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RESEARCH ARTICLE

ESTIMATION OF DENTIN SIALOPHOSPHOPROTEIN IN GINGIVAL CREVICULARFLUID DURING ORTHODONTIC INTRUSION USING RICKETTS' SIMULTANEOUS INTRUSION AND RETRACTION UTILITY ARCH

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ABSTRACT

Background: Apical root resorption is an unwanted effect associated with orthodontic tooth movement especially intrusion. Immuno analysis of Gingival Crevicular Fluid (GCF) has identified Dentin Sialo phospho protein (DSPP) to be present during root resorption. This study is aimed to identify and quantify DSPP, released into GCF during orthodontic intrusion using Ricketts' simultaneous intrusion and retraction utility arch and to investigate the potential of DSPP in GCF as a biomarker for root resorption.

Methods: GCF was taken from central and lateral incisors of 10 subjects(experimental group) undergoing fixed orthodontic treatment before intrusion and after 2 months of intrusion and 10 subjects with no history of orthodontic treatment (control group) using micro capillary tubes. These samples were analyzed and quantified for DSPP using ELISA. To determine differences between the means of the various experimental and control groups, data obtained were statistically analyzed using parametric t-test.

Results: DSPP in GCF was detected in both control and experimental subjects. There was a significant increase in DSPP levels in GCF, 2 months after intrusion. Significant differences were not found in DSPP levels between central and lateral incisors.

Conclusion: The results of the study confirm the presence of significant levels of DSPP in GCF during orthodontic intrusion. DSPP in GCF can be considered as a biomarker to monitor root resorption during orthodontic intrusion. Light continuous forces are recommended during intrusion mechanics. Early detection of DSPP in GCF in highly susceptible individuals for root resorption is beneficial so as to alter the treatment mechanics as needed.

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INTRODUCTION

Apical root resorption is a common, iatrogenic consequence associated with orthodontic tooth movement (Parker and Harris, 1998). Close radiographic examination of orthodontic ally treated individuals show some loss of root length in nearly every patient (Balducci *et al.,* 2007). Truly severe root resorption that threatens the longevity of the tooth or forces a halt to treatment is rare (Chandrasekar and Sridevi, 2013). For susceptible patients, root resorption may limit the outcome of successful orthodontic treatment. Intrusion is one of the specific types of tooth movement that has been suggested as a possible cause of root resorption (Costopoulos and Nanda, 1996).

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The tooth apex and associated periodontium can experience relatively high compression stresses when an intrusive force is applied to the crown. Incisors are the most susceptible because of their root characteristics, which concentrate greater forces on the apices (Levander *et al.,* 1998). Early detection of root resorption during orthodontic treatment is essential for identifying teeth at risk of severe root resorption. At present, detection of root resorption is done using radiographic techniques which are technique sensitive. They can detect resorption only after 60–70% of the mineralized tissue is lost. It cannot indicate if the process of root resorption is still active. Only after 5-6 months, a reliable radiographic diagnosis of apical root resorption can be performed. 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. Given these current limitations of radiographic methods, there is a need for

establishing a sensitive, safer, reliable alternative method to detect root resorption (Kereshanan *et al.,* 2008). Gingival Crevicular Fluid (GCF) is a serum transudate found in gingival sulcus that can be collected at the gingival margin. GCF is known to contain an array of biochemical and cellular factors that reflect the state of underlying periodontium (Kereshanan *et al.,* 2008). The site-specific nature of GCF means that it has great potential in containing factors that are specific for actions at a given site and might have diagnostic value to detect early stages of root resorption (Rody *et al.,* 2014). Among the dentin breakdown products, three dentin-specific non-collagenous proteins have been recognized: Dentin Matrix Protein1 (DMP1) and Dentin Phosphophoryn (DPP) and Dentin Sialoprotein (DSP). DPP and DSP are products of the same mRNA transcript and hence are portions of one expressed protein, now known as Dentin Sialophosphoprotein (DSPP)[6].Thus DSPP is the most abundant, non-collagenous protein within the dentin (Mah and Prasad, 2004). These proteins are not routinely released into the surrounding space as dentin does not undergo the process of remodeling as in bone. It is only in the presence of active external root resorption that these proteins are freed into the periodontal ligament space (Balducci *et al.,* 2007). DSPP has been detected in the GCF of patients with varying degrees of root resorption using immune analysis which suggests that DSPP can be a useful marker for root resorption.[2]Immunolocalization studies have shown that DSPP is localized within odontoblasts, the cells and the extracellular matrix of dental pulp, predentin and dentin. DSPP is not found in ameloblasts, bone, cartilage, soft tissues, or other components of the oral tissues, suggesting that it is highly dentin specific (Kereshanan *et al.,* 2008). Enzyme Linked Immunosorbent Assay (ELISA) is commonly employed to check the presence and quantify a particular protein present in a biological fluid, be it GCF, saliva or blood. In orthodontic research, ELISA is mainly performed for analysing the amount of inflammatory cytokines, root resorption markers, toxicity evaluations with GCF and salivary sample (Krishnan and Davidovitch, 2015). Many studies have been done proving DSPP in GCF as a biomarker for root resorption. In the present study, Dentin Sialophosphoprotein (DSPP) in GCF of maxillary central and lateral incisors for root resorption were estimated in subjects who were undergoing orthodontic intrusion using Ricketts Simultaneous Retraction and Intrusion Utility Arch, identifying at-risk individuals by getting information on the resorptive activity and severity, reducing the time between onset and usual clinical diagnosis of root resorption.

MATERIALS AND METHODS

Ethical clearance for the study was obtained from the institutional ethical and review board and written informed consent was obtained from subjects who agreed to participate voluntarily in this study. The study consisted of 20 subjects in the age group of 13-22 years. 10 control subjects with no history of orthodontic treatment and 10 experimental subjects undergoing orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopaedics with all first premolar extraction and using 0.022" x 0.028" MBT (Mc Laughlin Bennet Trevisi) prescription were selected. After leveling and aligning and separate canine retraction, Ricketts' simultaneous retraction and intrusion utility arch fabricated using 0.017"x0.025" TMA (Titanium Molybdenum Alloy) wire was used.60 grams of intrusive force measured using Dontrix gauge was applied.

In experimental group, GCF samples were taken at two time intervals. First, just before application of orthodontic intrusion force and second after 2 months of application of intrusion force. GCF was collected from the central and lateral incisors of maxillary arch, right and left side being randomly selected using disposable micro-capillary tubes of internal diameter 1.1mm, with a capacity of 5µL (Raghavendra *et al.,* 2012). (Ring Caps, Hirschmann Laborgerate, GmbH & Co.Germany). Approximately 2µL of GCF was collected from gingival sulcus of the teeth, over a period of 20 minutes. The micro-capillary tubes were carefully sealed off in tin foil paper and placed in plastic vials which were appropriately marked for identification and stored at -70^{o}C in refrigerator until the assay procedure. 1 µL 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 Dentin Sialophosphoprotein (DSPP) using Human Dentin Sialophosphoprotein Assay Kit (CRYSTAL DAY BIOTECH CO. LTD, SHANGHAI). The concentration of DSPP in GCF samples was then determined by comparing the Optical Density (O.D) of the samples to the standard curve. According to standards' concentration 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. All statistical analysis was performed by using SPSS software package (SPSS for windows version20.0, IBM, USA). Statistical analysis of mean values between experimental group and control group was carried out using parametric T test and within the experimental group, Pre and Post values were checked using Paired T test. P value < 0.05 was considered statistically significant.

RESULTS

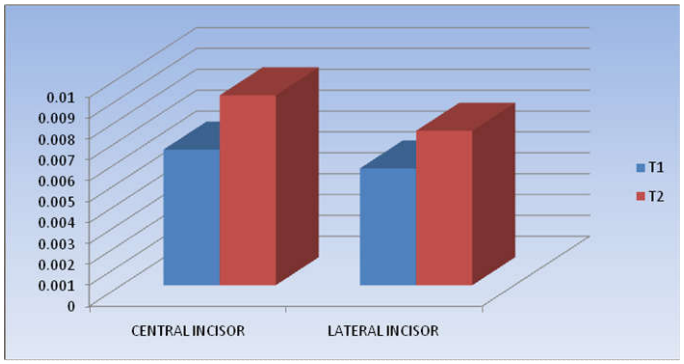
In the control group, DSPP was detected in GCF from 7 each of central and lateral incisors. The maximum value of DSPP for central incisors in control group was 0.002208ng/L and minimum value was 0.0.000125ng/L and the mean value was 0.0009ng/L. The maximum value for lateral incisors was 0.002458ng/L and minimum value was 0.001375ng/L and mean value 0.0016ng/L. In the experimental group, the maximum value of DSPP in GCF from central incisors just before intrusion was 0.01083ng/L, the minimum value was 0.004958ng/L and the mean was 0.0065ng/L. For lateral incisors, the maximum value was 0.00750ng/L, the minimum value was 0.00450ng/L and the mean value was 0.0056ng/L (Table –1) (Graph – 1).

Table 1. Comparision of DSPP values (ngL^{-1}) in GCF between experimental groups before intrusion AND 2 months after intrusion for central incisors ($T1_C$& $T2_C$) & lateral incisors

	N	Maximum	Minimum	Mean	SD	P value
$T1_C$	10	0.011	0.0049	0.0065	0.0019	0.006**
$T2_C$	10	0.013	0.0059	0.0091	0.0023	
$T1_L$	10	0.0075	0.0045	0.0056	0.0010	0.009**
$T2_L$	10	0.01	0.0057	0.0074	0.0018	

The maximum value of DSPP level in GCF from central incisors taken after 2 months of intrusion was 0.01337ng/L, the minimum value was 0.005958ng/L and the mean value was 0.0091ng/L. For lateral incisors, the maximum value was 0.0111ng/L, the minimum value was 0.0057ng/L and the mean value was 0.0074ng/L (Table -1) (Graph – 1). The mean DSPP level in GCF from central incisors ($T1_C$) before intrusion was

0.0065ng/L and after 2 months of intrusion ($T2_C$) was 0.009ng/L. There was an increase in mean DSPP level of 0.0026ng/L indicating that it is statistically highly significant (p = 0.006) (Table1) (Graph 1).

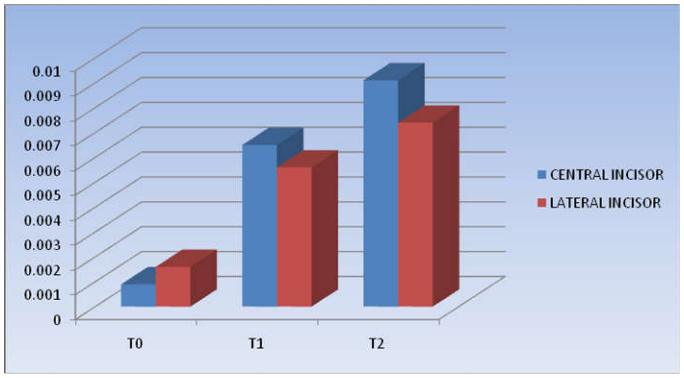


Graph 1. Comaparision of DSPP levels (ngL^{-1}) between experimental groups before intrusion and 2 months of intrusion for central incisors ($T1_C$& $T2_C$) & lateral incisors ($T1_L$& $T2_L$)

The mean DSPP level in GCF from lateral incisor ($T1_L$) before intrusion was 0.0056ng/L and after 2 months of intrusion ($T2_L$) was 0.0074ng/L. There was an increase in mean DSPP level of 0.0018ng/L indicating that it is statistically highly significant (p = 0.006) (Table 1) (Graph1). The mean difference in DSPP levels in GCF between central and lateral incisors within control group was 0.0008ng/L (p= 0.26), within experimental group before intrusion was 0.0056 (p = 0.1512) and after intrusion was 0.0074 (p =0.1374), indicating that it is not statistically significant (Table 2) (Graph 2).

Table 2. Comparision of DSPP values (ngL^{-1}) in GCF between central ($T0_C$) and lateral incisors ($T0_L$) withincontrol group, experimental group before intrusion ($T1_C$& $T1_L$) and after intrusion ($T2_C$& $T2_L$)

	N	Mean	SD	P value
$T0_C$	10	0.0009	0.0009	0.2649^{NS}
$T0_L$	10	0.0016	0.0017	
$T1_C$	10	0.0065	0.0019	
$T1_L$	10	0.0056	0.0010	0.1512^{NS}
$T2_C$	10	0.0091	0.0023	
$T2_L$	10	0.0074	0.0018	0.1374^{NS}



Graph 2. Comparision of DSPP levels (ngL^{-1}) between central and lateral incisors within control GROUP (T0), experimental group before intrusion (T1) and 2 months after intrusion (T2)

DISCUSSION

In the control subjects DSPP in GCF was detected in 7 each of the central and lateral incisors (70%) which was not anticipated. These values were not statistically significant when compared with those in the experimental subjects. Earlier studies by Mah *et al.,* 2004; Kereshanan *et al.,* 2008 and Balducci *et al.,* 2007 have also detected presence of DSPP in GCF of untreated subjects. Harris et al., 1998 and Wehrbein et al., 1995 in their studies have reported the presence of root resorption in permanent teeth in subjects with no history of orthodontic treatment. Histological studies show that roots of untreated permanent teeth also demonstrate sites of resorption, especially apical root areas on surfaces facing direction of physiological tooth movement. 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. These findings may reflect complex cellular and structural changes within the periodontium involved at the mineralization front as the maturation of the root takes place. Chang *et al.,* 1996 MacDougall *et al.,* 1997 in their study have shown that basal turnover of dentine proteins occurs during the maturation process of the root structures of the young permanent adult dentition and that DSPP may be liberated from pulpal cells as the roots of these teeth may have patent apices. Qin *et al.,* 2002 in a study found that the DSPP gene was expressed in osteoblast cells also and may take part in physiologic bone remodelling process.

The presence of root resorption before treatment is usually considered a strong predisposing factor for root resorption during treatment. Lupi *et al.,* 1996 and Linge *et al.,* 1983 have reported incidence of root resorption which was 15% before treatment, increasing to73% after treatment. Becks in a study found the incidence of root resorption to be 32% before treatment which increased to 94.6% after treatment. Massler and Malone *et al.,* 1954 in a study have found high correlation between the amount of root resorption present before and after treatment and therefore early diagnosis might serve for early risk management in such teeth. In the present study, there was significant levels of DSPP in GCF samples taken from experimental subjects before intrusion. This finding is in accordance with studies done by Mah *et al.,* 2004 and Kereshanan *et al.,* 2008. The study confirms that during the initial stages of fixed appliance therapy, DSPP is liberated into GCF. The dentin surfaces of the roots were being resorbed during the early stages of fixed appliance therapy i.e. aligning and leveling stage with light orthodontic forces and concludes that DSPP in GCF can be a potential biomarker for root resorption. DSPP levels increased in GCF samples from central incisors as compared to lateral incisors but the increase was not statistically significant as was also seen in studies conducted by Dermaut and DeMunck 1986 and Kaley and Philips 1991. There was a statistically significant increase in mean DSPP level in GCF of experimental subjects after 2 months of intrusion for both central incisors (0.0091ng/L) and lateral incisors (0.0074ng/L). This result is in accordance with earlier studies which have stated that intrusion is a technique that could increase the risk of apical root resorption (Dermaut and De Munck, 1986). Costopoulos *et al.,* 1996 have found that 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. Lopatiene *et al.,* 2008 in a study concluded that application of an additional upper utility arch for intrusion of maxillary incisors induces root resorption of maxillary central

incisors more often than by treating with straight arch. In the present study the DSPP levels in GCF increased significantly after 2 months of application of intrusion force in comparison with before intrusion which shows that there is more root resorption associated with intrusive tooth movement. These results indicate vertical tooth movement as a risk factor for apical root resorption. In the present study the DSPP levels in GCF samples from experimental subjects after intrusion of central incisors was slightly more than that of lateral incisors but was not statistically significant. This result confirms the study done by Dermaut and DeMunck 1986 which concludes that there is no difference in root resorption between central and lateral incisors during intrusive tooth movements. In the present study, Ricketts simultaneous retraction and intrusion utility arch was used and this combination of forces also may have resulted in increased levels of DSPP in GCF. All the experimental subjects were more or less equally affected by orthodontic treatment in general and intrusion in particular.

Conclusion

Significant amounts of DSPP was detected in GCF samples of subjects undergoing orthodontic treatment before intrusion and 2 months after intrusion and the amount of resorption can be assessed by evaluating the DSPP levels in GCF. After 2 months of intrusion the DSPP levels in GCF increased significantly as compared to GCF samples before intrusion, indicating that an increased root resorption is incident to intrusive tooth movement, even if it is applied for a shorter duration. There was no difference in susceptibility to root resorption between central and lateral incisors. DSPP present in GCF can be considered as a biomarker for root resorption since its levels significantly increased during orthodontic treatment and especially during intrusion. Use of light forces especially during intrusion of teeth is recommended for all patients. Early detection helps to identify highly susceptible individuals for root resorption, so that forces applied can be monitored and treatment alterations can be made. Development of a chair side bioassay based on this analytical biomarker would enable the clinician to monitor root resorption in day to day practice and initiate prompt alteration in treatment mechanics when there is suspicion of root resorption and thereby limit a common complication of orthodontic treatment.

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REFERENCES

Balducci, L., Ramachandran, A., Hao, J., Narayanan, K., Evans, C., George, A. 2007. Biological markers for evaluation of root resorption, *Arch Oral Biol.,* 52:203–208

Chandrasekar, R., Sridevi, K. S. 2013. Treatment of class 2 division 1 malocclusion with severe short roots of upper central incisors, *J. Pharm. Bioall. Sci.,* 5: 173-175.

Chang, S.R., Chiego, Jr, D., Clarkson, B. H. 1996. Characterization and identification of a human dentin phosphophoryn, *Calcif. Tissue Int.,* 59:149–153.

Costopoulos, G., Nanda, R. 1996. An evaluation of root resorption incident to Orthodontic Intrusion, *Am J Orthod.,* 109: 543-548.

Dermaut, L.R., De Munck, A. 1986. Apical root resorption of upper incisors caused by intrusive tooth movement: A radiographic study, *Am J Orthod.,* 90:321-326.

Kaley, J., Phillips, C. 1991. Factors related to root resorption in edgewise practice, *Angle Orthod.,* 61:125-32.

Kereshanan, S., Stephenson, P., Waddington, R. 2008. Identification of dentin sialophosphoprotein in gingival crevicular fluid during physiological root resorption and orthodontic tooth movement, *Eur J Orthod.,* 30:307-314

Krishnan, V., Davidovitch, Z. 2015. Biological mechanisms of tooth movement.2nd edn.

Levander, E., Bajka, R., Malmgren, O. 1998. Early radiographic signs of apical root resorption during orthodontic treatment: a study of maxillary incisors, *Eur J Orthod.,* 20:57-63.

Linge, B.O., Linge, L. 1983. Apical root resorption in upper anterior teeth, *Eur J Orthod.,* 5:173–183.

Lopatiene, K., Dumbravaite, A. 2008. Risk factors of root resorption after orthodontic treatment, Stomatologija, 10: 89–95.

Lupi, J.E., Handelman, C.S., Sadowsky, C. 1996. Prevalence and severity of apical root resorption and alveolar bone loss in orthodontically treated adults, *Am J Orthod.,* 109:28-37

MacDougall, M., Simmons, D., Luan, X., Nydegger, J., Feng, J., Gu, T.T. 1997. Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a single transcript coded by a gene on human chromosome 4, *J BiolChem.,* 272:835–842.

Mah, J., Prasad, N. 2004. Dentin phosphoproteins in gingival crevicular fluid during root resorption, *Eur J Orthod.,* 26:25-30

Massler, M., Malone, A.J. 1954. Root resorption in human permanent teeth, *Am J Orthod.,* 40:619–633.

Parker, R.J., Harris, E.F.1998. Directions of orthodontic tooth movements associated with external apical root resorption of the maxillary central incisor, *Am J Orthod.,* 114: 677-83.

Qin, C., Brunn, J.C., Cadena, E., Ridall, A., Tsujigiwa, H., Nagatsuka, H., Nagai, N. and Butler, W.T. 2002. The expression of dentin sialophosphoprotein gene in bone, *J. Dent. Res*., 81 : 392 – 394

Raghavendra, N.M., Pradeep, A.R., Kathariya, R., Sharma, A., Rao, N.S., Naik, S.B. 2012. Effect of non surgical periodontal therapy on gingival crevicular fluid and serum visfatin concentration in periodontal health and disease, *Disease Markers,* 32:383–388.

Rody, W.J. Jr, Holliday, L.S., McHugh, K.P., Wallet, S.M., Spicer, Krokhine O. 2014. Mass spectrometry analysis of gingival crevicular fluid in the presence of external root resorption, *Am J Orthod.,* 145:787-798.

Wehrbein, H., Fuhrmann, R.A., Diedrich P.R. 1995. Human histologic tissue response after long-term orthodontic tooth movement, *Am J Orthod.,* 107:360–371.

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