ORIGINAL ARTICLE

Volume 17 Issue 2 2022

DOI: 10.21315/aos2022.1702.OA07

ARTICLE INFO

Submitted: 17/12/2021
Accepted: 26/06/2022
Online: 22/12/2022

# Quantitative Assessment of Dentine Sialophosphoprotein, Aspartate Aminotransferase and Lactate Dehydrogenase in Gingival Crevicular Fluid of Teeth with Root Resorption

Noor Ayuni Ahmad Shafiai[a]*, Rohaya Megat Abdul Wahab[b]

[a]*Department of Dental Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia*

[b]*Department of Family Oral Health, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia*

*Corresponding author: noorayuni@usm.my

**To cite this article:** Shafiai NAA, Wahab RMA (2022). Quantitative assessment of dentine sialophosphoprotein, aspartate aminotransferase and lactate dehydrogenase in gingival crevicular fluid of teeth with root resorption. Arch Orofac Sci, 17(2): 237–246. https://doi.org/10.21315/aos2022.1702.OA07

**To link to this article:** https://doi.org/10.21315/aos2022.1702.OA07

## ABSTRACT

Root resorption is a shortening of root dentine which occurs physiologically in deciduous teeth. The present study aimed to quantify dentine sialophosphoprotein (DSPP), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in gingival crevicular fluid (GCF) during the physiological process of root resorption of deciduous teeth. A cross-sectional study was conducted with 25 children aged between 4 and 10 years old. GCF was collected from the gingival sulcus using periopaper strips from the upper first deciduous molar ($n$ = 45). The samples were divided equally into three groups, no resorption (R0), moderate resorption (RM) and severe resorption (RS), based on the existing radiographs taken. The GCF samples were then analysed using an enzyme-linked immunosorbent assay (ELISA) kit to determine the DSPP concentration levels and BioAssays System kit for AST and LDH. One-way ANOVA was used to determine the statistical differences between the means of the DSPP, AST and LDH concentration level in the three groups. A difference was considered significant when $p < 0.05$. High concentration levels of DSPP were significantly noted in RS ($p < 0.05$), compared to RM and R0. AST also portrayed significant high activity level ($p < 0.05$) similar to DSPP but LDH showed no significant changes between groups ($p > 0.05$). The high quantification of DSPP and AST levels in the severe and moderately resorbed roots indicated the potential use of this protein as a biomarker for detecting moderate-severe stages of root resorption.

***Keywords:*** *Aspartate aminotransferase; biomarker; dentine sialophosphoprotein; lactate dehydrogenase; root resorption*

 

## INTRODUCTION

Root resorption is a shortening of root dentine which occurs physiologically in deciduous teeth, while in permanent teeth, it is stimulus-based and initiated by injury. Traumatic dental injuries, such as intrusive luxation, extrusive luxation, lateral luxation, subluxation and concussion are considered the major potential predisposing factors for root resorption (de Souza *et al.*, 2020). Root resorption is also considered inevitable during orthodontic treatment where factors such as increase overjet, treatment with maxillary premolar extraction and lateral incisors teeth increases the risk factor for root resorption as they presume greater root displacement (Fernandes *et al.*, 2019). Some studies also suggested genetic predisposition which explain why one patient is more likely to develop root resorption than the others (Aminoshariae *et al.*, 2016; Nowrin *et al.*, 2018). The prevalence of root resorption during orthodontic treatment was reported as varying extensively, from 4% (Makedonas *et al.*, 2012) to 91% (Lund *et al.*, 2012), with few studies reporting root shortening of more than 4 mm (Maués *et al.*, 2015). Patients who are susceptible to root resorption at the beginning of treatment can progress into severe resorption during the treatment and this may limit the outcome of successful orthodontic treatment if not detected early. An early diagnosis of root resorption is the most critical factor in its management, because the earlier the intervention is initiated, the less severe the root resorption consequences will be.

Currently, radiograph has been the gold standard in detecting root resorption at orthodontic clinics. However, root resorption is usually discovered by incidental discovery or when a patient presents with signs and symptoms of tooth mobility or pain. When this occurs, the condition of resorption has usually become severe. A periapical radiograph is used as a tool for measuring root resorption as it provides less distortion and fewer superimposition errors compared to other 2D images, such as panoramic or lateral cephalometric radiographs (Ahuja *et al.*, 2017). The latest radiograph technology in dentistry, which uses cone beam computed tomography (CBCT), is a more reliable diagnostic tool to detect simulated external apical root resorption compared to periapical radiography (Ren *et al.*, 2013), as it provides the clinician with a detailed visualisation of the resorptive lesion extension. However, CBCT must only be used as an adjunct and only considered after a clinical and conventional radiographic examination (Aidos *et al.*, 2018).

Given these limitations, there is an indication of a safer, more sensitive and more prognostic diagnostic method for detecting root resorption, which is the quantification of biological markers (Yazid *et al.*, 2020). It is much safer as it does not involve radiation exposure to patients like the other 2D radiographs and CBCT. This approach is regarded as valuable as it increases the opportunity for a better understanding of the biological processes involved in root resorption. It will also help clinicians learn how to prevent, reduce and perhaps address root resorption in the future, following the development of therapies based on molecular biology and tissue engineering.

Dentine sialophosphoprotein (DSPP) consists of dentine phosphoprotein (DPP) as the C-terminal proteolytic cleavage and dentine sialoprotein (DSP) as the N-terminal. They form parts of the 10% of the organic matrix in dentine composition known as non-collagenous proteins. Several studies (Sha *et al.*, 2014; Uma & Ahmed, 2018; Zain *et al.* 2020; Mandour *et al.*, 2021) have highlighted the potential of using DSPP as a biological marker of root resorption, using samples from, for example, the permanent incisor and deciduous molar, but no research has reported on the quantification of DSPP based on the degree of root resorption severity on the same type of teeth. As mentioned, it is difficult to detect root resorption clinically, let alone to obtain permanent teeth with severe root resorption. Therefore most studies use samples with

different types of teeth; with the assumption that permanent teeth which undergo orthodontic treatment will suffer mild resorption, non-orthodontic/pre-orthodontic treatment has minimal/no root resorption (Sha *et al.*, 2014; Zain *et al.*, 2020) and the use of deciduous molar near its exfoliation time as indication of severe resorption (Mandour *et al.*, 2021). The severity of root resorption was also not confirmed with radiological investigation.

Other enzymes such as aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), are indicators of inflammatory cell death and cell necrosis respectively, and to this date, no studies have correlated these proteins to root resorption although their presence were detected in gingival crevicular fluid (GCF) during orthodontic tooth movement (Alfaqeeh & Anil, 2011; Alhadlaq, 2015) as well as periodontitis and gingival inflammation (Di Lenardo *et al.*, 2019).

Therefore, the aim of the present study was to quantify DSPP, AST and LDH in GCF in root resorption using the same type of tooth which was the upper deciduous molar, during the physiological root resorption, where the severity was confirmed by radiological findings.

## MATERIALS AND METHODS

### Subjects

The number of sample was calculated based from previous study (Kereshanan *et al.*, 2008) for 80% power (1-β error probability) and significance level of 5% (α-error probability). Twenty-five children aged between 4 and 10 years old seeking treatment at the paediatric clinic, Faculty of Dentistry, Universiti Kebangsaan Malaysia (UKM) were recruited in this cross-sectional study. The criteria for the subjects included those who had good general and periodontal health, sound upper first deciduous molar, no bleeding on probing and no plaque presents. Non-cooperative patient, consumption of anti-inflammatory drugs in the month preceding the study, teeth that were non-vital, root-treated, root fractured or ankylosed were excluded from the study. From these, 25 children, 45 sound upper first deciduous molar samples were involved, which were divided into three groups based on root resorption level: severe resorption ($n$ = 15) (RS, resorption halfway up the root to the amelocemental junction), moderate resorption ($n$ = 15) (RM, resorption from the apex up to half the root length) and no resorption ($n$ = 15) (R0, no loss of root structure) (Fig. 1). The level of resorption was determined based on existing radiographical evidence, such as an orthopantomogram, periapical radiographs or bitewing. The intraexaminer reliability of determining the root resorption level in this way was performed through re-assessments of 20 radiographs of upper first deciduous molar teeth over four weeks. The sample-taking appointments with the subjects were held not more than three months after the radiograph date.

Instructions on oral hygiene, which included presenting tooth brushing technique to the subjects and parents/guardians, were given prior to the start of the sample-taking. Plaque control was ensured in the screening visit before the sample-taking appointments were arranged. Informed consent was obtained from the parents/guardians prior to the study. The protocol was reviewed and approved by the University Research Ethical Committee of UKM (DD/2014/049(1)).

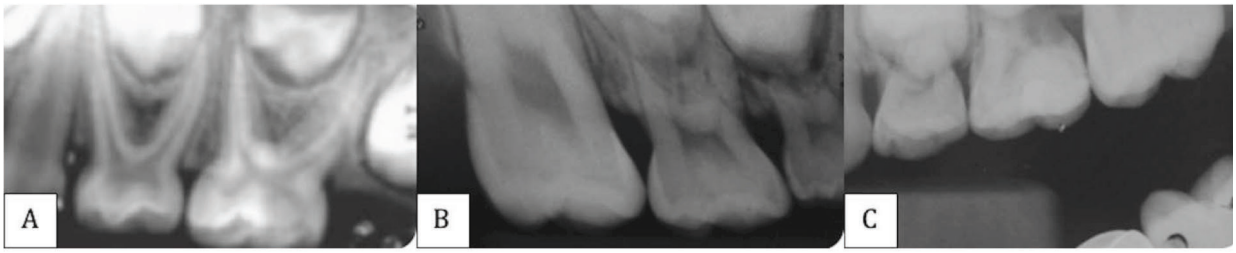


**Fig 1** Classification of root resorption severity: (A) control, no resorption (R0; intact root apex of upper first deciduous molar); (B) moderate resorption (RM; the root resorption from apex up to half the root length); (C) severe resorption (RS; the root resorption is halfway up the root to amelocemental junction).

## GCF Collection and Handling

The collection of GCF was performed by single person (NAAS). Each designated deciduous molar tooth was isolated with cotton roll and cleansed gently with gauze. Contamination of the GCF samples was prevented, as no bleeding or plaque would be allowed during the sample collection. Each crevicular sulcus on the test tooth was dried with cotton rolls and a saliva ejector was used to remove the remaining saliva. Samples of GCF were taken from the gingival crevicular sulcus at the mesial and distal sides of the teeth using periopaper strips (Periopaper; Proflow, USA). The samples were taken three times at the same side with a one-minute interval between each sampling. Each paper strip was inserted 1–2 mm into the gingival sulcus of the test teeth for 60 seconds. A total of three dipped paper strips were then placed into a 1.5 mL microcentrifuge tube containing 100 µL of protease inhibitor (20 µL/mL). A total of 400 µL of distilled water was added. The tube was then centrifuged at 4°C for 10 minutes at 400 g using a microcentrifuge (Hettich ZentrifugenMikro22R, Tuttlingen, Germany) to completely elute the GCF component from the paper strips.

## Assessment of Protein Concentration Levels

The DSPP analysis was performed according to an ELISA kit protocol by EIAb Science Co. Ltd. (China). A standard curve was constructed by plotting the log of the DSPP concentrations versus the log of the mean absorbance for each standard and the best fit line was determined by regression analysis (Microsoft Excel 2011). The DSPP concentration in each sample was determined by comparing the optical density (OD) of the samples to the standard curve provided by the equation in the regression analysis. A different standard curve was constructed for every DSPP analysis. The sensitivity detection range for the ELISA kit was between 0.31 and 20.0 ng/mL.

The analysis of AST activity on the other hand was according to protocol of BioAssay Systems EnzyChrom AST Assay Kit (Cat# EASTR-100)(USA) with sensitivity detection range between 2–100 U/L. For determination of the activity level of AST; firstly, the rate of nicotinamide adenine dinucleotide (NADH) consumption for each sample was calculated by subtracting the OD at 10 minutes from the OD at 5 minutes ($\Delta OD_S$). Similar calculation ($OD_{5\ min}–OD_{10\ min}$) was done to the rate of NADH standard ($\Delta OD_{NADH}$). The values were then incorporated into the equation according to the BioAssay Systems EnzyChrom AST Assay Kit protocol:

$$AST = 388 \times \frac{\Delta OD_S - \Delta OD_{NADH}\ (U/L)}{OD_{STD} - OD_{BLK}}$$

($OD_S$ = the rate of NADH of each sample; $OD_{NADH}$ = the rate of NADH standard;$OD_{STD} = OD_{340\ nm}$ values of NADH standard at 5 minutes; $OD_{BLK} = OD_{340\ nm}$ values of blank at 5 minutes; 388 = factor derived from calculation given in the kit)

The analysis of LDH activity was according to protocol of BioAssay Systems QuantiChrom LDH kit (DLDH-100)(USA). For measuring the LDH activity level, the assay kit provided a formula with unit of U/L, with definition of 1 unit of LDH will catalyse the conversion of 1 μmole of lactate to pyruvate per min at pH 8.2. The formula for LDH activity level for every sample was as follows:

$$\text{LDH activity} = \frac{OD_{S25} - OD_{S0}}{\varepsilon_{mtt} \cdot \iota} \times \frac{\text{Reaction Vol } (\mu L)}{\text{Time (min)} \cdot \text{Sample Vol } (\mu L)} \times n$$

$$= 43.68 \times \frac{OD_{S25} - OD_{S0}}{OD_{CAL} - OD_{H20}} \times n \ (\mu L)$$

($OD_{S25} = OD_{565\ nm}$ values of sample at 25 minutes; $OD_{S0} = OD_{565\ nm}$ values of sample at 0 minute; $\varepsilon_{mtt}$ = molar absorption coefficient of reduced MTT; $\iota$ = light path length which is calculated from the calibrator, $OD_{CAL} = OD_{565\ nm}$ values of the calibrator; $OD_{H2O} = OD_{565\ nm}$ values of the water)

Reaction Vol and Sample Vol are 200 μL and 10 μL, respectively, while n is the dilution factor. The detection limit for this kit is 2 U/L, linear up to 200 U/L.

### Statistical Analysis

All the data were analysed using SPSS® version 22.0. The Kappa for intraexaminer agreement for the root resorption level was determined. The data normality was tested using Shapiro-Wilk. Subsequently, the data were analysed with one-way ANOVA to determine the statistical differences between the means of the protein levels in various levels of root resorption severity. Differences were considered significant when $p < 0.05$.

## RESULTS

The study used 45 teeth from 25 recruited children aged between 4 and 10 years old (mean age 6.7±1.9). Of these, 53% were male and 47% were female. A Kappa intraexaminer of 0.76 was identified in the radiographic root resorption assessment, which indicated good agreement. The mean DSPP concentration level was 0.48±0.33 ng/mL in the R0 group, whereas a slightly higher mean concentration of DSPP level was recorded at RM, with 1.76±1.29 ng/mL. A mean concentration of DSPP level almost 3.4 times higher was noted in the RS group (6.07±2.22 ng/mL) (Fig. 2). The increased level in the RS group was significant when compared to the RM and R0 groups ($p < 0.05$). However, the difference between the mean concentration of DSPP levels in the RM and R0 groups was not significant ($p > 0.05$).

The AST activity levels in groups R0 was 3.90±2.68 U/L and RM 3.75±2.34 U/L; and they showed no significant difference between the groups ($p > 0.05$). However when comparison made to the RS group, they showed significant changes ($p < 0.05$). In the RS group, activity level of AST rose to 9.10±4.87 U/L, about 2.3 times higher than the R0 group ($p < 0.05$). Meanwhile for LDH activity, the R0 value of the LDH activity was 5.50±2.59 U/L and showed no significant difference ($p > 0.05$) when compared to RM (6.02±3.13 U/L). Although the amount of LDH activity in the RS (11.87±9.05 U/L) was increased about 2-fold from the R0, the elevation did not account for statistically significant ($p > 0.05$). Overall, activity level of the LDH showed no significant ($p > 0.05$) changes between various severity of root resorption (Fig. 2).

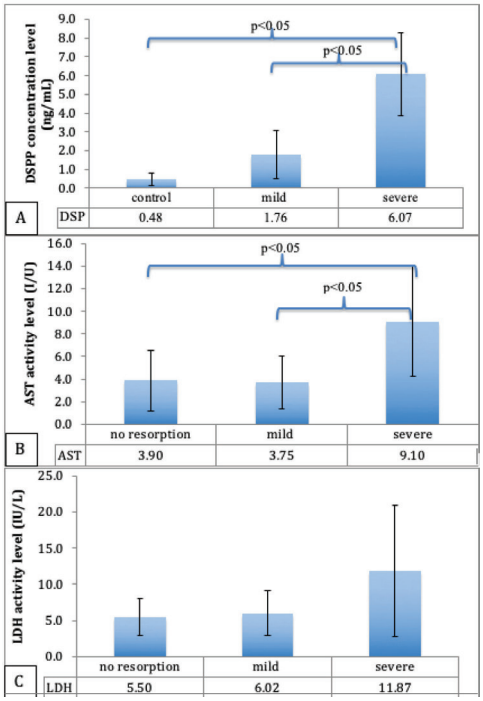


**Fig. 2** Comparison of (A) DSPP concentration level; (B) AST; (C) LDH activity level in various severity of root resorption using one-way ANOVA

Note: *Significant ($p < 0.05$), $n = 45$.

## DISCUSSION

Restriction of finding those subjects with severe root resorption during orthodontic treatment (Lund *et al.*, 2012; Maués *et al.*, 2015) leads to the present study where physiological root resorption would provide different categories of root resorption severity naturally and relate them to the level of studied proteins. The level of DSPP concentration was found to be high in the RM group and significantly higher in the RS group, as the severity of root resorption increased. This result was also found in other studies, which generally used permanent teeth before orthodontic treatment as a control, during orthodontic treatment as an indication of resorption (Sha *et al.*, 2014; Zain *et al.*, 2020), and the physiological resorption of deciduous teeth to indicate moderate/severe resorption (Mandour *et al.*, 2021). Uma & Ahmed (2018) found a significant increase in DSPP level after two months of orthodontic intrusion on maxillary central and lateral incisors.

Physiological root resorption is different from the pathologically induced as it is not associated with inflammatory mediators. However, when the root resorption is close to completion, acute and chronic inflammatory cells become evident infiltrating the dental pulp, and odontoclasts begin resorbing the exposing dentine and the predentin from the inner surface (Hassan *et al.*, 2019). Otherwise, both processes of resorption (physiological and pathological) were largely similar; which resulted from coordinated and complex interaction between cementoblasts, odontoblasts, macrophages and odontoclasts, depending on the performance of numerous systemic sites and regulatory factors. Researchers have accepted the use of physiological root resorption as a suitable model to study pathological root resorption (Tarallo *et al.*, 2019).

AST and LDH are known markers for inflammatory tissue destruction and cell necrosis (Zainal Ariffin *et al.*, 2011) and they were previously evaluated as markers in periodontitis (Di Lenardo *et al.*, 2019)

and orthodontic tooth movement (Alhadlaq, 2015). When comparing the activity levels of these two proteins against the various severity of root resorption, they both showed high level of activities in RS group, concurred with the evident of inflammatory cells during the late stage of physiological root resorption (Hassan *et al.*, 2019). As the root resorption became severe, subsequent amount of AST and LDH were released in the GCF as cell necrosis occurred during root resorption. However the changes in AST activity level were statistically significant while LDH was not. There could be some other unknown confounding factor that is affecting the level of LDH activity in root resorption which warrant further investigation.

The present study explored the DSPP, AST and LDH level in GCF using the same type of root resorption, which was the physiological root resorption on an upper first deciduous molar tooth. The upper first deciduous molar was the tooth of choice as its root completion occurs at the age of 2.5 years old. The estimated occurrence of root resorption of the tooth is between the duration of crown completion for the successor, which is around 5 to 6 years old, and the eruption time, which is around 10 to 11 years of age. Peretz *et al.* (2013) reported that lower first deciduous molars begin to resorb at 7 years old, from their observation of the radiographs of 217 patients aged between 5 and 12 years old. The factor of moist control was also considered when choosing the type of tooth to sample, as the second deciduous molar is located posteriorly further, while the lower first molar is harder to isolate as saliva tends to pool on the lower arch. It is very important to ensure the protocol of ensuring no contamination of GCF by saliva or blood were involved. Besides saliva control, these patients were examined for good oral hygiene and low gingival inflammation, to avoid collection of blood during GCF sampling which would cause invalid result and to minimise the repetition of sampling as the present study dealt with children.

The strength of the present study was the references for the root resorption categorisation; utilising the existing radiographs which included the periapical radiographs, the orthopantomogram and bitewing. Most studies of biomarkers of root resorption did not confirm the severity of root resorption. Ahuja *et al.* (2017) compared the amount of root resorption between the two types of radiographs of patients before and after orthodontic treatment, and they concluded that the orthopantomogram portrayed an exaggeration of root resorption. However, the significant difference was only noted in the lower incisors. Another study mentioned that more than 20% of the material loss in the root was noted in the orthopantomogram in comparison to the periapical radiographs, due to the difference in the position of the focal spot in relation to the root (Dindaroğlu & Doğan, 2016). However, it is not common for children to have periapical radiographs taken, hence the inclusion of the orthopantomogram in the present study. The assessment of the shape of the root was also reported to be more difficult with the orthopantomogram (Ahuja *et al.*, 2017). The present study, however, did not quantify the amount of root resorption, but rather categorised it into none, moderate and severe using the root length and amelocemental junctions as guidance. Murthy *et al.* (2020) used the remaining root length to classify the root resorption observed in extracted deciduous teeth as stage 1: resorption only in the apical third; stage 2: resorbed until the middle third of the root; and stage 3: resorbed until the cervical third of the root. The categorisation of root resorption in the present study would be more reliable if interexaminer calibration were involved, however 0.76 Kappa values of intraexaminer calibration indicated good reproducibility of these categorisation.

Two of the challenges in collecting the GCF samples from the children were, firstly, moisture control to ensure that the GCF samples were not contaminated with

saliva and blood and, secondly, keeping the children still for at least one minute. As a significant amount of GCF collection can be difficult to achieve, in the future, the use of an electrochemical ELISA for taking GCF samples with lower concentrations can be considered as this has a detection limit of 0.5 pg per millimetre, compared to the 5 pg per millimetre in a traditional spectrophotometric ELISA (Sha *et al.*, 2014).

DSPP was recommended (Zainal Ariffin *et al.*, 2011) as a potential marker of root resorption as it is released into the GCF as a consequence of dentine breakdown due to root resorption. The present study supports this recommendation, and it is proposed that DSPP and AST quantification from this study are added to the biomarker quantification for assessing the severity of root resorption where the higher the amount of DSPP and AST indicates much severe form of root resorption. The present study offers openings for further endeavours to find good candidate proteins to use in future monitoring kits for the identification of teeth at risk of root resorption, to be use clinically, without depending only on the radiological findings. It is recommended that a longitudinal study to be conducted to monitor the patterns of DSPP and AST with active root resorption severity changes.

## CONCLUSION

There were significant increase in the DSPP concentration level and AST activity level as root resorption became more severe while LDH activity level showed no significant changes.

## ACKNOWLEDGEMENTS

This work was supported by the Ministry of Higher Education, Malaysia, through Prototype Research Grant Scheme (PRGS) (PRGS/1/2018/SKK14/UKM/01/1).

## REFERENCES

Ahuja PD, Mhaske SP, Mishra G, Bhardwaj A, Dwivedi R, Mangalekar SB (2017). Assessment of root resorption and root shape by periapical and panoramic radiographs: A comparative study. *J Contemp Dent Pract*, **18(**6): 479–483. https://doi.org/10.5005/jp-journals-10024-2069

Aidos H, Diogo P, Santos JM (2018). Root resorption classifications: A narrative review and a clinical aid proposal for routine assessment. *Eur Endod J*, **3**(3): 134–145. https://doi.org/10.14744/eej.2018.33043

Alfaqeeh SA, Anil S (2011). Lactate dehydrogenase activity in gingival crevicular fluid as a marker in orthodontic tooth movement. *Open Dent J*, **5**: 105–109. https://doi.org/10.2174/1874210601105010105

Alhadlaq AM (2015). Biomarkers of orthodontic tooth movement in gingival crevicular fluid: A systematic review. *J Contemp Dent Pract*, **16**(7): 578–587. https://doi.org/10.5005/jp-journals-10024-1725

Aminoshariae A, Aminoshariae A, Valiathan M, Kulild JC (2016). Association of genetic polymorphism and external apical root resorption. *Angle Orthod*, **86**(6): 1042–1049. https://doi.org/10.2319/011916-50.1

de Souza BDM, Dutra KL, Reyes-Carmona J, Bortoluzzi EA, Kuntze MM, Teixeira CS *et al.* (2020). Incidence of root resorption after concussion, subluxation, lateral luxation, intrusion, and extrusion: A systematic review. *Clin Oral Investig*, **24**(3): 1101–1111. https://doi.org/10.1007/s00784-020-03199-3

Di Lenardo D, Silva FRPD, de Carvalho França LF, Carvalho JDS, Alves EHP, Vasconcelos DFP (2019). Evaluation of biochemical parameters present in the saliva of patients with chronic periodontitis: Results from a meta-analysis. *Genet Test Mol Biomarkers*, **23**(4): 255–263. https://doi.org/10.1089/gtmb.2017.0272

Dindaroğlu F, Doğan S (2016). Root resorption in orthodontics. *Turk J Orthod*, **29**(4): 103–108. https://doi.org/10.5152/TurkJOrthod.2016.16021

Fernandes LQP, Figueiredo NC, Montalvany Antonucci CC, Lages EMB, Andrade I Jr, Capelli Junior J (2019). Predisposing factors for external apical root resorption associated with orthodontic treatment. *Korean J Orthod*, **49**(5): 310–318. https://doi.org/10.4041/kjod.2019.49.5.310

Hassan GS, Zahran DH, Saleh RG (2019). Comparison of root resorption pattern in human primary teeth under physiologic and coexistent pathological conditions. *Egypt Dent J*, **65**(2): 1349–1358. https://doi.org/10.21608/EDJ.2019.72559

Kereshanan S, Stephenson P, Waddington R (2008). Identification of dentine sialoprotein in gingival crevicular fluid during physiological root resorption and orthodontic tooth movement. *Eur J Orthod*, **30**(3): 307–314. https://doi.org/10.1093/ejo/cjn024

Lund H, Gröndahl K, Hansen K, Gröndahl HG (2012). Apical root resorption during orthodontic treatment. *Angle Orthod*, **82**(3): 480–487. https://doi.org/10.2319/061311-390.1

Makedonas D, Lund H, Gröndahl K, Hansen K (2012). Root resorption diagnosed with cone beam computed tomography after 6 months of orthodontic treatment with fixed appliance and the relation to risk factors. *Angle Orthod*, **82**(2): 196–201. https://doi.org/10.2319/112810-691.1

Mandour KAA, Tawfeek MA, Montasser MA (2021). Expression of biological markers in gingival crevicular fluid of teeth with orthodontically induced root resorption. *J Orofac Orthop*, **82**(5): 313–320. https://doi.org/10.1007/s00056-020-00267-x

Maués CP, do Nascimento RR, Vilella Ode V (2015). Severe root resorption resulting from orthodontic treatment: Prevalence and risk factors. *Dental Press J Orthod*, **20**(1): 52–58. https://doi.org/10.1590/2176-9451.20.1.052-058.oar

Murthy P, Bhojraj N, Hegde U (2020). Pattern of physiologic root resorption in deciduous teeth at different stages. *J Clin Diagnostic Res*, **14**(6): ZC18–ZC22. https://doi.org/10.7860/JCDR/2020/44517.13751

Nowrin SA, Jaafar S, Ab Rahman N, Basri R, Alam MK, Shahid F (2018). Association between genetic polymorphisms and external apical root resorption: A systematic review and meta-analysis. *Korean J Orthod*, **48**(6): 395–404. https://doi.org/10.4041/kjod.2018.48.6.395

Peretz B, Nisan S, Herteanu L, Blumer S (2013). Root resorption patterns of primary mandibular molars and location of the premolar successors: A radiographic evaluation. *Pediatr Dent*, **35**(5): 426–429.

Ren H, Chen J, Deng F, Zheng L, Liu X, Dong Y (2013). Comparison of cone-beam computed tomography and periapical radiography for detecting simulated apical root resorption. *Angle Orthod*, **83**(2): 189–195. https://doi.org/10.2319/050512-372.1

Sha H, Bai Y, Li S, Wang X, Yin Y (2014). Comparison between electrochemical ELISA and spectrophotometric ELISA for the detection of dentine sialophosphoprotein for root resorption. *Am J Orthod Dentofacial Orthop*, **145**(1): 36–40. https://doi.org/10.1016/j.ajodo.2013.09.008

Tarallo F, Chimenti C, Paiella G, Cordaro M, Tepedino M (2019). Biomarkers in the gingival crevicular fluid used to detect root resorption in patients undergoing orthodontic treatment: A systematic review. *Orthod Craniofac Res*, **22**(4): 236–247. https://doi.org/10.1111/ocr.12329

Uma HL, Ahmed N (2018). Identification of dentine sialophosphoprotein in gingival crevicular fluid to assess root resorption using three piece base arch. *IOSR J Dent Med Sci*, **17**(1): 56–63.

Yazid F, Teh Y, Ashari A, Ariffin SHZ, Wahab RMA (2020). Detection methods of orthodontically induced inflammatory root resorption (OIIRR): A review. *Australas Orthod J*, **36**(1): 101–107. https://doi.org/10.21307/aoj-2020-012

Zain MNM, Yusof ZM, Yazid F, Ashari A, Wong KSH, Lee WJ *et al.* (2020). Absorption spectrum analysis of dentine sialophosphoprotein (DSPP) in orthodontic patient. *AIP Conf Proc*, **2203**: 020007. https://doi.org/10.1063/1.5142099

Zainal Ariffin SH, Yamamoto Z, Zainol Abidin IZ, Megat Abdul Wahab R, Zainal Ariffin Z (2011). Cellular and molecular changes in orthodontic tooth movement. *ScientificWorldJournal*, **11**: 1788–1803. https://doi.org/10.1100/2011/761768