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Anti-dentine antibodies with root resorption during orthodontic treatment

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SUMMARY The aim of this study was to analyse 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. Fifty orthodontic patients were selected, 19 males (15.6 ± 8.5 years) and 31 females (21.4 ± 11.2 years), 19 in the mixed dentition (10.3 ± 1.9 years) and 31 in the permanent dentition (24.6 ± 9.9 years). Fifty individuals not undergoing orthodontic treatment matched by gender and age were selected as the controls. Periapical radiographs of the upper central incisors and saliva sampling were obtained of all patients at T0 and T6. Serum samples were collected from the permanent dentition patients (n = 31). Antibody levels were determined by means of immunoenzyme assay. At T6, root resorption was classified as grade 0 (no resorption), grade 1 (slight resorption), and grade 2 (moderate to severe resorption). Differences between antibody levels at T0 and T6 and among different grades of resorption were determined by paired t- and Kruskal–Wallis tests, respectively. Spearman's rank correlation coefficient was applied to detect correlation between sIgA and IgG levels, and logistic regression to determine the association of root resorption grade and the studied variables. Differences were considered significant at $P < 0.05$.

Serum anti-HDE IgG levels decreased ($P < 0.01$) in grade 2 root resorption patients during treatment and was not correlated to salivary sIgA levels or other variables. Patients who had grade 2 root resorption at T6 showed higher levels of anti-HDE sIgA ($P < 0.001$). Anti-HDE sIgA levels at T0 and root shape were the main factors associated with the degree of root resorption. The results suggest that variations to systemic and local humoural immune response to dentine antigens may occur during orthodontic treatment. High levels of salivary sIgA before treatment were associated with more advanced lesions after 6 months of treatment.

Introduction

Root resorption is found in at least 90 per cent of orthodontic patients, although the majority of cases do not compromise dental functions. Nevertheless, 25–30 per cent of patients develop moderate lesions (greater than 2 mm) and 0.5–0.7 per cent have been considered advanced (Apajalahti and Peltola, 2007; Mohandesan *et al.*, 2007; Årtun *et al.*, 2009). The most affected teeth are the upper incisors (Mirabella and Årtun, 1995a; Levander and Malmgren, 2000). Risk factors related to orthodontic root resorption include patient age, root shape, malocclusion severity, orthodontic appliances, treatment duration, length of root displacement, magnitude and direction of force, tongue and lip habits, previous dental trauma or endodontic treatment, use of intermaxillary elastics, and genetic polymorphisms (Linge and Linge, 1991; Mirabella and Årtun, 1995a,b; Taner *et al.*, 1999; Sameshima and Sinclair, 2004; Segal *et al.*, 2004; Apajalahti and Peltola, 2007; de Freitas *et al.*, 2007; Mohandesan *et al.*, 2007; Årtun *et al.*, 2009). The most significant factors in multifactorial analysis are the treatment time to correct the malocclusion and root morphology (Sameshima and Sinclair, 2004; Segal *et al.*, 2004; Apajalahti and Peltola, 2007). The known risk factors explain only about 20–30 per cent of the expected variation in the degree of root resorption, suggesting that none of these risk factors are good predictors (Baumrind *et al.*, 1996; Apajalahti and Peltola, 2007). The weak association of treatment variables and patient characteristics with the degree of root resorption suggests that an individual factor may be determinant in the development of severe root resorption. Radiographic signals of accelerated root resorption can be detected early during orthodontic treatment, after 6 months of force application, and increases the risk of severe lesions during overall treatment (Levander *et al.*, 1998; Årtun *et al.*, 2009). Unfortunately, radiographs show root resorption only after it has destroyed large

amounts of cement and dentine and resulted in permanent loss of dental structure (Levander *et al.*, 1998).

Orthodontic forces induce an inflammatory cell infiltration on periodontal tissues that produce signals and cytokines for differentiation and activation of clast cells (Hellsing and Hammarström, 1996; Kurol and Owman-Moll, 1998; Lee *et al.*, 2004; Consolaro, 2005; Başaran *et al.*, 2006; George and Evans, 2009). The chronic inflammatory process may aid the presentation of autoantigens to the immune system and the breakdown of immunological tolerance (Goldsby *et al.*, 2002; Abbas *et al.*, 2005). 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 (Haug *et al.*, 2003; Alhashimi *et al.*, 2004). 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 (Hidalgo *et al.*, 2005).

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 (Goldsby *et al.*, 2002; Abbas *et al.*, 2005; Avery, 2005; Karolewska *et al.*, 2008). sIgA represents the local response of adaptative immune systems to environmental antigens found in the digestive and upper respiratory tract (Avery, 2005; Neville *et al.*, 2008; Nogueira *et al.*, 2008). Alterations of the salivary levels of sIgA autoantibodies may represent a local imbalance of the immune response in the oral cavity (Savage *et al.*, 2004). Autoantibodies (sIgA) can be detected in saliva samples of patients with digestive tract autoimmune diseases, such as Sjögren syndrome, cirrhosis, and coeliac disease (Reynoso-Paz *et al.*, 2000; Tanaka *et al.*, 2000; Berra *et al.*, 2002; Bonamico *et al.*, 2008). Currently, no 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.

The aim of present study was to investigate salivary sIgA and serum IgG in human dentine extract (HDE) before and 6 months after orthodontic treatment. The analysis of these antibodies may have a diagnostic value and may also help elucidate the immunological mechanisms involved in root resorption.

Subjects and methods

All procedures were performed after informed consent was given by the individuals or by a parent/legal guardian and were approved by the Research Ethics Committee for Human Experiments at Londrina State University. The study group comprised 50 orthodontic patients, mean age 19.2 ± 10.5 years, 31 (62 per cent) females (mean age 21.4 ± 11.2 years) and 19 (38 per cent) males (mean age 15.6 ± 8.5 years). Nineteen patients were in the mixed dentition (mean age 9.3 ± 1.0 years) and 31 in the permanent dentition (mean age 25.9 ± 9.1 years). The degree of upper central incisor resorption and sIgA levels were analysed in all patients before (T0) and 6 months after (T6) treatment with orthodontic appliances. For ethical reasons, IgG levels were only investigated in patients in the permanent dentition. Twenty-five patients had a Class I (50 per cent) and 25 a Class II malocclusion, treated with edgewise or straightwire fixed orthodontic appliances with 0.018 × 0.025 inch bracket slots. Patients who had premolar extractions before or during treatment were excluded.

The control group comprised 50 volunteers who had not undergone orthodontic treatment, 36 (72 per cent) females (mean age 26.4 ± 8.1 years) and 14 (28 per cent) males (mean age 17.6 ±10.0 years). Twenty-four (48 per cent) were in the mixed (mean age 9.4 ± 1.2 years) and 26 in the permanent (mean age 23.5 ± 6.4 years) dentition.

None of the patients or controls reported previous trauma of the primary or permanent dentition, autoimmune or chronic inflammatory disease, or the use of steroidal and non-steroidal anti-inflammatory drugs for at least 1 month before sampling. They did not show clinical or radiographic signs of periodontal disease, periapical lesions, or root resorption at T0. Patients with active caries or oral mucosa lesions were excluded.

Saliva and blood samples

Saliva and blood samples were collected at T0 and T6. Unstimulated whole saliva samples (2 ml) were collected by expectoration into sterilized vials after the subjects had rinsed their mouth twice with water. To avoid the effect of the circadian cycle in sIgA secretion into saliva, samples were obtained between 10:00 and 16:00. Saliva samples were centrifuged at 12 000 rpm for 10 minutes and then the supernatants were stored at −20°C until use. Blood samples (5 ml) were collected by venipuncture, allowed to clot, and then centrifuged at 1500 rpm for 4 minutes. The serum samples were stored at −20°C until use.

Radiographs

Periapical radiographs were obtained for all subjects 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. Three trained examiners blind to the investigation (two orthodontic specialists and one radiologist) evaluated each radiograph. Kappa values for intra-examiner variation ranged from 0.85 to 0.9. The most resorbed incisor was considered for analysis.

The degree of root resorption was classified using the criteria described by Malmgren *et al.* (1982). Tooth length was measured from the incisal edge to the apex. The measurements were made with a pachymeter (0.02 mm precision; Mitutoyo Sul Americana, São Paulo, Brazil) placed parallel to the pulp

canal. Root and crown length was measured from the incisal edge to the apex using the 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 per cent. Root resorption was graded from 0 to 2, where 0 = no discernable root resorption; 1 = slight root resorption (less than 2 mm); and 2 = moderate to severe resorption (more than or equal to 2 mm).

Root shape was classified using criteria described by Mirabella and Årtun (1995a). Root morphology was subjectively classified on periapical radiographs as normal or abnormal (pointed, deviated, blunt, eroded, or pipette/bottle shaped). Panoramic radiographs of the controls and patients at T0 were examined to screen for root resorption, periodontal disease, and periapical lesions.

Antigen preparation

HDE, a crude extract containing the organic material of the dentine matrix, was used as the antigen. HDE was obtained through a modification of the technique described by Wheeler and Stroup (1993) using third molars donated by patients in whom extractions were indicated. The dentine was drilled out using a high-speed bit. The precipitate was placed in a demineralizing solution diluted 1:1 (guanidine–HCl 5 M, 10 per cent enzyme-linked immunosorbant assay (EDTA), 5 µM phenylmetilsulfonylfluoride, pH 5.0) for 14 days at 4°C and then centrifuged at 12 000 rpm for 20 minutes. The supernatant was dialysed overnight against phosphate-buffered saline (PBS; pH 7.2) at 4°C. Protein concentration (ranging from 300 to 400 µg/ml) was determined using the Folin method (Lowry *et al.*, 1951). HDE was stored at −20°C until use.

ELISA for detection of serum anti-HDE IgG

HDE (100 µg protein/ml) in carbonate–bicarbonate buffer (Na_2CO_3 1.59 g, $NaHCO_3$ 2.93 g, distilled water qsp 1000 ml, pH 9.6) was used to coat 96-well immunoplates (Techno Plastic Products, Zurich, Switzerland) for 1 hour at 37°C and then stored overnight at 4°C. The plates were washed four times with PBS containing 0.05 per cent Tween 20 (PBS-T) blocked with PBS-T-5 per cent skimmed milk for 1 hour at room temperature. After washing, the serum samples (1/10 in PBS) were incubated at 37°C for 1 hour, washed four times, incubated with goat anti-human IgG labelled with peroxidase (A8775; Sigma–Aldrich, St Louis, USA) diluted 1:4000 at 37°C for 1 hour. After washing, 100 µl of substrate solution was added (5 mg ortho-phenylenediamine, 10 ml of 0.1 M citrate buffer, pH 4.5, and 5 µl H_2O_2). After 15 minutes, the reaction was stopped with 50 µl H_2SO_4 4 N and the absorbance was read in a Multiskan EX reader (Lab Systems, Helsinki, Finland) at 492 nm. Antibody levels were expressed as absorbance in optical density (OD) units.

ELISA for detection of salivary anti-HDE sIgA

Immunoplates were sensitized and blocked as described above. After washing, undiluted saliva samples were incubated at 37°C for 2 hours, rewashed, and incubated with mouse monoclonal IgG to human secretory chain (I6635; Sigma–Aldrich, St Louis, USA) diluted 1:4000 at 37°C for 2 hours. After washing, the plates were incubated with goat anti-mouse IgG labelled with peroxidase (A2554; Sigma–Aldrich, St Louis, USA) and diluted 1:4000 at 37°C for 1 hour. After the process, absorbance was read.

Statistical analysis

Bartlett's test was used to test normality, parametric tests (Student's *t*-, ANOVA/Tukey), and non-parametric tests (Mann–Whitney and Kruskal–Wallis/Dunn's tests) were applied to detect differences in ELISA absorbance (antibody levels expressed as OD). The paired *t*-test was utilized to determine differences in absorbance between T0 and T6. Spearman's rank correlation coefficient was used to detect correlations between IgG and sIgA levels. Logistic regression analysis was performed with the degree of root resorption as the outcome variable. *P* values less than 0.05 were considered to be statistically significant.

Results

Root resorption

The frequency of root resorption by degree at T6 and its association with other study variables are shown in Table 1. The degree of root resorption was associated with age and the presence of an abnormal root shape. The mixed dentition variable was not included in the regression model because it is related to age.

Anti-HDE IgG in serum

The IgG levels in the control group (0.224 ± 0.072 OD) did not differ from those of patients at T0 (0.209 ± 0.083 OD; $P > 0.05$, Student's *t*-test). The mean difference in patients' IgG levels from T0 to T6 was not significant, except for the grade 2 root resorption group (Figure 1). No association between the degree of root resorption and IgG levels at T0 and T6 in the permanent dentition patients was found (Table 2).

Anti-HDE sIgA in saliva

Anti-HDE sIgA levels did not differ between the patients (0.208 ± 0.144 OD) or controls (0.177 ± 0.110 OD; $P > 0.05$, Student's *t*-test) at T0. Patients' salivary sIgA levels at T0 (0.209 ± 0.146 OD) did not differ from those at T6 (0.196 ± 0.134 OD; $P > 0.05$, paired *t*-test). Pearson's correlation coefficient did not show a significant correlation between IgG and sIgA levels at T0 ($P = 0.59$; $r^2 = 0.01$) or T6 ($P = 0.09$; $r^2 = 0.10$).

Salivary sIgA levels from T0 to T6 showed different profiles when stratified by the degree of root resorption

Table 1 Frequency and logistic regression analysis of root resorption by degree of root resorption after 6 months of orthodontic treatment in relation to other study variables.

	Root resorption degree			Logistic regression*		
	Grade 0	Grade 1	Grade 2	Odds ratio	95% confidence interval	*P* value
Mean (±SD)						
Age (years)	15.1 ± 8.50	20.5 ± 11.0	24.5 ± 11.8	1.11	1.02–1.20	0.02
Initial root length (mm)	13.7 ± 2.00	14.3 ± 2.00	14.6 ± 1.70	1.20	0.78–1.84	0.39
Frequency (%)						
Dentition						
Mixed	08 (42.1)	08 (42.1)	03 (15.8)			
Permanent	10 (32.3)	16 (51.6)	05 (16.1)	N/A[2]	N/A[2]	N/A[2]
Gender						
Male	08 (42.1)	07 (36.8)	04 (21.1)			
Female	10 (32.3)	17 (54.8)	04 (12.9)	1.32	0.22–7.82	0.76
Ethnicity						
White	14 (33.3)	22 (52.4)	06 (14.3)			
Asian	02 (100)	—	—	—		
Black*	02 (33.3)	02 (33.3)	02 (33.3)	0.58	0.06–5.25	0.63
Root morphology						
Normal	16 (43.2)	15 (40.5)	06 (16.2)			
Atypical	02 (15.4)	09 (69.2)	02 (15.4)	0.11	0.02–0.79	0.02[1]
Pointed	02 (28.6)	04 (57.1)	01 (14.3)			
Deviated	—	02 (66.7)	01 (33.3)			
Blunt	—	01 (100)	—			
Eroded	—	01 (100)	—			
Pipette (bottle)	—	01 (100)	—			
Malocclusion						
Class I	08 (32)	14 (56)	03 (12)			
Class II	10 (40)	10 (40)	05 (20)	0.85	0.19–3.78	0.83
Respiratory allergy**						
No	11 (39.3)	11 (39.3)	06 (21.4)			
Yes	07 (31.8)	13 (59.1)	02 (09.1)	2.25	0.53–9.45	0.26
Total	18 (36.0)	24 (48.0)	08 (16.0)			

*Included two patients classified as mullato.
**Included 11 subjects of rhinitis, 1 with allergic sinusitis, and 10 with bronchitis. No patient was undergoing corticosteroid therapy.
[1]Fisher's exact test.
[2]Not included in the regression model because it is related to age.

(Figure 2). Patients who developed grade 2 root resorption at T6 presented increased sIgA levels at T0 (0.434 ± 0.203 OD) in comparison with grade 1 ($P < 0.001$; 0.188 ± 0.095 OD) and grade 0 ($P < 0.001$; 0.136 ± 0.051 OD) patients. The degree of root resorption at T6 was associated with sIgA levels at T0 and with root shape (Table 3).

As physiological root resorption in the primary dentition can be a source of antigen stimulation, salivary sIgA levels in the mixed and permanent dentition patients were analysed. No differences in T0 sIgA levels between the mixed (Student's *t*-test, $P > 0.05$, 0.198 ± 0.141 OD) and permanent (0.207 ± 0.148 OD) dentition patients were observed. At T6, differences in sIgA levels between the mixed ($P > 0.05$; 221 ± 0.117 OD) and permanent (0.189 ± 0.129 OD) dentition patients also did not show statistical significance.

Discussion

Root resorption is a multifactorial occurrence but the variation in the susceptibility could be caused by an individual's unknown predisposing factors (Linge and Linge, 1991; Sameshima and Sinclair, 2004; Apajalahti and Peltola, 2007). 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 (Wheeler and Stroup, 1993; Consolaro, 2005; Hidalgo *et al.*, 2005). 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 (Gillespie, 2007; Takayanagi, 2007).

The presence of a systemic humoural immune response (Th2 response) was first assessed through the detection of circulating anti-HDE IgG. No relationship between IgG levels and the severity of lesions was found. However, low levels of anti-HDE antibodies were observed in the majority of patients at T0. Unspecific binding of serum proteins or the presence of IgG in HDE antigen could not be attributed to the observed absorbance because some sera samples presented

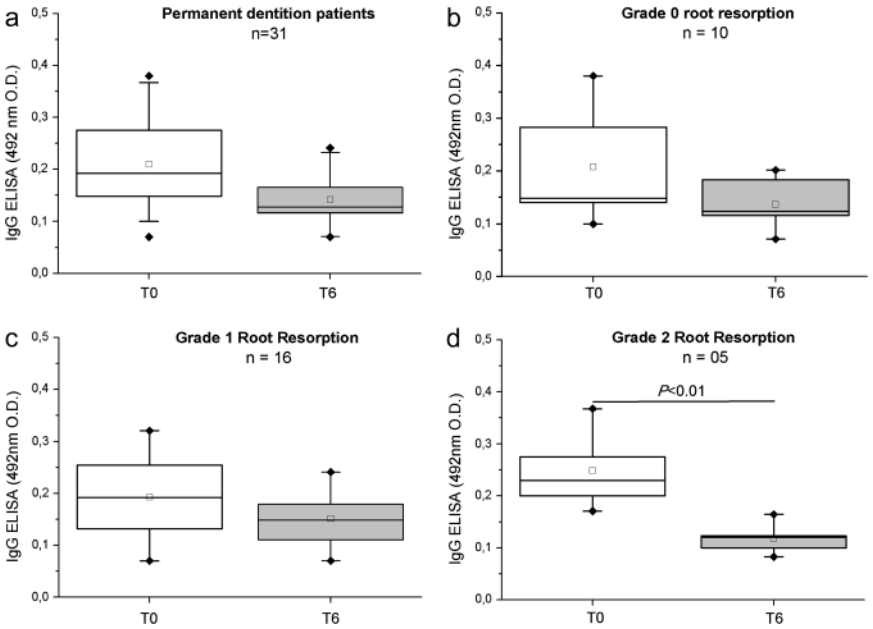

Figure 1 Analysis of serum IgG levels before (T0) and 6 months after (T6) orthodontic treatment. (a) All patients. (b) Patients without radiographic signs of root resorption (grade 0) at T6. (c) Patients with slight root resorption (grade 1) at T6. (d) Patients with moderate to severe root resorption (grade 2) at T6. Differences between the IgG levels at T0 and T6 in patients with different degrees of resorption were tested by paired *t*-test, $P < 0.05$ was considered significant. The box represents 25–75 per cent of the values of optical densities (OD) at 492 nm and the horizontal bar the median. Open square represents the mean OD, the vertical bars 1–99 per cent of the OD values, and closed diamond extreme values.

Table 2 Logistic regression analysis of root resorption degree to serum IgG levels and other study variables.

	Odds ratio	95% confidence interval	*P* value
IgG level before treatment	0.99	0.98–1.01	0.98
IgG levels after 6 months of treatment	0.99	0.96–1.02	0.58
Age	1.25	1.00–1.59	0.05
Gender (male/female)	0.65	0.20–2.13	0.48
Ethnicity (White/Asian/Black)	1.14	0.18–6.94	0.88
Root morphology (normal/abnormal)	0.24	0.01–12.69	0.48
Root length	2.35	0.73–7.52	0.15
Malocclusion (I/II)	0.46	0.02–10.87	0.63
Respiratory allergy (yes/no)	5.18	0.16–16.44	0.35
Constant	—	—	0.17

The presence of physiological root resorption was not analysed because sera were collected only from permanent dentition patients.

the same absorbance values as negative control wells (PBS control, 0.050 ± 0.018 OD) in ELISA plates. A significant decrease in IgG levels was found in patients with at least grade 2 resorption. In mice, IgG levels also decrease during inflammatory root resorption suggesting that the formation of immunecomplexes may be responsible for such a difference. In murine experiments, the presence of IgG antibodies may be a protective factor because dentine extract from immunized mice presented fewer root resorption craters than control animals (Wheeler and Stroup, 1993). The same association could not be demonstrated in the present study.

A significant association was found between T0 anti-HDE sIgA levels and the degree of root resorption. This suggests that a local immune response to dentine antigens is present and may play a role in root resorption. The expected variance in these findings in relation to known risk factors is in agreement with previous studies that included analysis of age, gender, root shape, root length, malocclusion Class and other individual and treatment variables (Linge and Linge, 1991; Mirabella and Årtun, 1995a,b; Baumrind *et al.*, 1996; Kurol and Owman-Moll, 1998; Taner *et al.*, 1999; Sameshima and Sinclair, 2004; Segal *et al.*, 2004; Årtun *et al.*,2005; Apajalahti and Peltola, 2007; de Freitas *et al.*, 2007; Mohandesan *et al.*, 2007). The inclusion of anti-HDE sIgA levels in the regression analysis suggests that these autoantibodies may represent a significant marker or a risk factor for root resorption. Detection of sIgA antibodies in saliva may help to identify susceptible patients before development of orthodontically induced root resorption. Saliva is easily sampled and non-invasive, making this approach more acceptable to patients. Saliva sampling must be carefully carried out in order to avoid interpretation errors. The amount of secreted sIgA into saliva is decreased during early morning by cortisol variation during the circadian circle (Hucklebridge *et al.*, 1998), method of sampling (Chang *et al.*, 2009), passive or active smoking (Barton *et al.*, 1990; Avşar *et al.*, 2009), response to stress (Phillips *et al.*, 2006; Moreira *et al.*, 2008), and after acute or intense exercise (Nieman *et al.*, 2006; Neville *et al.*, 2008). Inflammatory process, salivary sIgA secretion, and saliva

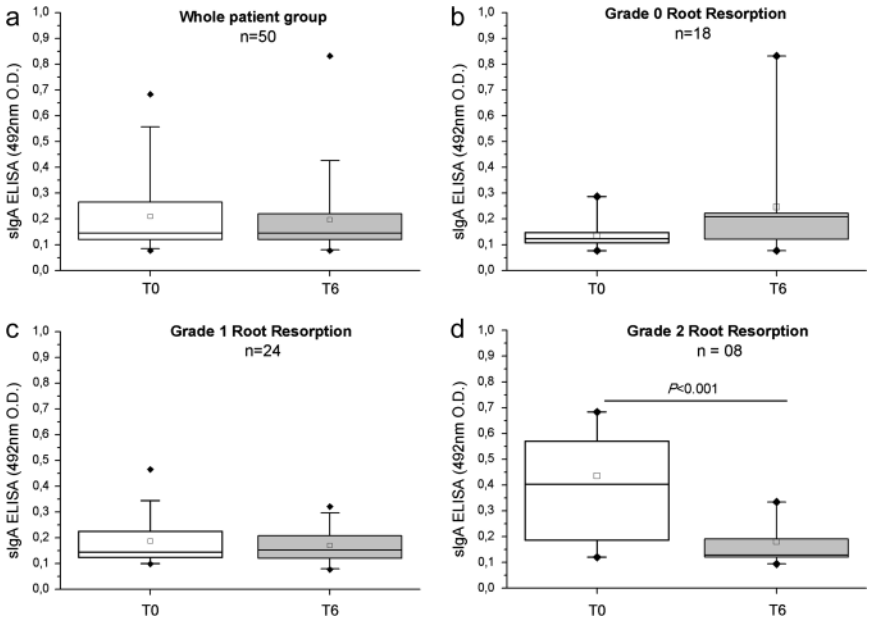

Figure 2 Analysis of salivary secretory IgA (sIgA) levels before (T0) and 6 months after (T6) orthodontic treatment. (a) All patients. (b) Patients without radiographic signs of root resorption (grade 0) at T6. (c) Patients with slight root resorption (grade 1) at T6. (d) Patients with moderate to severe root resorption (grade 2) at T6. Differences between the sIgA levels at T0 and T6 in patients with different degrees of resorption were tested by paired *t*-test, $P < 0.05$ was considered significant. The box represents 25–75 per cent of the values of optical densities (OD) at 492 nm and the horizontal bar represents the median. Open square represents the mean OD, the vertical bars 1–99 per cent of the OD values, and closed diamond extreme values.

Table 3 Logistic regression analysis of the degree of root resorption to salivary secretory IgA (sIgA) levels and other study variables.

	Odds ratio	95% confidence interval	*P* value
sIgA levels before treatment	1.05	1.01–1.20	0.01
sIgA levels at 6 months of treatment	0.99	0.98–1.01	0.07
Age	1.09	0.91–1.22	0.07
Gender (male/female)	1.67	0.17–15.9	0.65
Ethnicity (White/Asian/Black)	0.21	0.01–4.00	0.30
Root morphology (normal/abnormal)	0.05	0.01–1.59	0.02
Root length	1.42	0.86–2.35	0.16
Malocclusion (I/II)	0.38	0.05–2.17	0.24
Respiratory allergy (yes/no)	1.38	0.42–4.48	0.58
Constant	—	—	0.29

flow rate are under autonomous nervous system control and are factors that may alter sIgA concentration in saliva (Avery, 2005; Haensel *et al.*, 2008; Yoshino *et al.*, 2009). In the present study, patients with chronic inflammatory disease, oral lesions, under anti-inflammatory therapy, or taking drugs that affected autonomic regulation were excluded.

The presence of autoantibodies may not cause root resorption, whereas autoimmune aggression occurs when the tissue antigens are accessible to specific receptors of the immune system and there are costimulatory stimuli (Janeway *et al.*, 2007). Compression areas and hyaline necrosis in the periodontium may damage the cementum layer and expose the dentine matrix (Consolaro, 2005). The resulting inflammation caused by damaged periodontal tissue can result in the recruitment of antigen-presenting cells (Vandevska-Radunovic *et al.*, 1997) and can also induce the expression of costimulatory molecules that favour lymphocyte activation (Alhashimi *et al.*, 2004). Yet, how could the presence of these antibodies be explained before orthodontic treatment and in healthy individuals? Anti-HDE antibody levels in saliva and serum detected in both the patient and control groups at T0 should not be attributed to other local inflammatory conditions (periodontal disease, caries, and trauma) because they were excluded from the study during anamnesis and initial radiographic observations. Moreover, sIgA levels do not appear to be affected by a high response to dentine antigens during physiological resorption of primary teeth. The organic matrix of dentine shares common components with bone matrix proteins, especially type I collagen, as well as non-collagenous proteins and serum components (Qin *et al.*, 2001). Recent evidence has 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 (Qin *et al.*, 2002; Baba *et al.*, 2004). However, the mean concentration of some of these dentine proteins (dentine matrix protein-1, dentine sialoprotein, and dentine phosphoprotein) is higher in the dentine matrix (Qin *et al.*, 2002, 2003). 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. No correlation between salivary sIgA and serum IgG levels was found, suggesting a different behaviour of local and systemic immune response to dentine antigens. These results also suggest that an unusual local response against dentine antigens, highlighted by high anti-HDE sIgA levels, may be present in susceptible patients.

The resorption of mineralized tissues by clast cells is influenced by cytokines and co-stimulatory molecules produced by lymphocytes (Gillespie, 2007; Takayanagi, 2007). The effect of anti-dentine lymphocyte activation upon clast activity and root resorption is not known. The present results suggest that a local and systemic immunomodulation of specific B lymphocytes to dentine proteins 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 (Gillespie, 2007; Takayanagi, 2007). It is possible that suppression of the humoural and systemic response is caused by the breakdown of oral tolerance to dentine antigens or an immune deviation in susceptible patients. New studies characterizing T-cell subsets involved in this response are required to answer this question.

Some cytokines of the innate immune response may affect the production and delivery of sIgA on the mucosal surface. Tumour necrosis factor-alpha (TNFα) and interleukin-1 (IL-1) are inflammatory cytokines of the innate immune response induced by orthodontic force (Lee *et al.*, 2004; Jäger *et al.*, 2005; Bletsa *et al.*, 2006; Maeda *et al.*, 2007). Both can stimulate sIgA transportation throughout the epithelial barriers and stimulate clast differentiation and activation (Gillespie, 2007; Liu *et al.*, 2007). However, patients with significant degrees of 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 (Maeda *et al.*, 2007). In the present study, unspecific inflammatory conditions, such as respiratory allergy, were included in regression analysis but did not correlate with sIgA levels or the degree of root resorption of the examined teeth. Respiratory allergy included in the investigation because it is an unspecific source of local inflammatory mediators (Abbas *et al.*, 2005; Janeway *et al.*, 2007). Nishioka *et al.* (2006), demonstrated a link between the allergy process and root resorption.

Th2 responses favours the production of antibodies and can produce cytokines, such as IL-4, IL-5, IL-10, and TGF-β, favouring antibody production (Abbas *et al.*, 2005; Janeway *et al.*, 2007) and inhibiting clast activation (Gillespie, 2007). Salivary sIgA may be a hallmark of local autoimmunity to dentine and a bias to the local Th2 response could control local clast activation. However, a local inflammatory response could evoke an imbalance in this autoimmune response and may favour activation of clast cells.

Conclusions

The finding of the present research demonstrated increased sIgA levels in saliva at the beginning of therapy in patients who later showed moderate to severe resorption after 6 months of treatment. The presence of an abnormal root shape and initial levels of anti-HDE sIgA in saliva are associated with the degree of upper central incisor root resorption. The findings also suggest that analysis of serum IgG anti-HDE during orthodontic treatment does not correlate with lesion severity but may help to explain some of the immunopathological mechanisms of the process.

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References

Abbas A K, Lichtman A H, Pilai S 2005 Cellular and molecular immunology. Saunders, Philadelphia

Alhashimi N, Frithiof L, Brudvik P, Bakhiet M 2004 CD40-CD40L expression during orthodontic tooth movement in rats. Angle Orthodontist 74: 100–105

Apajalahti S, Peltola J S 2007 Apical root resorption after orthodontic treatment—a retrospective study. European Journal of Orthodontics 29: 408–412

Årtun J, Hullenaar R V, Doppel D, Kuijpers-Jagtman A M 2009 Identification of orthodontic patients at risk of severe apical root resorption. American Journal of Orthodontics and Dentofacial Orthopedics 135: 448–455

Årtun J, Smale I, Behbehani F, Doppel D, Van 't Hof M, Kuijpers-Jagtman A M 2005 Apical root resorption six and 12 months after initiation of fixed orthodontic appliance therapy. Angle Orthodontist 75: 919–926

Avery J K 2005 Oral development and histology. Santos, Brazil

Avşar A, Darka O, Bodrumlu E H, Bek Y 2009 Evaluation of the relationship between passive smoking and salivary electrolytes, protein, secretory IgA, sialic acid and amylase in young children. Archives of Oral Biology 54: 457–463

Baba O *et al.* 2004 Detection of dentin sialoprotein in rat periodontium. European Journal of Oral Sciences 112: 163–170

Barton J R, Riad M A, Gaze M N, Maran A G, Ferguson A 1990 Mucosal immunodeficiency in smokers, and in patients with epithelial head and neck tumours. Gut 31: 378–382

Başaran G, Ozer T, Kaya F A, Hamamci O 2006 Interleukins 2, 6, and 8 levels in human gingival sulcus during orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics 130: 7.e1–7.e6

Baumrind S, Korn E L, Boyd R L 1996 Apical root resorption in orthodontically treated adults. American Journal of Orthodontics and Dentofacial Orthopedics 110: 311–320

Berra A, Sterin-Borda L, Bacman S, Borda E 2002 Role of salivary IgA in the pathogenesis of Sjögren syndrome. Clinical Immunology 104: 49–57

Bletsa A, Berggreen E, Brudvik P 2006 Interleukin-1 alpha and tumor necrosis factor-alpha expression during the early phases of orthodontic tooth movement in rats. European Journal of Oral Sciences 114: 423–429

Bonamico M *et al.* 2008 Radioimmunological detection of anti-transglutaminase autoantibodies in human saliva: a useful test to monitor coeliac disease follow-up. Alimentary Pharmacology and Therapeutics 28: 364–370

Chang C K, Cohen M E, Bienek D R 2009 Efficiency of oral fluid collection devices in extracting antibodies. Oral Microbiology and Immunology 24: 231–235

Consolaro A 2005 Reabsorções dentárias. Dental Press, Maringá

de Freitas M R, Beltrão R T, Janson G, Henriques J F, Chiqueto K 2007 Evaluation of root resorption after open bite treatment with and without extractions. American Journal of Orthodontics and Dentofacial Orthopedics 132: 143.e15–143.e22

George A, Evans C A 2009 Detection of root resorption using dentin and bone markers. Orthodontics and Craniofacial Research 12: 229–235

Gillespie M T 2007 Impact of cytokines and T lymphocytes upon osteoclast differentiation and function. Arthritis Research and Therapy 9: 103

Goldsby R A, Kindt T J, Osborne B A 2002 Kuby imunologia. Revinter, São Paulo

Haensel A, Mills P J, Nelesen R A, Ziegler M G, Dimsdale J E 2008 The relationship between heart rate variability and inflammatory markers in cardiovascular diseases. Psychoneuroendocrinology 33: 1305–1312

Haug S R, Brudvik P, Fristad I, Heyeraas K J 2003 Sympathectomy causes increased root resorption after orthodontic tooth movement in rats: immunohistochemical study. Cell and Tissue Research 313: 167–175

Hellsing E, Hammarström L 1996 The hyaline zone and associated root surface changes in experimental orthodontics in rats: a light and scanning electron microscope study. European Journal of Orthodontics 18: 11–18

Hidalgo M M, Itano E N, Consolaro A 2005 Humoral immune response of patients with dental trauma and consequent replacement resorption. Dental Traumatology 21: 218–221

Hucklebridge F, Clow A, Evans P 1998 The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. International Journal Psychophysiology 31: 69–76

Jäger A *et al.* 2005 Soluble cytokine receptor treatment in experimental orthodontic tooth movement in the rat. European Journal of Orthodontics 27: 1–11

Janeway C A, Travers P, Walport M 2007 Imunobiologia. Artmed, Porto Alegre

Karolewska E, Konopka T, Pupek M, Chybicka A, Mendak M 2008 Antibacterial potential of saliva in children with leukemia. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 105: 739–744

Kurol J, Owman-Moll P 1998 Hyalinization and root resorption during early orthodontic tooth movement in adolescents. Angle Orthodontist 68: 161–165

Lee K J, Park Y C, Yu H S, Choi S H, Yoo Y J 2004 Effects of continuous and interrupted orthodontic force on interleukin-1beta and prostaglandin E2 production in gingival crevicular fluid. American Journal of Orthodontics and Dentofacial Orthopedics 125: 168–177

Levander E, Bajka R, Malmgren O 1998 Early radiographic diagnosis of apical root resorption during orthodontic treatment: a study of maxillary incisors. European Journal of Orthodontics 20: 57–63

Levander E, Malmgren O 2000 Long-term follow-up of maxillary incisors with severe apical root resorption. European Journal of Orthodontics 22: 85–92

Linge L, Linge B O 1991 Patient characteristics and treatment variables associated with apical root resorption during orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics 99: 35–43

Liu D Y, Wang X L, Liu P 2007 Tumor necrosis factor-alpha upregulates the expression of immunoglobulin secretory component. Journal of Investigational Allergology and Clinical Immunology 17: 101–106

Lowry O H, Rosebrough N J, Farr A L, Randall R J 1951 Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265–275

Maeda A *et al.* 2007 Force-induced IL-8 from periodontal ligament cells requires IL-1 beta. Journal of Dental Research 86: 629–634

Malmgren O, Goldson L, Hill C, Orwin A, Petrini L, Lundberg M 1982 Root resorption after orthodontic treatment of traumatized teeth. American Journal of Orthodontics 82: 487–491

Mirabella A D, Årtun J 1995a Prevalence and severity of apical root resorption of maxillary anterior teeth in adult orthodontic patients. European Journal of Orthodontics 17: 93–99

Mirabella A D, Årtun J 1995b Risk factors for apical root resorption of maxillary anterior teeth in adult orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopedics 108: 48–55

Mohandesan H, Ravanmehr H, Valaei N 2007 A radiographic analysis of external apical root resorption of maxillary incisors during active orthodontic treatment. European Journal of Orthodontics 29: 134–139

Moreira A *et al.* 2008 The impact of a 17-day training period for an international championship on mucosal immune parameters in top-level basketball players and staff members. European Journal of Oral Sciences 116: 431–437

Neville V, Gleeson M, Folland J P 2008 Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. Medicine and Science in Sports and Exercise 40: 1228–1236

Nieman D C, Henson D A, Dumke C L, Lind R H, Shooter L R, Gross S J 2006 Relationship between salivary IgA secretion and upper respiratory tract infection following a 160-km race. Journal of Sports Medicine and Physical Fitness 46: 158–162

Nishioka M, Loi H, Nakata S, Nakasima A, Counts A 2006 Root resorption and immune system factors in the Japanese. Angle Orthodontist 76: 103–108

Nogueira R D *et al.* 2008 Mutans streptococcal infection induces salivary antibody to virulence proteins and associated functional domains. Infection and Immunity 8: 3606–3613

Phillips A C *et al.* 2006 Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. Brain, Behavior and Immunity 20: 191–197

Qin C *et al.* 2001 A comparative study of sialic acid-rich proteins in rat bone and dentin. European Journal of Oral Sciences 109: 133–141

Qin C *et al.* 2002 The expression of dentin sialophosphoprotein gene in bone. Journal of Dental Research 81: 392–394

Qin C, Brunn J C, Cadena E, Ridall A, Butler W T 2003 Dentin sialoprotein in bone and dentin sialophosphoprotein gene expressed by osteoblasts. Connective Tissue Research 44: 179–183

Reynoso-Paz S *et al.* 2000 Evidence for a locally driven mucosal response and the presence of mitochondrial antigens in saliva in primary biliary cirrhosis. Hepatology 31: 24–29

Sameshima G T, Sinclair P M 2004 Characteristics of patients with severe root resorption. Orthodontics and Craniofacial Research 7: 108–114

Savage N W *et al.* 2004 Serum and salivary IgA antibody responses to *Saccharomyces cerevisiae*, *Candida albicans* and *Streptococcus mutans* in orofacial granulomatosis and Crohn's disease. Clinical and Experimental Immunology 135: 483–489

Segal G R, Schiffman P H, Tuncay O C 2004 Meta analysis of the treatment-related factors of external apical root resorption. Orthodontics and Craniofacial Research 7: 71–78

Takayanagi H 2007 Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nature Reviews. Immunology 7: 292–304

Tanaka A *et al.* 2000 Mucosal immunity and primary biliary cirrhosis: presence of antimitochondrial antibodies in urine. Hepatology 32: 910–915

Taner T, Ciğer S, Sençift Y 1999 Evaluation of apical root resorption following extraction therapy in subjects with Class I and Class II malocclusions. European Journal of Orthodontics 21: 491–496

Vandevska-Radunovic V, Kvinnsland I H, Kvinnsland S, Jonsson R 1997 Immunocompetent cells in rat periodontal ligament and their recruitment incident to experimental orthodontic tooth movement. European Journal of Oral Sciences 105: 36–44

Wheeler T T, Stroup S E 1993 Traumatic root resorption in dentine immunized mice. American Journal of Orthodontics and Dentofacial Orthopedics 104: 352–357

Yoshino Y, Yamane A, Suzuki M, Nakagawa Y 2009 Availability of saliva for the assessment of alterations in the autonomic nervous system caused by physical exercise training. Archives of Oral Biology 54: 977–985