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Dentine sialoprotein expression in gingival crevicular fluid during trauma-induced root resorption

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Abstract

Kumar V, Logani A, Shah N. Dentine sialoprotein expression in gingival crevicular fluid during trauma-induced root resorption. *International Endodontic Journal*

Aim To detect and quantify dentine sialoprotein (DSP) in the gingival crevicular fluid (GCF) of luxated teeth.

Methodology Eighteen subjects were enroled and distributed as follows. Group I ($n = 6$, positive control): subjects with primary second molar teeth undergoing physiological root resorption. Group II ($n = 6$, negative control): subjects with permanent mature maxillary central incisors. Subjects with a recent history (<1 week) of luxation injury were included in group III ($n = 6$, test group) and standardized digital radiographs with a superimposed mesh gauge were exposed at various time intervals. Percentage of radiographic root resorption (%RRR) was calculated. GCF was collected using microcapillary pipettes. DSP in the GCF was quantified using enzyme-linked immunosorbant assay. Group III was subjected to Spearman's rank test to establish the correlation between the concentration of DSP and %RRR at 6 weeks, 3 and 6 months.

Results Quantifiable amounts of DSP were released in the GCF of subjects in Group I and III. However, the protein was not detected in Group II. Detectable quantities of DSP were observed in the GCF of luxated teeth before any radiographic evidence of root resorption (base line radiograph). A positive correlation was established at 6 weeks ($r = 0.795$), 3 ($r = 0.755$) and 6 month ($r = 0.837$) between the release of DSP and %RRR ($P < 0.05$).

Conclusion Dentine sialoprotein was released in the GCF of luxated teeth and its concentration correlated with the active and remission phases of this pathological process. Further investigation is required to establish a potentially noninvasive aid for diagnosing and monitoring root resorption.

Keywords: dentine sialoprotein, gingival crevicular fluid, luxation injury, root resorption.

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Introduction

Luxation injuries account for 15–61% of all traumatic dental injuries (TDI) to the permanent teeth (Addo *et al.* 2007). Depending on the force and direction of impact, injuries may occur ranging from concussion to intrusive luxation. These result in damage to the attachment apparatus and subsequent root resorption, the severity of which is dependent on the type of luxation injury sustained. This can manifest as 'self-limiting' transient surface or even catastrophic replacement root resorption (Andreasen & Bakland 2008). Its accurate diagnosis and appropriate early treatment is essential for a successful long-term outcome.

Root resorption is routinely diagnosed on the basis of radiographic evidence. However 60–70% of

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mineralized tissue loss is required for the lesion to be discernible on radiographs (Andreasen *et al.* 1987). Moreover, radiographs provide a two-dimensional static representation of a three-dimensional dynamic resorptive process and are subjected to a high degree of inter and intraobserver variability (Ono *et al.* 2011). Computerized tomography and cone beam computed tomography have a high sensitivity and specificity for diagnosing resorptive defects, but their routine use in endodontic practice remains limited because of high cost and radiation concerns (Nakata *et al.* 2009, Lermen *et al.* 2010). Given the limitations of the currently available imaging techniques, a safer and more reliable alternative method to clinically diagnose root resorption is desirable.

Dentine is the principal mineralized tissue of the tooth. It is composed of 70% mineral, 20% organic matter and 10% water as a percentage of wet weight (Mah & Prasad 2004). The organic matrix consists of type I (86%), III, V collagen, type I trimer and several noncollagenous proteins (NCP) secreted by the odontoblasts. Dentine sialoprotein (DSP) is the second most abundant NCP after dentine phosphoprotein (DPP), accounting for 5–8% of the dentine extracellular matrix (DECM) proteins (Butler 1992). This highly tooth-specific protein is released in gingival crevicular fluid (GCF) during orthodontically induced root resorption and has been used as a biomarker for its diagnosis and monitoring (Balducci *et al.* 2007). Root resorption is also a sequel to luxation injuries and hence DSP can be expected to be released in the GCF of such traumatized teeth. This study aimed to detect and quantify DSP in the GCF of luxated teeth for its potential use as a diagnostic and prognostic biomarker of trauma-induced root resorption. The null hypothesis was that DSP would not be released in the GCF during root resorption of luxated teeth.

Materials and methods

Ethical clearance from the Institute Research Ethics Committee was obtained. Eighteen subjects were enroled and distributed. Group I (positive control, $n = 6$) comprised of subjects between the age group of 9–12 years who had their primary second molars undergoing physiological root resorption as confirmed by intraoral periapical radiographs. They were further subdivided into the apical (R_a, $n = 3$) and the coronal group (R_c, $n = 3$) based on the extent of resorption, that is, from the apex to half of the root length and from the mid-root to the cementoenamel junction (CEJ), respectively. Group II (negative control, $n = 6$) consisted of subjects between the age group of 18–25 years with permanent healthy dentition without any prior history of orthodontic treatment, trauma or radiographic evidence of root resorption. Six subjects (twelve teeth, three males, three females, age group of 14–25 years) with a recent history (<1 week duration) of luxation injury were included in group III (test group, $n = 6$). The diagnosis of the type of luxation injury sustained to teeth, concussion (2), subluxation (7), extrusion (2) and intrusion (1) was made on the basis of clinical/radiographic presentation, and appropriate treatment was provided as per the guidelines of International Association of Dental Traumatology (IADT-2007, www.iadt-dentaltrauma.org). Accordingly, endodontic therapy was immediately initiated for the teeth with intrusive luxation and at 6-week follow-up for four subluxated and two extruded teeth where the pulp became necrotic. Three teeth with subluxation and two with concussion injury maintained pulp vitality, and only symptomatic treatment was rendered.

Percentage of radiographic root resorption

A mesh gauge (Dentech Corporation, Tokyo, Japan) with squares of 1 mm^2 was adapted over a size-2 CMOS sensor (KODAK RVG digital radiography system; Eastman Kodak Company, Marne-la-Vallée, France). This was held on a RINN sensor-positioning device (RINN XCP-ORA; Dentsply Int., Elgin, IL, USA). Images were exposed with an X-ray unit (ENDO-ACP; Villa system medical, Milan, Italy) set at 70 kV and 7 mA using a long-cone paralleling technique. These images were taken at day one for Group I and II and at 2 and 6 weeks, 3- and 6-month time intervals for Group III.

All images were stored in a JPEG format and evaluated by two endodontists at 100% magnification using the Microsoft office manager (version-11.5510.5606; Microsoft, Redmond, WA, USA). Two points, one representing the root end and the other the CEJ were identified on the images. Total root surface area was calculated by counting the number of squares covering it. A full, 3/4th, ½th and 1/4th square coverage was given a score of 1, 0.75, 0.5 and 0.25, respectively. Total scores for each root were added, and percentage of RRR was calculated as follows:

$$\%\mathrm{RRR} = \frac{\text{Total scores for resorptive area}}{\text{Total scores for the root surface (Apical to CEJ)}} \times 100.$$

Dentine sialoprotein estimation

Collection of GCF

Thorough oral prophylaxis and strict oral hygiene instructions were given to all the subjects. Two millilitres of 0.2% Chlorhexidine gluconate (Clohex Plus, Dr. Reddy's, Hyderabad, India, twice a day for 2 weeks) was prescribed to ensure optimal plaque control. Prior to sample collection, the subjects were instructed to vigorously rinse with a glass of water. A cheek retractor was placed and the identified/traumatized tooth was isolated with cotton rolls. A 1–5 μL calibrated volumetric microcapillary pipette (Sigma Aldrich Chemicals Company Limited, Bangalore, India) was placed extracrevicularly. One microlitre of GCF was collected from the mesial and distal aspect of the primary second molar, maxillary central incisor and the luxated tooth. For groups I and II (positive and negative control) the samples were collected once only. For group III (test), GCF collection was performed at 2 and 6 weeks, 3 and 6 months. The samples were stored at −22 °C (Deep freezer Model no. H4-220; Blue star, New Delhi, India).

Enzyme-linked immunosorbant assay for DSP

The Enzyme-linked immunosorbant assay (ELISA) kit for DSP was obtained from Bluegene (Catalogue no. E01D0269; Wuhan, China). It consisted of 96 wells pre-coated with polyclonal antibody specific to human DSP. The procedure was performed following the manufacturer's instructions. All the samples were diluted with 100 μL of phosphate-buffered solution. The samples and standards were assayed in duplicates. The optical density (OD) of each well was noted with a spectrophotometer (FLUO star Omega; Bmg Labtech, Cary, NC, USA) set at 450 nm. The DSP concentration of test samples was calculated.

$$\text{DSP Concentration}\,(\text{ng mL}^{-1}) = \frac{\text{OD of test}}{\text{OD of standard}} \times \text{Concentration of standard} \times \text{Dilution factor.}$$

The three groups were subjected to statistical analysis using Student *t*-test. A P value of <0.05 was set for the difference to be accepted as statistically significant.

Results

Quantifiable amounts of DSP were released in the GCF of subjects in Group I (positive control). Higher mean concentrations of this protein were observed in the R_c subgroup (coronal resorption) in comparison with the R_a subgroup (apical resorption) and this was significant ($P = 0.02$). However, this protein was not detected in the GCF of subjects in Group II (negative control). For group III (test group), DSP was released in quantifiable amounts in the GCF at the 2-week interval and were higher in comparison with the R_c subgroup, but was not statistically significant ($P = 0.11$, Fig. 1). For this group, there were detectable quantities of this protein in the GCF in the absence of any radiographic evidence of resorption at 2 weeks.

A positive correlation was established at all subsequent follow-ups between the DSP concentration and %RRR using the Spearman's rank correlation test. (Table 1, Figs 2, 3 and 4).

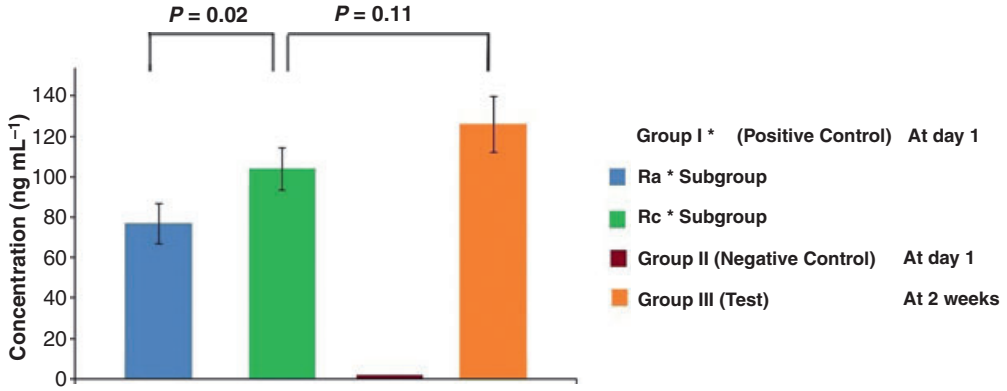

Figure 1 Base line mean dentine sialoprotein (ng mL^{-1}) concentrations in gingival crevicular fluid of group I ($n = 6$), II ($n = 6$) and III ($n = 12$). #Bar shows the standard deviation.

Table 1 Dentine sialoprotein concentrations (ng mL^{-1}) in gingival crevicular fluid and %RRR in group III

		Follow-up							
		2 Weeks		6 Weeks		3 Months		6 Months	
Tooth no.	Diagnosis	DSP (ng mL^{-1})	%RRR	DSP (ng mL^{-1})	%RRR	DSP (ng mL^{-1})	%RRR	DSP (ng mL^{-1})	%RRR
12	Concussion[a]	130	NA	170	8.53	74	6.66	50	6.25
11	Concussion [b]	111	NA	130	3.13	56	2.7	ND	1.2
21	Extrusion [b]	137	NA	170	5.71	78	4.05	ND	3.1
11	Extrusion [c]	138	NA	163	3.6	181	6.8	186	9.76
11	Subluxation [a]	147	NA	165	5.55	64	3.22	42	2.94
11	Subluxation	115	NA	125	NA	ND	NA	ND	NA
11	Subluxation	129	NA	136	1.8	63	1	ND	1
42	Subluxation	127	NA	159	1.6	75	1.6	ND	0.5
32	Subluxation	137	NA	164	2	95	2	ND	1
41	Subluxation	113	NA	138	3.03	195	5.9	103	4.1
31	Subluxation	103	NA	138	2	171	4.36	127	3.77
21	Intrusion	139	NA	106	NA	ND	NA	ND	NA

NA, not appreciable; ND, not detected; DSP, dentine sialoprotein; %RRR, percentage of radiographic root resorption.
[a]Figure 5.
[b]Figure 6.
[c]Figure 7.

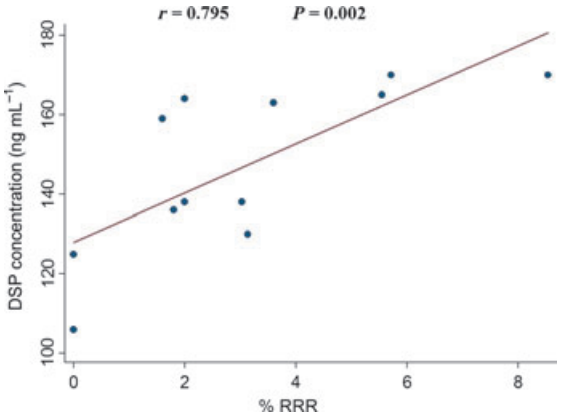

Figure 2 Correlation between dentine sialoprotein (DSP, ng mL^{-1}) in gingival crevicular fluid and percentage of radiographic root resorption (%RRR) at 6-week follow-up.

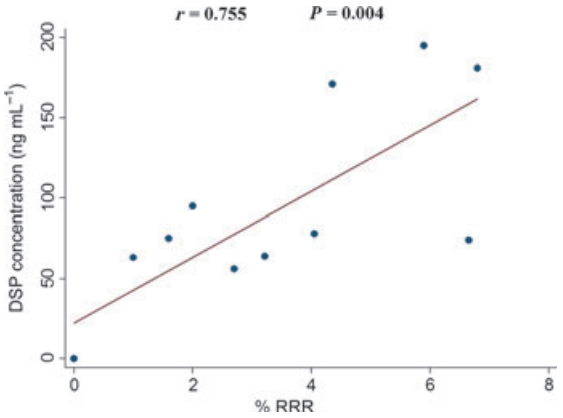

Figure 3 Correlation between dentine sialoprotein (DSP, ng mL^{-1}) in gingival crevicular fluid and percentage of radiographic root resorption (%RRR) at 3-month follow-up.

Discussion

Dentine sialoprotein is an N-terminal proteolytic cleavage product of its precursor dentine sialophosphoprotein (DSPP, Butler 1992). It helps in transport of the protein precursors to the site of proteolytic processing and controls the activity of DPP, a nucleator of hydroxyapatite crystals (Butler *et al.* 2003). It is an important marker of the odontoblast phenotype because it appears in the terminally differentiated odontoblasts just prior to the onset of mineralization (D'Souza *et al.* 1997). Immunolocalisation studies have shown DSP to be incorporated into the mineral-collagen structure during mineralization and remain confined to the DECM (Baba *et al.* 2004). Only during the process of active root resorption that dentine demineralization occurs by clastic activity, and DSP is released into the adjacent tissues (Butler & Ritchie 1995).

Gingival crevicular fluid is an osmotically mediated transudate that emerges between the surface of the tooth and the epithelial integument (Griffiths 2003). It is 'site specific' and contains an array of biochemical and cellular factors, which can be of immense diagnostic value. Calibrated volumetric microcapillary pipettes were used for its collection, as they provide an undiluted sample of the 'native' GCF (Griffiths 2003).

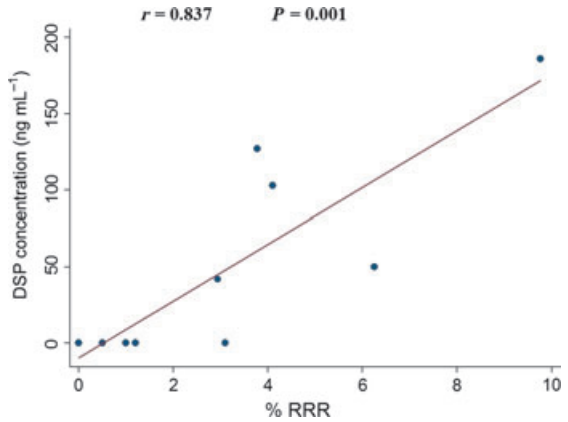

Figure 4 Correlation between dentine sialoprotein (DSP, ng mL^{-1}) in gingival crevicular fluid and percentage of radiographic root resorption (%RRR) at 6-month follow-up.

Oral prophylaxis and plaque control measures were undertaken. Initial sample collection in the test group was performed at 2-week intervals to allow for healing of the traumatized periodontal tissues. The site was isolated with cotton rolls to prevent contamination and dilution with saliva. An extracrevicular method was preferred to collect the GCF as intracrevicular placement of micropipettes could have triggered the flow of inflammatory exudate. Samples contaminated with either blood or saliva were discarded. One μL of GCF was diluted with 100 μL of the phosphate-buffered solution. Use of similar quantities for the detection of dentin NCP has been reported for sandwich ELISA (Mah & Prasad 2004, Balducci *et al.* 2007, Kereshanan *et al.* 2008). This is a rapid and convenient method, which has the ability to detect the desired proteins in nanograms (10^{-9} g, Berg *et al.* 2002). The microtiter plates pre-coated with specific human DSP were used in the present study to avoid cross-reaction with other proteins.

The resorption of primary teeth is a physiological process, which starts immediately after root completion. Detectable quantities of DSP were released in the GCF of group-I subjects. Mah & Prasad (2004) and Kereshanan *et al.* (2008) reported the release of these proteins in the GCF of primary teeth and attributed their findings to the degradation of DECM during exfoliation. In the present study, significantly higher concentrations of this protein were observed in the R_c when compared with the R_a subgroup. These findings were expected and consistent with the results of scanning electron microscope (Sasaki *et al.* 1988) and histological (Francini *et al.* 1992) studies that revealed presence of deep resorption lacunae and clastic cells with well-defined ruffled borders, signifying faster resorption during late stages of root resorption (more than 1/3rd of the root length).

Dentine sialoprotein was not detected in the GCF of subjects of Group II. Under normal physiologic conditions, permanent teeth are resistant to resorption. Periodontal ligament (PDL), cementoblasts and cementoid form a protective covering (Heithersay 2007) by providing a zone of nonmineralized matrix

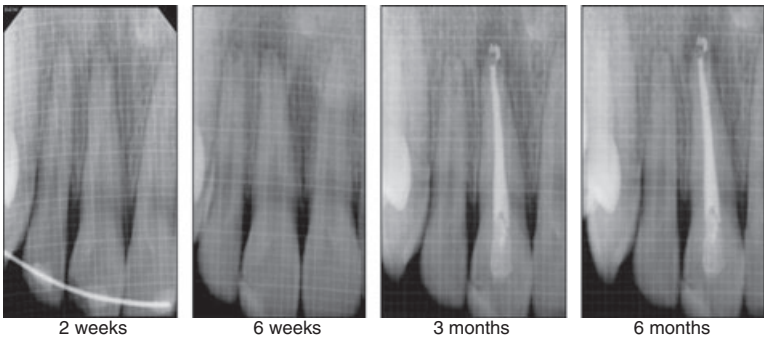

Figure 5 A healthy 16-year-old male was referred for the evaluation of his broken front teeth subsequent to a fall 2 days before. Intra-oral examination revealed an uncomplicated crown fracture with subluxation and concussion in relation to tooth 11 and 12, respectively. Both teeth were tender to percussion, and an Electric pulp test (EPT) resulted in a positive response. Base line intra oral periapical radiographs (IOPA) revealed apical periodontal ligament space widening of tooth 11. Teeth were stabilized with a 24 gauge orthodontic wire-composite splint for 2 weeks. IOPA at 6 weeks showed periapical radiolucency in relation to tooth 11 and apical root resorption of tooth 12. Root canal treatment was initiated in tooth 11 and a calcium hydroxide intracanal medicament placed for 2 weeks and the root canal subsequently filled. Follow-up IOPA at 3 months showed blunting of the root apex of tooth 12. A positive EPT response was elicited at 6 months.

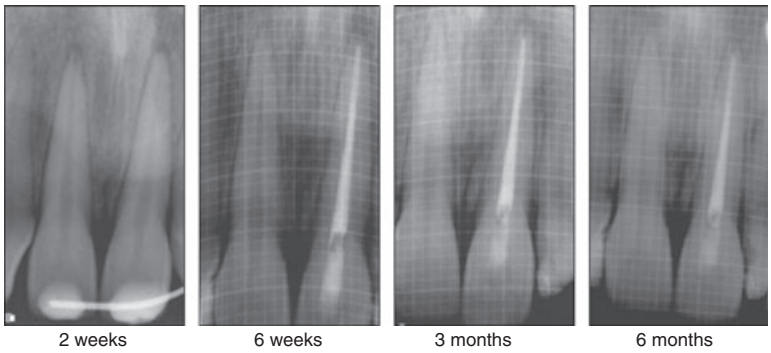

Figure 6 A 17-year-old female with a noncontributory medical history was referred for the evaluation of a dental injury sustained in a road traffic accident a day before. Intra-oral examination revealed a 2 mm extrusion and concussion of tooth 21 and 11, respectively. EPT was negative for the extruded tooth and a positive response was noted in tooth 11. IOPA revealed widened apical periodontal ligament spaces. Repositioning and stabilization was achieved with a 24 gauge orthodontic wire-composite splint for 2 weeks. At 6 weeks a periapical radiolucency in relation to tooth 21 and apical root resorption in tooth 11 was noted. Root canal treatment of the left maxillary central incisor was initiated, a calcium hydroxide intracanal medicament was placed and subsequently the root canal filled. IOPA at 3 months and 6 months showed minimal blunting of the root apex with positive EPT response of tooth 11.

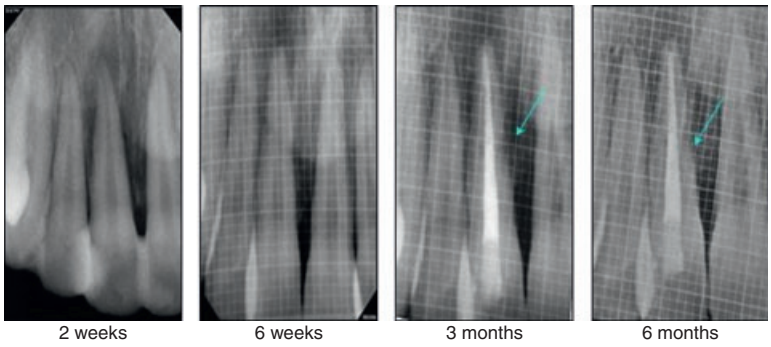

Figure 7 A 14-year-old male was referred for the management of his maxillary central incisor. He had a history of fall 1 week prior for which he had sought treatment at a private clinic. His referring dentist's records revealed extrusion of tooth 11, which had been repositioned. Intraoral examination revealed an inter dental composite splint with the adjacent teeth. EPT showed a negative response. Loss of periodontal ligament space was evident on radiographic examination. At 6 weeks, a periapical radiolucency and lateral replacement resorption developed. Root canal treatment was initiated in tooth 11 and calcium hydroxide intracanal medicament placed for 2 weeks and the root canal filled. At 3 months and 6 months follow-up periapical healing was evident; however, lateral replacement root resorption progressed.

that prevents the adherence of clastic cells (Trope 2002). Also, the preferential expression of osteoprotegerin by PDL cells inhibits osteoclasts formation (Harokopakis-Hajishengallis 2007) and this coupled with the presence of an anti-invasion factor in the cementum, and the PDL (Lindskog & Hammarström 1980) tends to further protect against resorption. However, Kereshanan *et al.* (2008) utilizing the slot blot analysis, demonstrated the presence of DSP in the GCF of 50% of their controls. These findings may be related to the fact that the test samples were collected from second premolars of patients (age group 11–15 year) undergoing root maturation, which may have reflected the complex cellular and structural changes within the periodontium involved at the mineralization front during this stage. In contrast, the GCF evaluated in the present study was of permanent maxillary central incisors in which root maturation had been completed, and hence the release of this protein was not observed.

At 2 weeks, DSP was detected in quantifiable amounts in the GCF of all the teeth in group III and

was released in a higher concentration in comparison with the R_c subgroup. Although the difference was not significant ($P = 0.11$), its presence suggests that irrespective of the type of luxation injury sustained, all teeth underwent early root resorption at a high rate. Andreasen (1980), in a histological study on replanted monkey teeth, demonstrated surface and inflammatory root resorption as early as 1 week. However, replacement resorption was noted only at 2 weeks. Keeping the results of the study as the basis, the 2-week time period (baseline) after injury was considered optimal for the first GCF sample collection in group III.

The concentration of protein in GCF was correlated with %RRR. Radiographs were standardized to diagnose, quantify and follow-up root resorption using commercially available film holders to minimize projection geometry errors. The mesh gauge was placed on the RVG sensor to incorporate the pattern on to the radiographic image. Its use has been previously documented to compare bone fill and alveolar crest resorption in infrabony defects after bone graft procedures (Thomas & Subbaiah 2011) and for monitoring of root resorption during orthodontic therapy (Hölttä *et al.* 2004). This is an acceptable method for accurate radiographic measurements as the distance between the two grid lines on the radiograph remains constant, even if the image is foreshortened or elongated (Krithika *et al.* 2008). This was modified and adapted in the present study.

For group III, there were detectable quantities of DSP in the GCF before any evidence of resorption was discernible on the baseline radiograph (2 weeks, Fig. 5). A positive correlation between the GCF concentration of DSP and radiographic percentage of root resorption was established at all subsequent follow-ups. At 6 weeks, the concentration of this protein increased with the appearance of radiographic evidence of resorption (Fig. 5). This probably indicated an active resorptive process. At 3 and 6 months, the concentration of protein in the GCF regressed for the teeth, which were endodontically treated, demonstrating the success of the therapy in arresting the inflammatory resorptive process (Fig. 6). It also declined in cases with vital pulps, suggesting the transient and self-arresting nature of the apical resorption (Fig. 5). In cases where there was radiographic evidence of replacement root resorption, the levels of DSP tended to increase in spite of endodontic intervention, confirming the progressive nature of this pathological process (Fig. 7).

Conclusion

Detectable quantities of DSP were released in the GCF of traumatized teeth before any radiographic evidence of root resorption. Further clinical trials are necessary to understand the real potential of the results obtained in the present study. Quantification of this protein in the GCF could be a noninvasive, site specific, sensitive and futuristic objective method to diagnose and provide the 'real-time' measure of root resorption.

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