IgM compared with those obtained from healthy controls ^{23,24} or between diabetic patients of different glycemic controls²⁵,

assuming that possible immune-inflammatory abnormalities were the underlying cause for the elevated immunoglobulins in patients with type 1 diabetes mellitus and its complication, and that these abnormalities might have either a role in the genesis of type I diabetes and its complications or may themselves be a complication thereof.^{24,26} While in other studies, these immunoglobulins didn't differ between diabetic patients and healthy controls.^{27,28}

Other studies on other immune parameters were assessed in type I diabetic patients treated with insulin and the control healthy group. The complement C3 and C4, which are the major plasma proteins of the immune system complement pathways. These studies reported that synthesis of serum complement protein C3 increased in response to inflammation and metabolic disorders, diabetes C3 levels elevated in IDDM ^{29,30} . Zhain et al. ³¹ reported that serum C3 and C4 of T1DM patients is significantly lower than corresponding value of healthy subjects and the lowest value of C3 was observed in patients with HbA1c >11% . The C3 levels were tended to reduce after improving glycaemia . Insulin has potential anti-inflammatory effects ³² .Insulin closely related with C3 and plays a vital role in alteration of fasting glucose metabolism ³³. While in other studies plasma levels of C3 and C4 were nearly normal during insulin treatment in type 1 diabetes.^{27,28} ; thus it is of interest to see if there is any difference in the immunoglobulins levels and complement components (C3, C4) in type 1 diabetic patients who wearing orthodontic appliances comparing them with non- wearing group and what will happen if these patients suffer from root resorption after 6 months orthodontic treatment.

Material and Methods

Sixty well- controlled type I diabetic patients(HbA1c <8.5) , who were attending to Al-mustansiriya National Diabetes Center from April to October in 2012,were participating in this study. none of these participants reported history of other autoimmune diseases or chronic

inflammatory diseases, or the use of steroidal and non-steroidal anti-inflammatory drugs for at least one month before sampling. They didn't have history of previous orthodontic treatment or previous trauma of dentition, and they also didn't show clinical or radiographical signs of periapical diseases, periapical lesions, or root resorption before starting this study. Patients with active caries or oral mucosa lesions were excluded. Thirty cases of them (16 male and 14 females) were wearing fixed orthodontic appliance because these patients had class I malocclusion (crowding in the upper anterior teeth need to be treated without premolar extraction) by using straight wire fixed orthodontic appliances with 0.018×0.025 inch bracket slots, and the other thirty cases didn't wear orthodontic appliances and considered as the controls (12 male and 18 females). The mean age of study groups was (15±1 SD) year.

The degree of upper central incisor root resorption of all patients were recorded before (T0) and 6 months after (T6) orthodontic treatment through using periapical radiographs (70 kV, 10 mA, exposure time 0.7 seconds) with 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. (1982)³⁴. 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, and 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).

The level of IgG, IgA, IgM, C3, and C4 in serum were analyzed after 6 months orthodontic treatment. (5 ml) of blood samples were collected from well-

controlled type I diabetic patients of both groups by venipuncture after an overnight fast, 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.

The quantitative estimation of serum immunoglobulins (IgG, IgA, and IgM), and complement components (C3, C4) was done using single radial immunodiffusion (SRID) method as follows^{35,36}:-

1-Principle of the test:

The concentration of serum immunoglobulins (IgG, IgA, and IgM), and complement components (C3, C4) were measured by a single radial immunodiffusion method (SRID) in which equal volumes of reference sera and test samples were added to wells in a gelose containing monospecific anti-sera. The sample diffuses radially through this gel and the substance being assayed from precipitin ring with the monospecific anti-sera. Ring diameter were measured and a reference curve is constructed on a graph paper. Unknown concentration is determined from the reference standard curve. This work was done in Immunology Unit at Alkaramah teaching Hospital in October 2012.

2- The procedure:-

Before starting the procedure, the plates were opened and left for 5 minutes at room temperature for evaporation of any water (if present in the wells due to storage at 4°C). (5µl) of each serum sample was dispensed by Hamilton syringe into one well of each plate (containing 16 wells) for three classes of immunoglobulins and two types of complement components. The plates were left opened for (10-20) minutes then covered and left at (10° -20)°C for three to four days for precipitin ring to be formed. The diameter at each immune precipitating using formed around each well was measured in millimeter by immune viewer and the concentration of each class of immunoglobulins and complement level was calculated from standard curve. All

results were in mg/dl for serum concentration of immunoglobulins , and complement components.

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.^{37,38}

Results

The results of this study showed that there were no statistical significant differences between the two study groups according to age and gender as seen in Table-1.

The results also showed that there were significant statistical differences between the two study groups(wearing and non-wearing groups) in the mean serum level of study immune parameters (IgG ,IgA, IgM ,C3), there were an increase in the serum level of (IgG ,IgA) , and a decrease in the serum level of (IgM, C3,and C4)in the wearing group comparing with non-wearing group as shown in Table-2.

Regarding the statistical analysis of association between the two study groups and root resorption grades by using chi square ,it reported (36.7%) root resorption grade 1(R1) and (63.3%) no root resorption grade 0 (R0) in the wearing group, with no root resorption (100% R0) in the non-wearing group as seen in Table -3 .

The statistical analysis between the different studied parameters and two root resorption grades (R0 and R1) in wearing group which showed a high mean of serum level of IgG and IgA in R1 grade than R0 grade ,while low mean of serum level of IgM,C3,and C4 were reported in R1 grade than R0 grade as shown in Table -4 .

Discussion

Disturbances of immune system may be the cause and/or associated with diabetes mellitus. Type 1 Diabetes mellitus is the

result of a breakdown in immune regulation that leads to expansion of autoreactive CD₄ and CD₈T cells, autoantibody-producing B lymphocytes and activation of the innate immune system.^{39,40}

The innate and adaptive immune responses are components of an integrated system of host defense in which numerous cells and molecules function cooperatively .The mechanisms of innate immunity provide an effective initial defense against infections .However, many pathogenic microbes have evolved to resist innate immunity , and their elimination requires the more powerful mechanisms of adaptive immunity. The innate immune response to pathogen also stimulates adaptive immune responses and influences the nature of the adaptive responses which consist of lymphocytes and antibodies .The adaptive immune response is initiated by the recognition of foreign antigens by specific lymphocytes which producing antibodies in response to exposure to these foreign structures .^{21,22}

The antigen –antibody complexes activate the complement system , which is a system of serum and cell surface proteins ,that interact with one another and with other molecules of the immune system to generate important effectors of innate and adaptive immune responses .The complement system serves as an important mediator of the humoral responses by amplifying the response and converting it into an effective defense mechanism to destroy the invading pathogen , participating in the inflammatory responses ,opsonization of antigen, and clearance of immune complexes .^{21,22}

In the present study ,it can be seen that these type I diabetic patients who wearing orthodontic appliance have high serum levels of IgG,and IgA, and low serum levels of IgM,C3,and C4 comparing with non wearing group and also in root resorption grade 1 (R1) comparing with root resorption grade 0(R0) , suggesting that tooth movement may be considered an inflammatory process ,thus antibodies levels of (IgG and IgA)increased ,while the level of IgM decreased due to their short life because it is the first antibody appearing in response to initial exposure to

antigen then decreased after 5-6 days from exposure²¹. These findings agreed with exposure the patients for pathological root resorption, the presence of antibodies against dentine antigens, causing increased serum IgG, and low levels of IgM suggests that an autoimmune reaction may present and induce of primary immune response.¹⁴

The orthodontically induced inflammatory root resorption is a inflammatory process that is extremely complex and composed of various disparate components including forces, tooth roots, bone, cells, surrounding matrix, and certain known biologic messengers⁴¹. It is therefore not surprising to find that terms such as individual susceptibility^{42,43}, genetics⁴⁴, and systemic factors⁴⁵ are being discussed when damage is evident after otherwise successful orthodontic treatment.

The degree of resorption can be very variable, highlighting the importance of individual susceptibility over and above other risk factors⁴⁶. 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^{12,13,14}. Immune 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^{19,20}.

Orthodontic forces induce an inflammatory cell infiltration on periodontal tissues that produce signals and cytokines for differentiation and activation of clast cells^{47,48,49,13,50,51}.

The chronic inflammatory process may aid the presentation of autoantigens to the immune system and the breakdown of immunological tolerance^{52,53}. Leading to 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 and stimuli for all non specific immune response including immunoglobulins and complement^{54,17}.

Low serum levels of C3,C4 can be also found in high level in the wearing group comparing with non wearing group and in serum of patients with root resorption grade 1 (R1) comparing with root resorption grade 0(R0),suggesting that the complement concentration can be useful in diagnosis and monitoring progress of inflammation. When low concentrations are present indicate increased consumption or decreased synthesis of the complement components. The decrease of serum levels of C3,C4 indicated that innate and adaptive immunity are declined in type 1 diabetic patients^{55,56}. On the other hand, the decrease of serum C4 level pointed out the existence of immune complex diseases which clearly observed in type 1 diabetes mellitus⁵⁷

Conclusion

There were significant differences in the mean of serum levels of IgG, IgA, IgM, C3, and C4 between wearing orthodontic appliance and non- wearing groups of well controlled type I diabetic patients and also between root resorption grade 1 comparing with root resorption grade 0 in the wearing group.

Table- 1: Distribution of study groups according to gender

Study groups	gender				p-value
	Male		Female		
	No.	%	No.	%	
<i>Wearing orthodontic appliance</i>	16	53.3	14	46.7	NS
<i>wearing -Non orthodontic appliance</i>	12	40.0	18	60.0	NS
Total	28	46.7	32	53.3	NS

NS: Highly Significance differences

Table-2: Mean level of study parameters in both wearing and non-wearing orthodontic appliance groups

Study Variables	Ortho-status	N	Mean	Std. Deviation	T Test	P value
<i>IgG</i>	<i>Non-wearing</i>	30	1293.8333	92.88490	-9.080	*HS
	<i>Wearing</i>	30	1755.0200	262.23992		
<i>IgA</i>	<i>Non-wearing</i>	30	112.8067	17.39568	-13.050	*HS
	<i>Wearing</i>	30	258.6300	58.68152		
<i>IgM</i>	<i>Non-wearing</i>	30	155.8000	13.77011	3.278	*0.002
	<i>Wearing</i>	30	132.9100	35.68168		
<i>C3</i>	<i>Non-wearing</i>	30	114.0800	8.85587	1.452	*0.152
	<i>Wearing</i>	30	110.4900	10.24992		
<i>C4</i>	<i>Non-wearing</i>	30	32.2167	2.73547	3.100	*0.004
	<i>Wearing</i>	30	27.0567	8.69719		

HS: Highly Significance differences (*)

Table –3: Distribution of study groups according to root resorption grades.

Study groups		ROOT RESPRPTION		Total	X2	P-VALUE
		R 0	R 1			
<i>Non-Wearing orthodontic appliance group</i>	<i>No.</i>	30	0	30	13.46	*0.001
	<i>% within ORTHO</i>	100.0	0.0	100.0		
<i>Wearing orthodontic appliance group</i>	<i>No.</i>	19	11	30		
	<i>% within ORTHO</i>	63.3	36.7	100.0		
Total	<i>No.</i>	49	11	60		
	<i>% within ORTHO</i>	81.7	18.3	100.0		

Table -4: Mean of study variables according to the grade of root resorption in wearing orthodontic appliance group

<i>Variables</i>	Root resorption	No.	Mean	Std. Deviation	T TEST	P Value
<i>IgG</i>	R 0	19	1572.811	99.7344	13.260	HS
	R 1	11	2069.745	97.4164		
<i>IgA</i>	R 0	19	227.189	49.8228	-5.430	HS
	R 1	11	312.936	19.8943		
<i>IgM</i>	R 0	19	158.984	7.6381	24.138	HS
	R 1	11	87.873	8.0176		
<i>C3</i>	R 0	19	115.316	6.1894	4.285	HS
	R 1	11	102.155	10.7259		
<i>C4</i>	R 0	19	33.063	2.3538	12.709	HS
	R 1	11	16.682	4.7368		

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