Identification of Dentine Sialophosphoprotein In Gingival Crevicular Fluid To Assess Root Resorption Using Three Piece Base Arch

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Abstract

Introduction: Apical root resorption is a common side effect of orthodontic treatment. Intrusion is suggested as a possible cause of root resorption affecting the prognosis of the patient. Diagnosis is usually done by radiographs. Dentine sialophosphoprotein (DSPP), a non-collagenous protein is released into gingival crevicular fluid during active root resorption. This study is aimed to identify the role of DSPP in root resorption during orthodontic intrusion using three piece base arch.

Materials and method: GCF was collected from permanent maxillary central and lateral incisors of 10 control subjects with no history of orthodontic treatment and 10 experimental subjects before intrusion and 2 months after intrusion. DSPP was measured in GCF using ELISA method.

Results: Statistical analysis showed there was highly significant increase in DSPP levels after 2 months of intrusion. Low levels of DSPP were detected in control subjects. There was no statistically significant difference between central and lateral incisors for the presence of DSPP in all groups.

Conclusion: DSPP was present in GCF in increased levels in subjects undergoing intrusion with three piece intrusion base arch. DSPP can be considered as a biomarker for root resorption. It helps in monitoring root resorption during orthodontic treatment especially during intrusion.

Keywords: root resorption, intrusion, gingival crevicular fluid, dentine sialophosphoprotein

I. Introduction

The most common and undesirable side effect of orthodontic treatment is external root resorption. Most patients with root resorption experience it to a mild degree, an amount that does not compromise the dentition. However there are some patients who experience severe root resorption affecting the long term prognosis of the dentition. [1]It has been accepted that the reported incidence of root resorption after orthodontic treatment is about 1-2%. [2]It is important to identify which treatment factors contribute to external apical root resorption, so that the detrimental effects can be minimized. Intrusion is one of the specific types of tooth movement that has been suggested as a possible cause of root resorption. The tooth apex and associated periodontium can experience relatively high compression stresses when an intrusive force is applied to the crown. [3] The upper incisors are more susceptible, the morphology of the roots of the incisors being the catalyst for root resorption. [4] At present, clinical diagnosis of root resorption is largely radiographic examination. It offers wide accessibility, ease of use and cost effectiveness. But problems of technique, standardization, limited projection views and radiation exposure persist. Radiographs do not allow accurate identification of root resorption in the early stages and often fail to reveal surface resorption on the lingual and buccal aspects of the roots. They are technique sensitive and can detect resorption only after 60-70% of the mineralized tissue is lost and provide only two-dimensional information. Radiographs cannot indicate if the process of root resorption is still active and require additional
radiation exposure to monitor progress of root resorption. [5] Computerized tomography and cone beam volumetric imaging have been shown to increase sensitivity in detecting root resorption. However the cost and high radiation exposure make it impracticable for routine use in dentistry. There is a need for establishing a safer, reliable alternative method to clinically diagnose root resorption at early stages. Gingival Creviccular Fluid (GCF) is the inflammatory transudate that flows out via the gingival crevice. The quantity and composition of the fluid varies depending on the health of the periodontium. GCF is known to contain an array of biochemical and cellular factors that reflect the state of the underlying periodontium. These biomolecules are now finding value as diagnostic or prognostic biomarkers of periodontal health. Among the dentin breakdown products, three dentin-specific non-collagenous proteins have been recognized: dentin matrix protein 1 (DMP1), dentine phosphoprotein (DPP), and dentin sialoprotein (DSP). DMP1 is found both in dentin and bone. The latter two proteins, DPP and DSP are products of the same mRNA transcript and hence are portions of one expressed protein, now known as dentine sialophosphoprotein (DSPP). [2] DSPP is released into the GCF during active external root resorption. The ability to detect the presence of dentinal proteins early during orthodontic treatment induced external root resorption will aid the clinician to instigate prompt treatment if there is a suspicion of root resorption. GCF analysis provides a safe, sensitive, non-invasive method wherein it is possible to identify at-risk individuals for root resorption, predicting subsequent clinical course and prognosis and implement alterations in therapy. DSPP in GCF has been measured using Enzyme linked immunosorbent immunoassay (ELISA). It is a traditional method used in biochemical and clinical practice. It is easy to use and suitable for most findings. The present study is designed to assess the potential role of DSPP in GCF in root resorption during orthodontic intrusion using BhavnaShroff's Three Piece Base Arch. [6]

II. Materials And Method

Ethical clearance was obtained from the institutional ethical committee and review board GDCRI, Bangalore. 20 subjects, of whom 10 were undergoing orthodontic treatment with all first premolar extraction (0.022”x0.028”MBT prescription) and in need of intrusion (experimental group) and 10 subjects who had no history of orthodontic treatment (control group) were selected based on the power of the study and the confidence interval of 95% (p<0.05). The subjects were explained regarding the study procedure and written informed consent was obtained.

2.1 Inclusion criteria

Age group between 16-22 years. Patients with overbite of >5mm and in need of incisor intrusion. No radiographic evidence of apical root resorption. No history of orthodontic treatment and periodontal disease.

GCF was collected from the central and lateral incisors of maxillary arch, right and left side being randomly selected. In experimental group, GCF samples were taken at two time intervals. First, after levelling, aligning and separate canine retraction, before application of orthodontic intrusion force and second after 2 months of application of intrusion force. Three Piece Base arch fabricated using 0.017”x0.025” TMA wire was used with 30gms/side of intrusion force and 90gms/side of retraction force.

2.2 study design

2.2.1 Control group

T0_C: GCF samples collected from gingival sulcus of central incisors.
T0_L: GCF samples collected from gingival sulcus of lateral incisors.

2.2.2 Experimental Group:

T1_C: GCF samples collected from gingival sulcus of central incisors just before application of intrusion force.
T1_L: GCF samples collected from gingival sulcus of lateral incisors just before application of intrusion force.
T2_C: GCF samples collected from gingival sulcus of central incisors after 2 months of intrusion.
T2_L: GCF samples collected from gingival sulcus of lateral incisors after 2 months of intrusion.
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2.3 GCF collection:
After oral prophylaxis, the teeth were rinsed with water, dried and isolated with cotton rolls to avoid saliva contamination. GCF was collected using calibrated volumetric disposable microcapillary tube of internal diameter 1.1mm, with a capacity of 5μl (Ring Caps, HirschmannLaborgerate, GmbH & Co. Germany). Approximately 2μL of GCF was collected from gingival sulcus of the central and lateral incisors respectively over a period of 20 minutes. Microcapillary tubes contaminated with blood and saliva were discarded and sample was collected again. The microcapillary tubes were carefully sealed off in tin foil paper and placed in plastic vial which were appropriately marked for identification and kept at -70oC in refrigerator until the assay procedure. [7]1μl of GCF from each sample was added to a sterile eppendorff vial containing 99μl of phosphate buffer saline (1:100 dilutions) at the time of assay procedure. The samples were then assayed for Dentine Sialophosphoprotein using Human Dentin Sialophosphoprotein (DSPP) Assay Kit (crystal day, biotech co. ltd, shanghai). Samples were analysed using Enzyme linked immunosorbent assay (ELISA) method. The concentration of DSPP in the samples was then determined by comparing the Optical Density (O.D) of the samples to the standard curve. According to standards’ concentrations and the corresponding OD values, the linear regression equation of the standard curve was calculated. Then according to the OD value of samples, the concentration of the corresponding sample was calculated.

2.4 Statistical analysis
All statistical analysis was performed using SPSS software package. (SPSS for windows version 20.0, IBM, USA); The normality assumption of the data was checked using Shapiro-Wilk test and the data was found to be normal. Statistical analysis of mean values between experimental group and control group was carried out using parametric T test and within the group Pre and Post values was checked using Paired T test. P value < 0.05 was considered statistically significant.

III. Results
In the control group (T0), GCF samples of 7 out of 10 central incisors and 7 out of 10 lateral incisors showed the presence of DSPP indicating some activity of root resorption. The maximum, minimum and mean value for central and lateral incisors of all the groups is shown in (Table1).

Table 1: Maximum, minimum and mean values (ngl⁻¹) of DSPP in GCF of central and lateral incisors of control and experimental groups

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Mean</th>
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<tbody>
<tr>
<td>T0c</td>
<td>10</td>
<td>0.0022</td>
<td>0.00013</td>
<td>0.0009</td>
</tr>
<tr>
<td>T0l</td>
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<td>0.0025</td>
<td>0.0014</td>
<td>0.0016</td>
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<tr>
<td>T1c</td>
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<td>0.0042</td>
<td>0.0048</td>
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<tr>
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<td>0.0051</td>
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<tr>
<td>T2c</td>
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<td>0.0075</td>
<td>0.0047</td>
<td>0.0055</td>
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<tr>
<td>T2l</td>
<td>10</td>
<td>0.01</td>
<td>0.0043</td>
<td>0.006</td>
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</table>

Low levels of DSPP were observed in GCF samples at T0. In the experimental group, both just before intrusion (T1) and 2 months after intrusion (T2), the GCF collected from central and lateral incisors showed highly significant increase in DSPP levels (Table 2). Table 2: Comparison of DSPP values (ngl⁻¹) in GCF between experimental groups before intrusion and 2 months after intrusion for central incisors (T1c & T2c) & lateral incisors (T1l & T2l)
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Analysis of data indicated statistically very highly significant increase in the levels of DSPP in GCF between T0 and T1 (Table 3) and between T0 and T2 (Table 4). In the present study, the lateral incisors showed more DSPP in GCF than central incisors both in the experimental groups and control group. But it was not statistically significant (Table 5, Graph 3).

Table 3: Comparison of DSPP values (ng/l) in GCF between control group and experimental group before intrusion for central incisors (t0c & t1c) & lateral incisors (t0l & t1l)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Maximum</th>
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<th>Mean</th>
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<tr>
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<td>0.0042</td>
<td>0.0048</td>
<td>0.0018</td>
<td>0.006**</td>
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<td>0.006</td>
<td>0.0022</td>
<td>0.001**</td>
</tr>
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</table>

p value <0.05- significant*  p value <0.01 - highly significant**  p value <0.001- very highly significant***

Table 4: Comparison of DSPP values (ng/l) in GCF between control groups and experimental group 2 months after intrusion for central incisors (t0c & t2c) & lateral incisors (t0l & t2l)

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<thead>
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<th>N</th>
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<th>Minimum</th>
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<th>SD</th>
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<tr>
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<td>&lt;0.001***</td>
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<tr>
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<td>0.0016</td>
<td>0.0017</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>T1l</td>
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<td>0.0041</td>
<td>0.0051</td>
<td>0.0015</td>
<td></td>
</tr>
</tbody>
</table>

p value <0.05- significant*  p value <0.01 - highly significant**  p value <0.001- very highly significant***

Table 5: Comparison of DSPP values (ng/l) in GCF between central (t0c) and lateral incisors (t0l) within control group, experimental group before intrusion (T1c & T1l) and after intrusion (T2c & T2l)

<table>
<thead>
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<th>N</th>
<th>mean</th>
<th>sd</th>
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</tr>
</thead>
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<td></td>
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<tr>
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<td>0.0048</td>
<td>0.0018</td>
<td></td>
</tr>
<tr>
<td>T1l</td>
<td>10</td>
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<td>0.1462NS</td>
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<tr>
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<td>0.0024</td>
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<td>T2l</td>
<td>10</td>
<td>0.006</td>
<td>0.0022</td>
<td>0.1794NS</td>
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P value > 0.05 not significant NS

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IV. Discussion

In the present study, DSPP in GCF of control subjects was detected in 70% of central and lateral incisors which was not anticipated. The mean DSPP concentration for central incisors was 0.0009ng/L and for lateral incisors 0.0016ng/L. However these values are very low. Earlier studies have also detected presence of DSPP in GCF of untreated subjects.[1,2,5] Wehrbein et al[8] in their histological studies have demonstrated that even untreated teeth are affected by root resorption, especially apical root area. The presence of DSPP in the untreated group could be suggestive of more subtle changes taking place at a structural level due to physiological root resorption. Odontoblasts and odontoclasts might have a similar function as osteoblasts and osteoclasts of bone to resorb, remodel and maintain the root surface.[1] There are some suggestions that DSPP may not be entirely dentin specific.[9] From the ages of 16–32 years, the incidence of physiologic root resorption was 86.4%, and from 32 to 50 years it was 96.4%. The presence of root resorption before treatment is usually considered a strong predisposing factor for root resorption during treatment. Lupi et al. [10] and Linge et al. [11] have reported a 15% incidence of root resorption before treatment and 73% after treatment. Becks [12] found the incidence of root resorption to be 32% before treatment and 94.6% after treatment. Minor root resorption or an irregular root contour detected 6-9 months from the beginning of orthodontic treatment seemed to have a high risk for further root resorption.[13] Ruo-ping Jiang et
al [14] in a study using panoramic radiographs have found that degree of pre-treatment root resorption seems to influence root resorption during orthodontic treatment. If root resorption fails to occur after 6-9 months of orthodontic treatment then no severe root resorption will occur at the end of the treatment. It was found that less resorption was seen in younger children and that the chances of resorption increase when orthodontic movements take place after the root is completed. [11]

There was a very highly significant increase in the mean DSPP levels in GCF samples taken from experimental group before intrusion, when compared to the control group for both central and lateral incisors. Jon Artun et al [15] and IsoldeSmale et al [16] in their study have found significant root resorption in patients after 6 months and 12 months of orthodontic therapy by means of periapical radiographs. Root resorption seems to begin in the early aligning and levelling stages of orthodontic treatment. Alexander Dudic et al [17] in a CBCT study found root resorption in 69% of the patients at the end of orthodontic treatment. DimitriosMakedonas et al [18] in a CBCT study found that after 6 months of treatment, clinically significant resorption was diagnosed in only 4% of the patients. The radiographic examination did not reveal any clinically significant information in 96% of the patients and concluded that early monitoring for root resorption after 3 months and 6 months have little significance on predicting root resorption at the end of treatment.

Kereshanan et al [2] in a study have found statistically significant increase in levels of DSPP in GCF of subjects after 12 weeks of commencement of orthodontic treatment when compared to untreated controls. Their study indicates that during the initial stages of fixed appliance therapy, DSPP is liberated into GCF as the dentin matrices of the permanent roots undergo intermittent phases of surface resorption and repair. In the present study there was a significant increase in the mean DSPP levels in GCF samples taken from experimental group before intrusion (0.0048ng/L for centrals and 0.0051ng/L for laterals) when compared to the control group (0.0009ng/L for centrals and 0.0016ng/L for laterals). This finding is in accordance with other studies. The mean levels of DSPP estimated in the present study before intrusion for central incisors and lateral incisors which when compared to earlier studies is very low. This difference may be due to the fact that in the present study the GCF samples were taken at approximately 6 months of commencement of orthodontic treatment after levelling and aligning while in other studies the GCF samples were taken after 1 year of orthodontic treatment. Studies have shown that as duration of orthodontic treatment increases, the amount of root resorption increases and levels of DSPP in GCF also increases.

There was a significant increase in mean DSPP levels in GCF of experimental subjects after 2 months of intrusion for both central (0.0055ng/L) and lateral incisors (0.0058ng/L). This result is in accordance with earlier studies which have stated that intrusion is a technique that logically could increase the risk of apical root resorption. In a study by Costopoulos et al [3] after a period of approximately 4 months, intrusive tooth movement caused slightly more root resorption than the controls, 0.6 mm versus 0.2 mm which was statistically significant. Dermaut and DeMunc [19] stated that intrusion seemed to cause more resorption than overall orthodontic treatment. No correlation was found between the amount and duration of intrusion and root resorption. They attributed higher resorption observed in their study to the use of higher force (25g) on each maxillary incisor whereas Costopoulos et al used 15gms per tooth Han et al [20] have found significant increase in root resorption associated with intrusive tooth movement as compared with extrusive tooth movement concluding that intrusion of teeth causes about four times more root resorption than extrusion. Lopatiene et al [21] in a study concluded that application of an upper utility arch for intrusion of maxillary incisors induces root resorption of maxillary central incisors more often than by treating with straight arch. Balducci et al [5] explored the presence of DSPP and DMP-1 in GCF of patients diagnosed with mild and severe resorption after 1 year of fixed appliance therapy in comparison with untreated controls as confirmed by intraoral periapical radiographs. They concluded that the use of DSPP as biomarker were suitable alternatives for monitoring root resorption during orthodontic tooth movement. DSPP levels (0.0055ng/L for centrals and 0.0058ng/L for laterals) increased significantly after 2 months of application of intrusion force in comparison with before intrusion (0.0048ng/L for centrals and 0.0051ng/L for laterals). Paired T tests showed p values 0.006 for centrals and 0.001 for laterals respectively, indicating that the results are statistically highly significant and that there is more root resorption associated with intrusive forces. As three piece simultaneous intrusion and retraction base arch was used in the present study, the significant increase
in the amount of DSPP in GCF after 2 months of intrusive force application may be due to a combination of forces. In the present study DSPP levels increased in GCF samples from lateral incisors as compared to central incisors but the increase was not statistically significant. Previous studies have also found maxillary lateral incisors to be more affected by root resorption during orthodontic treatment.[16,22,23] Mohandesan et al [23] have also found that 82% lateral incisors and 74% of central incisors are affected by root resorption during orthodontic treatment

V. Conclusion

Intrusion of incisors is an important treatment modality and one of the factors for root resorption. In the present study, DSPP levels in GCF of maxillary incisors increased significantly after 2 months of application of intrusion forces with the three piece base arch compared to before intrusion. There was no significant difference between central and lateral incisors for the presence of DSPP. An increased level of DSPP in GCF detects the process of root resorption earlier than the x-ray results and the clinical manifestations. Thereby root resorption can be intercepted and alterations in treatment mechanics can be done. Use of light forces during orthodontic treatment especially intrusion is recommended. Considering its significant presence in GCF during root resorption, DSPP can be considered as a biomarker for root resorption. GCF analysis is safe and non-invasive and site-specific. There is need to develop a chair-side assessment where it can be easily implemented in the clinic. A baseline value of DSPP in GCF for root resorption due to orthodontic treatment has not been established. Further, a comparative study between radiographic and biochemical method for root resorption in the same individuals need to be done.

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