ASSOCIATION OF INTERLEUKIN GENE POLYMORPHISM AND CLINICAL VARIABLES WITH EXTERNAL APICAL ROOT RESORPTION AFTER INITIAL STAGE OF ORTHODONTIC TREATMENT AMONG MALAY POPULATION

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A	Adenine
AD	Alzheimer's disease
BMMs	Bone marrow macrophages
bp	Base pair
C	Cytosine
C_1	Crown length before starting the treatment
C_2	Crown length after levelling and alignment stage of the treatment
CASP1	Caspase 1
CB	Conventional bracket
CBCT	Cone beam computed tomography
CD	Cluster of differentiation
CEJ	Cemento-enamel junction
CF	Correction factor
CP	Chronic periodontitis
CT	Computed tomography
df	Degrees of freedom
dH_2O	Distilled water
ddH_2O	Double distilled water
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphate
EARR	External apical root resorption

EDTA	Ethylenediaminetetraacetic acid
ERR	External root resorption
FDI	Federation Dentaire Internationale
G	Guanine
HCl	Hydrogen chloride
ICC	Intra-class correlation
IL	Interleukin
IL-1A	Interleukin-1A
IL-1B	Interleukin-1B
IL-1RN	Interleukin-1RN
IL-1ß	Interleukin-1 beta
IL-1α	Interleukin-1 alpha
IL-ra	Interleukin-1 receptor antagonist
IQR	Interquartile range
IRR	Internal root resorption
IκB	Inhibitor of kappa B
JEPeM	Jawatankuasa Etika Penyelidikan Manusia (Human Research Ethics Committee)
K	Kappa score
Kb	Kilobase
Lat. Ceph	Lateral cephalogram
M1	Activated macrophage
M2	Alternatively activated macrophage
$MgCl_2$	Magnesium chloride
ml	Millilitre

mM	Millimolar
mm	Millimetre
mRNA	Messenger ribonucleic acid
n	Total number
NFKB	Nuclear factor κ B
NO	Nitric oxide
ºC	Degree celsius
OPN	Osteopontin
OPG	Orthopantomogram
OR	Odds ratio
P	P value
P′	Anticipated population proportion
P2RX7	Purinergic receptor P2X 7
Pa	Periapical radiographs
PCR	Polymerase chain reaction
PCR-RFLP	polymerase chain reaction - restriction fragment length polymorphism
PDL	Periodontal ligament
pH	Power of hydrogen
R_1	Root length before starting the treatment
R_2	Root length after levelling and alignment stage of the treatment
RANK	Receptor activator for nuclear factor kappa B
RANKL	Receptor activator for nuclear factor kappa B ligand
RCT	Randomised control trial

RE	Restriction enzyme
RNase A	Ribonuclease A
SD	Standard deviation
SL	Self-ligating bracket
SNP	Single nucleotide polymorphism
S-W	Shapiro-Wilk
T	Thymine
TBE	Tris-Borate- Ethylenediaminetetraacetic acid
Th1	T Helper cell Type 1
Th2	T Helper cell Type 2
TNFRSF11A	Tumour necrosis factor receptor superfamily member 11a
TNF-α	Tumour necrosis factor-alpha
TNSALP	Tissue-nonspecific alkaline phosphatase
TRAP	Tartrate resistant acid phosphatase
Tris-HCL	(hydroxymethyl) aminomethane hydrochloride
U	Unit
VDR	Vitamin D receptor
VNTR	Variable number of tandem repeat
γ	Gamma
%	Percent
µl	Microlitre
2D	Two dimension
3D	Three dimension

KAITAN ANTARA POLIMORFISME GEN INTERLEUKIN DAN VARIASI KLINIKAL DENGAN RESORPSI AKAR APIKAL LUARAN SELEPAS PERINGKAT AWAL RAWATAN ORTODONTIK DALAM KALANGAN POPULASI MELAYU

ABSTRAK

Pelbagai manfaat boleh didapati daripada rawatan ortodontik dan dalam kebanyakan kes, manfaatnya mengatasi kelemahan yang mungkin ada. Resorpsi akar apikal luaran (EARR) merupakan satu fenomena buruk yang lazim berkait dengan rawatan ortodontik. Kejadian ini boleh berlaku disebabkan oleh beberapa faktor mekanikal seperti daya retraksi yang kuat, ekstrusi-intrusi atau tempoh rawatan yang lama. Bagi faktor biologi pula, kerentanan genetik seperti polimorfisme dalam kluster gen interleukin-1 (IL-1) dikatakan dapat meningkatkan risiko EARR. Oleh demikian, kajian ini menyelidik perkaitan gen polimorfisme IL-1A, IL-1B dan IL-1RN dengan EARR selepas peringkat pertama rawatan ortodontik berakhir. Seramai 44 subjek (min umur 21.79±3.83 tahun) yang terlibat dalam kajian ini merupakan pesakit yang menjalani rawatan ortodontik menggunakan dua jenis sistem braket yang berlainan. Sapuan oral diambil untuk pengenotipan dan semua subjek digenotip menggunakan tindak balas reaksi rantai polimerase - polimorfisme kepanjangan serpihan pembatasan (PCR-RFLP) untuk gen IL-1A dan IL-1B dan tindak balas reaksi rantai polimerase konvensional untuk gen IL-1RN. EARR pesakit yang terlibat dalam kajian ini diukur daripada radiograf pancaran kon tomografi berkomputer yang diambil pada peringkat awal dan selepas peringkat pertama rawatan ortodontik. Kolerasi antara kumpulan (ICC) dan ujian kappa masing-masing dijalankan untuk memastikan

kebolehpercayaan ukuran EARR dan pengenalan genotip. Statistik deskriptif digunakan untuk merumus frekuensi relatif dan mutlak EARR dalam setiap gigi anterior. EARR untuk gigi kontralateral dan ipsilateral dibandingkan dengan menggunakan ujian Wilcoxon. Sementara itu, ujian Mann-Whitney pula dilakukan untuk membanding bacaan EARR daripada dua kumpulan berbeza: jantina, sistem braket, klasifikasi skeletal dan EARR kumpulan kawalan. Regressi logistik binari digunakan untuk menilai gangguan daripada faktor pembauran klinikal seperti umur, modaliti rawatan, jantina, tempoh rawatan dan hubungan skeletal. Taburan genotip dan frekuensi allelic IL-1A, IL-1B dan IL-1RN gen polimorfisme dalam subjek kedua kumpulan EARR dan kumpulan kawalan dianalisis menggunakan ujian Chi-square. Regresi logistik univariat dan multivariat digunakan untuk mengenalpasti perkaitan genetik dengan EARR dalam rawatan ortodontik. Keputusan ICC dan ujian kappa menunjukkan terdapat korelasi yang kuat antara pemeriksaan dan intra pemeriksaan mengikut ukuran EARR dan pengecaman genotip. Keputusan analisis statistik diskriptif menunjukkan terdapat 96.9% daripada semua akar anterior menunjukkan resorpsi akar selepas peringkat pertama rawatan. Sebanyak 9.7% gigi anterior pada kesemua subjek mempunyai EARR ≥2 mm setelah peringkat awal rawatan. Ujian Wilcoxon menunjukkan tidak terdapat perbezaan secara statistik antara EARR sebelah kiri dan kanan. Namun begitu, gigi kacip lateral kanan menunjukkan perbezaan yang signifikan apabila dibandingkan dengan gigi ipsilateral. Ujian Mann-Whitney tidak menunjukkan perbezaan yang signifikan dalam ukuran EARR antara jantina dan sistem braket. Kajian juga mendapati bahawa gigi taring bawah kiri menunjukkan peningkatan dalam EARR dalam hubungan dengan skeletal kelas I berbanding hubungan skeletal kelas II. Selain itu, juga didapati gigi kacip lateral kanan, gigi kacip tengah bawah kiri dan gigi taring mempunyai penyerapan EARR lebih besar ≥2 mm

daripada kumpulan kawalan (EARR <2 mm). Analisis chi-square dan regresi logistik dalam kohort kajian ini menunjukkan tidak terdapat perkaitan signifikan antara polimorfisme gen dan EARR yang menandakan bahawa polimorfisme gen IL-1A, IL-1B dan IL-1RN tidak menyumbang secara langsung kepada kecenderungan EARR. Faktor risiko keupayaan untuk mendapat EARR semasa rawatan ortodontik tidak menunjukkan perkaitan dengan gen IL-1A, IL-1B dan IL-1RN dalam populasi kajian ini.

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ABSTRACT

Orthodontic treatment brings numerous benefits and in most cases, the benefits outweigh the possible disadvantages. External apical root resorption (EARR) is a common adverse phenomenon associated with orthodontic treatment. It can be caused by some mechanical factors such as heavy retraction force, extrusion-intrusion or prolonged treatment duration. For biological factors, a genetic susceptibility such as polymorphisms in the interleukin-1 (IL-1) gene cluster have been implicated to increase the risk of EARR. Therefore, this study investigated the association of IL-1A, IL-1B and IL-1RN gene polymorphisms and EARR after finishing the initial (levelling and alignment) stage of orthodontic treatment. A total of 44 subjects (mean age 21.79 ± 3.83 years) who were undergoing orthodontic treatment with two different types of bracket system (conventional and self-ligating) were involved in this current study. Buccal cell swab was taken for genotyping and all subjects were genotyped by using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) for IL-1A and IL-1B genes and conventional PCR for IL-1RN gene. EARR in patients recruited for this study were measured from the cone beam computed tomography (CBCT) radiographs that were taken at the beginning of orthodontic procedures and after finishing the initial stage of orthodontic treatment. Intra-class correlation (ICC) and kappa test were then performed to ascertain the reliability of these EARR

measurements and genotypes identification, respectively. Descriptive statistics were used for summarising the absolute and relative frequency of EARR in each anterior tooth. The EARR of contralateral and ipsilateral tooth was then compared using the Wilcoxon test. Meanwhile, Mann-Whitney test was performed to compare the EARR of two independent groups; gender, bracket system, skeletal classification and EARR versus control group. Binary logistic regression was used to assess interference from clinical confounding factors such as age, treatment modalities, sex, treatment duration and skeletal relationship. The distribution of genotypes and the allelic frequency of IL-1A, IL-1B and IL-1RN gene polymorphisms in the subjects of both EARR and control group was analysed using Chi-square test. Univariate and multivariate logistic regression were used to identify the genetic association with EARR in orthodontic treatment. Results of ICC and kappa test showed strong correlations between inter-examiner and intra-examiner in regards to EARR measurements and genotypes identification. The outcome of descriptive statistics analysis showed that a total of 96.9% of all anterior teeth manifested the root resorption after initial stage of the treatment. A total of 9.7% of the anterior teeth among all subjects possessed EARR of ≥2 mm after the initial stage of treatment. Wilcoxon test showed no statistical difference between the EARR of left and right side. However, right lateral incisor showed a significant difference when compared to the ipsilateral tooth. Mann-Whitney test did not show any significant differences between gender and between bracket system of EARR measurements. It was noted that lower left canine displayed increased EARR in Class I skeletal relation than the Class II skeletal relation. Moreover, it was also noted that right lateral incisors, lower left central incisor and canines possessed greater EARR resorptions of ≥2 mm than the control group (EARR <2 mm). Chi-square and logistic regression analyses within this study cohort showed no significant

association between gene polymorphisms and EARR indicated that IL-1A, IL-1B and IL-1RN gene polymorphisms did not directly contribute to predisposition of EARR. The potential risk factors for EARR during orthodontic treatment is not associated with the IL-1A, IL-1B and IL-1RN gene in this study population.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Malocclusion is a common phenomenon which could be seen in most of the people around the world. Incorrect relationship between the teeth of both arches and/or misalignment of the teeth is considered as a malocclusion (Proffit *et al.*, 1992; Sarver *et al.*, 2000). The classification of malocclusion was introduced in the 1899's which made a significant influence in the development of orthodontics treatment based on the malocclusion. According to the classification of malocclusion, when mesiobuccal cusp of upper first permanent molar occludes with the mesiobuccal groove of lower permanent first molar it is termed as normal or ideal occlusion. Any variation from this state will cause different types of malocclusion (Proffit *et al.*, 2006). This condition usually could be recognised at an early age and gradually manifests with growth which forces an individual to seek an orthodontic treatment. People having malposition of the teeth might affect the self-confidence which then disturb their career and personality as an individual. Therefore, the primary concern of the orthodontic treatment is to improve the dentofacial aesthetics (Ackerman *et al.*, 2007).

Orthodontic treatments based on the status of malocclusion are widely recommended and easily available due to the rising awareness of people towards the correction of malposition of teeth. The average duration for orthodontic treatment with fixed braces is about 2 to 3 years (Tsichlaki *et al.*, 2016). However, subjects who are

undergoing orthodontic treatment do not partake with the patience of continuing treatment for more than 1.5 years (Sayers and Newton, 2007). Moreover, longer treatment duration is unfavourable for patients according to the British National Health Service (General Dental Services) (Turbill *et al.*, 2001). Just like many other treatments, orthodontic intervention with fixed braces pose some innate risks and complications. Excessive force application to the tooth during treatment in order to achieve the desired tooth movement causes some undesirable outcomes, such as root resorption, delayed tooth movement, pain, mobility and sometimes loss of vitality of the tooth (Talic, 2011).

External apical root resorption (EARR) is one of the most common adverse effects of all types of orthodontic treatment which might start in any stage of the treatment such as initial, retraction and finishing phase. Root resorption might commence about 7 weeks of treatment; however, it takes 5-6 months to be identified in any radiographs (Gülden *et al.*, 2009). Although many aspects of this undesirable effect remain unclear, it is a complex biological process which happens when the force generation at the root apex area exceeds the resistance and reparative aptitude of the periapical tissues (Brezniak and Wasserstein, 2002). Genetic influence is one of the most important risk factors attributed to the root resorption during an orthodontic treatment (Ngan, 2003; Al-Qawasmi *et al.*, 2004; Hartsfield *et al.*, 2004).

Genetic evaluation is required in order to identify the exact aetiology of root resorption. Many studies have been carried out to examine the genetic influence in the development of EARR in subjects undergoing orthodontic treatment. In addition, a few clinical studies confirmed the association between genetic factor and EARR (Al-Qawasmi *et al.*, 2003b; Fontana *et al.*, 2012; Gülden *et al.*, 2009; Guo *et al.*, 2016;

Iglesias-Linares *et al.*, 2012; Lages *et al.*, 2009; Linhartova *et al.*, 2013; Sharab *et al.*, 2015). Various genes and their polymorphisms might play crucial roles in some extent to the severity and susceptibility of EARR (Wakeland *et al.*, 2001).

Various studies were conducted on different gene polymorphisms such as interleukins (ILs), Vitamin D receptor (VDR), Tumour Necrosis Factor-alpha (TNF-α) and Cluster of Differentiation (CD) genes which might be associated with periodontitis in certain populations (Vijayalakshmi *et al.*, 2010). In spite of the different etiological factors, periodontitis and EARR share similar cellular and molecular pathways which involve bone metabolism and inflammation, specifically those dependent on IL cytokines. Among the 11 members of IL-1 cytokine family, IL-1A and IL-1B are the most frequently studied in periodontal diseases (Netea *et al.*, 2015). IL-1 cytokines partake in host response of infection such as endogenous molecule which is released by dead cells. These are concerned with the aetiology of both periodontitis and EARR (Dinarello *et al.*, 2012). As EARR and periodontitis co-related with each other, therefore, genes related with periodontitis are frequently found associated with EARR. Some studies revealed that polymorphism of genes which were associated with periodontitis such as IL-1A (-889) and IL-1B (+3954) genes also increase the risk of EARR in the orthodontic subjects treated with fixed orthodontic appliances (Al-Qawasmi *et al.*, 2003b; Iglesias-Linares *et al.*, 2012; Lages *et al.*, 2009).

The IL-1 gene cluster on human chromosome 2q13 consists of three genes. Two genes (IL-1A and IL-1B) encode pro-inflammatory cytokine proteins Interleukin-1 alpha (IL-1α) and Interleukin-1 beta (IL-1ß), respectively. Moreover, the third gene Interleukin-1RN (IL-1RN) encodes an associated protein Interleukin-1 receptor

antagonist (IL-1ra) which acts as a receptor antagonist (Linhartova *et al.*, 2013). All three genes, IL-1A, IL-1B and IL-1RN are located in the chromosome 2 in 7.5 kb region, whereas IL-1A and IL-1B are composed of seven exons and IL-1RN is composed of eleven exons. Translation and transcription length of IL-1B gene is 269 residues and 1,498 bps, respectively (Giraldo *et al.*, 2009). During the orthodontic tooth movement, the existence of IL-1 in the periodontal tissue additionally increases the tissue resorption (Hartsfield Jr, 2009; Iglesias-Linares and Hartsfield Jr, 2017; Kalra *et al.*, 2020). Furthermore, IL is an effective bone-resorptive cytokine which is a major factor of the multifaceted signalling pathways leading to the resorption of roots and IL-1B acts as a decisive role in the progression of any periapical lesions (Hartsfield Jr, 2009; Iglesias-Linares and Hartsfield Jr, 2017). Therefore, many studies have been conducted on these IL-1 genes to identify its association with EARR which is a result from orthodontic tooth movement. However, the outcomes were not consistent and contradictory. While most of the studies showed a positive association with the IL-1 gene in different populations, many other investigations indicated a negative association.

It is widely known that frequencies of single nucleotide polymorphism (SNP) vary among different populations and is mostly ethnicity dependent (Wang *et al.*, 2008). Therefore, the ethnic factor is the most important to evaluate the predisposition of any disease (Sameshima and Sinclair, 2001a; Wang *et al.*, 2008). Many polymorphisms have been defined in IL-1 genes where many of them might contribute to the initiation of EARR. A previous study reported that messenger ribonucleic acid (mRNA) and IL-1α protein levels in the plasma increased due to the increased transcriptional activity of the homozygous genotype of the IL-1A gene (Dominici *et al.*, 2002). Similarly, a specific allele of SNP at the positioned +3954 in the IL-1B gene

also increased the production of IL-1ß cytokine (Linhartova *et al.*, 2013). On the other hand, for IL-1RN gene, functional polymorphism has been recognised to be associated with various copies of 86 nucleotides repeat sequences situated in the chromosome 2. A total of five alleles containing two to six repeats of nucleotide can be defined and resulting in either short alleles with two repeats or long allele with three to six repeats. However, conflicting results have been observed from this polymorphism where one copy of allele 2 of IL-1RN gene has been associated with both increase and decrease of the secretion of IL protein (Rafiq *et al.*, 2007; Vamvakopoulos *et al.*, 2002). An investigation on the IL-1RN gene stated that not only the variable number of tandem repeat (VNTR) in its intron 2 but also many other variants in the IL-1RN gene is responsible for increasing the IL-1ra production (Rafiq *et al.*, 2007).

It was noted that various studies have been performed to identify the genetic association and EARR in many different populations at the end of orthodontic treatment (Al-Qawasmi *et al.*, 2003b; Fontana *et al.*, 2012; Gülden *et al.*, 2009; Guo *et al.*, 2016; Iglesias-Linares *et al.*, 2012; Lages *et al.*, 2009; Linhartova *et al.*, 2013; Sharab *et al.*, 2015). However, different stages of orthodontic treatment might contribute differently on EARR development. Due to these inconsistent findings of the IL-1 gene association with EARR, additional studies are warranted especially in different populations or ethnic groups. Therefore, in the current study, the association between a polymorphism in the IL-1A (-889), IL-1B (+3954) and IL-1RN (VNTR) genes and EARR after levelling and alignment stage of orthodontic treatment was conducted.

1.2 Justification of the study

Many studies have demonstrated that EARR is strongly influenced by genetic factors along with several clinical and environmental factors (Al-Qawasmi *et al.*, 2003b; Harris *et al.*, 1997; Lages *et al.*, 2009; Nieto-Nieto *et al.*, 2017). Moreover, other investigations have proposed that positive association exist between gene polymorphisms and EARR in orthodontic treatment. However, various mechanical factors might also involve in EARR during orthodontic treatment such as excessive force, intrusion, extrusion and root tipping. These mechanical factors usually applied during the retraction stage or finishing stage of orthodontic treatment which might overstated the development of root resorption and the genetic associations. Therefore, to distinguish the genetic effects and mechanical effects on EARR, genetic studies should be conducted at a stage during orthodontic treatment where the mechanical factors presumably have less effect and interference to the outcome of genetic investigation. Perhaps, at the initial stage or the levelling and alignment stage of orthodontic treatment, a mechanism that leads to enhancing the EARR such as the application of heavy force is not much applicable. Hence, after finishing the levelling and alignment of orthodontic treatment, genetic factors can be notably seen and its associations with EARR can be established as minimal mechanical factors are involved. Therefore, genetic analysis in orthodontic patients until levelling and alignment stage of orthodontic treatment has been selected for this current investigation. As mentioned earlier, the genetic association is considered as ethnicity dependent. To the best of our knowledge, no study has ever been conducted in Malaysian population in relation to gene polymorphism and EARR during orthodontic treatment.

Apart from genetic and environmental influences, clinical variables such as age, gender, ethnic background, tooth extraction pattern, types of bracket system during orthodontic treatment, skeletal pattern, and duration of orthodontic treatment also play important roles in the development of EARR (Castro *et al*., 2012; Jiang *et al*., 2010; Lund *et al*., 2011; Gay *et al*., 2017; Pandis *et al*., 2008; Mohandesan *et al*., 2007; Chen *et al*., 2015). Therefore, study on the association of both genetic and clinical variables with EARR provides better understanding on the factors that influence the development of EARR during orthodontic treatment.

Identification of the positive association between IL-1 genes and EARR will provide a positive impact to both the scientific and clinical contexts of EARR. For the orthodontists, better understanding of the aetiology of EARR could facilitate them to modify treatment plan to minimise unwanted outcomes in relation to EARR during orthodontic treatment. Moreover, the effects of clinical confounding factors such as age, gender, duration of the treatment, skeletal classification and bracket systems on EARR would also enhance the understanding of the EARR development and can benefit the treatment of orthodontic. As EARR measurement in different stages of orthodontic treatment predicted to show different degrees of EARR, assessing the EARR after the initial stage would provide the important information to what extend the EARR develop in other stages of orthodontic treatment. Apart from that, it has been reported that the susceptibility of certain condition is varying within different ethnic backgrounds, it is hoped that this current study could establish the contribution of IL-1 genes on EARR in Malaysian population.

1.3 Objectives

1.3.1 General objective

To investigate the association of IL-1A (-889), IL-1B (+3954) and IL-1RN (VNTR) gene polymorphism and clinical variables with EARR until the initial stage in patients who are undertaking orthodontic treatment.

1.3.2 Specific objectives

1. To describe the incidence of EARR and association of clinical variables (age, gender, skeletal classification, bracket system and duration of finishing the initial stage) in patients who are undertaking orthodontic treatment.
2. To study the genotype and allelic frequency of IL-1A (-889) gene polymorphism and its association in patients with EARR who are undertaking orthodontic treatment.
3. To study the genotype and allelic frequency of IL-1B (+3954) gene polymorphism and its association in patients with EARR who are undertaking orthodontic treatment.
4. To study the frequency of genotype and allelic frequency of IL-1RN (VNTR) gene polymorphism and its association in patients with EARR who are undertaking orthodontic treatment.

1.4 Research questions

1. What is the incidence of EARR and association of clinical variables (age, gender, skeletal classification, bracket system and duration of finishing initial stage) in patients who are undertaking orthodontic treatment?
2. What is the genotype and allelic frequency of IL-1A (-889) gene polymorphism and its association in patients with EARR who are undertaking orthodontic treatment?
3. What is the genotype and allelic frequency of IL-1B (+3954) gene polymorphism and its association in patients with EARR who are undertaking orthodontic treatment?
4. What is the genotype and allelic frequency of IL-1RN (VNTR) gene polymorphism and its association in patients with EARR and who are undertaking orthodontic treatment?

1.5 Research hypothesis

1. There is a specific distribution of EARR and association of clinical variables (age, gender, skeletal classification, bracket system and duration of finishing initial stage) in patients who are undertaking orthodontic treatment.
2. There is a specific distribution of genotype and allelic frequency of IL-1A (-889) gene polymorphism and an association in patients with EARR who are undertaking orthodontic treatment.

3. There is a specific distribution of genotype and allelic frequency of IL-1B (+3954) gene polymorphism and an association in patients with EARR and who are undertaking orthodontic treatment.

4. There is a specific distribution of genotype and allelic frequency of IL-1RN (VNTR) gene polymorphism and an association in patients with EARR and who are undertaking orthodontic treatment.

CHAPTER 2

LITERATURE REVIEW

2.1 Orthodontic treatment for malocclusions

Orthodontic treatment is one of the foremost solutions for correcting the malocclusion regardless of its types and classifications. However, different types of indices were already established with the intention of classifying malocclusion to many groups depending on the need and urgency of the treatment (Brook and Shaw, 1989). During orthodontic treatment, the continual and balanced process of resorption and deposition of alveolar bone occurs surrounding the tooth; in response to the force applied. The applied force causes compression of periodontal ligament (PDL) on one side, stretch or tension in the PDL of the opposite side (Dolce *et al.*, 2002). Ultimately, this result in remodelling of the dental tissues and its surrounding supporting tissues including pulp, periodontal ligament, alveolar bone, and gingiva (Zainal Ariffin *et al.*, 2011).

Most of the patients with malocclusion are treated with fixed orthodontic treatment. Orthodontic treatment can be relied upon to achieve a good outcome for most patients with mild to severe skeletal bases. Management of any malocclusion depends on three important factors: age of the patient, the nature and severity of the problem and the underlying etiologic factors from the clinical and functional examination. Therefore, the management of malocclusions can have three approaches, such as anticipation, preventing the malocclusion from developing, interceptive treatment of the developing malocclusion and management of an already developed

malocclusion (Ackerman *et al.*, 2007). The standard treatment of a moderate or severe type of malocclusion in the adolescent stage is usually orthognathic surgery (Yen, 2011). Orthodontic camouflage treatment can also be attempted in most of the cases. However, in younger ages, before puberty, orthopaedic treatment is the treatment of choice, especially in early mixed dentition years (Feng *et al.*, 2012).

The first phase of orthodontic treatment is called an initial phase which started 24 hours to two days within the socket after force application. At this stage, the acute inflammatory response leads to vasodilation and migration of leukocytes, which release cytokine cell signalling molecules. After that, the arrest phase started where tooth movement stops after 20 to 30 days of the force application. In the second phase, treatment-related chronic inflammation occurs with the continuation of migration of leukocytes and periodontal remodelling happen (Burstone, 1989). The third phase or acceleration phase occurs after 40 days of accelerated tooth movement after the initial force of application. This phase leads to another phase of acute inflammation. Moreover, this third phase of orthodontic treatment is a linear phase where orthodontic tooth movements take place. The recruitments of macrophages, fibroblasts, osteoblasts, osteoclast and alkaline phosphatase activity, lead to tooth movement (Dolce *et al.*, 2002; Kato *et al.*, 1996; Zainal Ariffin *et al.*, 2011).

Similar to other treatments, orthodontic treatment also poses some drawbacks such as root resorption, pain, periodontal disease, pulp vitality and temporomandibular dysfunction (Talic, 2011). Among all the side effects, root resorption is one of the most controversial which has been assessed by many groups of researchers in orthodontic treatment, though this phenomenon is considered to be a non-invasive effect.

2.2 Root resorption

Root resorption is a condition where the loss of dental hard tissues occurs due to the clastic activity of bone (Patel and Ford, 2007). This phenomenon may also result as a pathological or physiological process. However, root resorption is considered as a normal physiological process in deciduous teeth unless it occurs prematurely (Bille *et al.*, 2007; Bille *et al.*, 2008). Although, the aetiological factors which involve in physiological root resorption in primary teeth are not entirely understood, the whole process controlled as similar to bone remodelling by transcription factors and cytokines (Harokopakis-Hajishengallis, 2007; Yildirim *et al.*, 2008). Root resorption in the permanent tooth is usually caused by inflammation, contrasting with bone remodelling which endures throughout life. Therefore, root resorption is known as a pathological event in permanent teeth which might lead to the premature loss of affected teeth if untreated.

There are two types of root resorption commonly found based on the surface of the root where resorption occurs i.e. internal root resorption and external root resorption (Tronstad, 1988; Yildirim *et al.*, 2008). Between both types of root resorption, external root resorptions are most commonly observed compared to the internal resorption. The internal and external root resorptions are further divided into different types. Regardless of the types, injury and stimulation are the main etiological factors for developing root resorption (Tronstad, 1988; Trope, 1998). There are many other clinical factors such as age, gender, ethnic background, tooth and root morphology which are also causing root resorption (Sameshima and Sinclair, 2001a; Årtun *et al.*, 2005; Mohandesan *et al.*, 2007; Dudic *et al.*, 2009; Lund *et al.*, 2011). Orthodontic treatment also plays a considerable role in developing root resorption.

Moreover, different factors during orthodontic treatment such as skeletal relationship, bracket system, duration of the treatment and extraction pattern would also influence the manifestation of root resorption (Iglesias Linares *et al.*, 2013; Iglesias-Linares *et al.*, 2012; Årtun *et al.*, 2005; Jacobs *et al.*, 2014; Kreich *et al.*, 2016; Mohandesan *et al.*, 2007; Chen *et al.*, 2015; Jacobs *et al.*, 2014).

Pre-cementum and pre-dentin are non-mineralized tissues that cover the external and internal parts of the root, respectively is related to the injury. Multinucleated cells colonize the denuded mineralized tissue which usually initiates the resorption process. However, the occurrence of inflammation stops if the stimulation is withdrawn. If the resorption is not confining the large area, then cementum like tissue form in the resorbed surface within 2-3 weeks. Nevertheless, if the resorption area is large, bone cells form surrounding the resorbed area before cementum could produce cells and ankylosis occur (Fuss *et al.*, 2003). On the other hand, the resorption process is also responsible for the continuous stimulations on osteoclast cells such as pressure or infection. However, the origin is different is each type of root resorption (Fuss *et al.*, 2003).

2.2.1 Internal root resorption

Internal root resorption (IRR) is a process that occurs within the canal in the intra-radicular or apical area. The phenomenon is commonly associated with periapical pathology (Tronstad, 1988). It might observe in the progression of the external inflammatory resorption in the canal, especially in the apical area (Vier and Figueiredo, 2004). IRR could be seen more in males compared to females (Calişkan

and Türkün, 1997; Goultschin *et al.*, 1982). However, the exact aetiology and mechanisms of internal root resorption were poorly understood (Feiglin, 1986).

Internal resorption can be identified in radiographs as a round uniform and oval radiolucent finding in the canal. Most of the cases, tooth with IRR showed no symptoms (Figure 2.1). Generally, IRR occurred in the cervical region, however it might also occur in all root canal system areas (Heithersay, 1985). The main subcategory of IRR is internal surface resorption, internal inflammatory resorption and internal replacement root resorption.

Internal surface resorption is a process that is similar to the external surface resorption. Although osteoclasts activity starts the resorption, however it eventually resolves the cavity if stimulation is withdrawn (Wedenberg and Lindskog, 1985). Internal inflammatory resorption can be found as an ovoid enlargement of the pulp chamber or root canal in the radiographs. It usually enlarges in lateral and apical direction and might be due to chronic pulpal inflammation (Patel *et al.*, 2010). Internal replacement root resorption is a scarce kind of IRR which is often found as an enlarged canal space. It displays in a diffuse area of both radio-opaque and radio-lucent reflection (Wedenberg and Zetterqvist, 1987). Although the physiology of this process is unknown, it might be the reason for osteoid material production by the pulp stem cells in order to repair the inflammation, trauma and bacteria (Patel *et al.*, 2010).

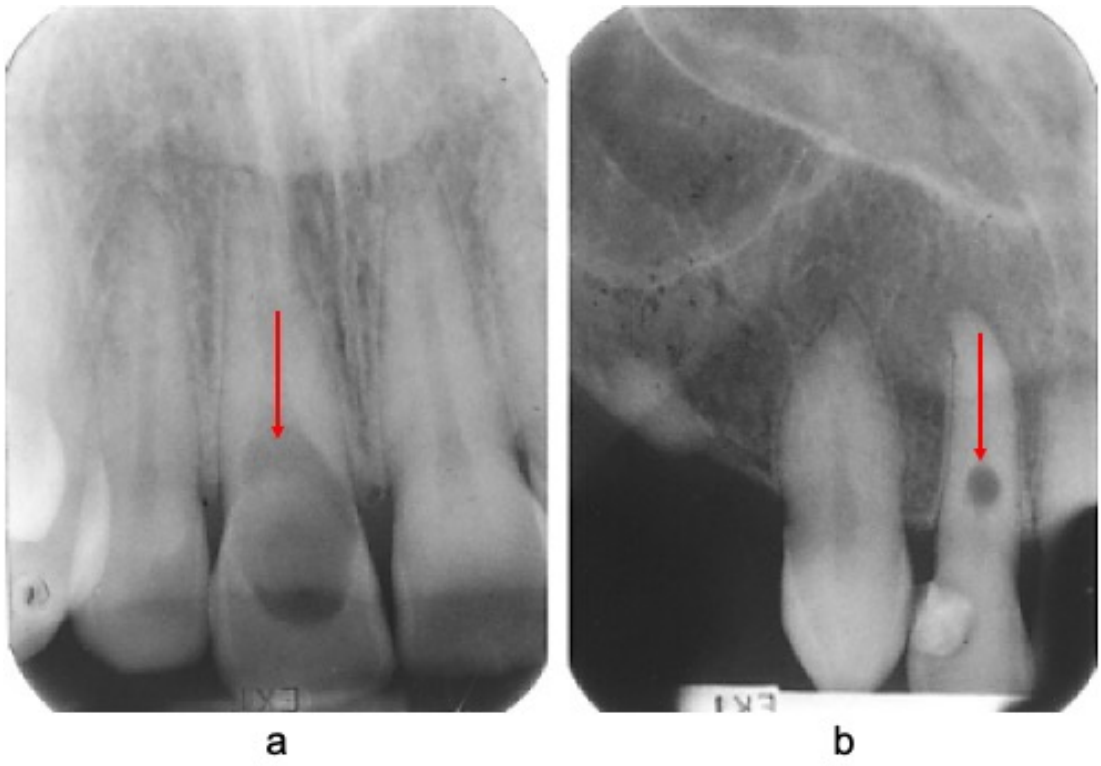

Figure 2.1: Internal root resorption

Figures show internal root resorption ~~i~~n different area of the tooth (a) Internal resorption appears radiographically as a round to oval radiolucent enlargement at the cervical region of the tooth (arrow) and (b) Internal resorption appears radiographically as a uniform, round to oval radiolucent enlargement at the root canal in the middle of the root (arrow)

(Photos adapted from Gunraj *et al.,* 1999)

2.2.2 External root resorption

External root resorption (ERR) is the most common type of resorption usually observed. Resorption at first starts to develop in the external surface of the root. Later on, it progresses towards the dentine of the root canal or pulp chamber (Darcey and Qualtrough, 2013). ERR is also classified as external surface resorption, external inflammatory resorption, external cervical resorption and external replacement resorption.

External surface resorption is a result of injury to the periodontium and its surroundings (Majorana *et al.*, 2003). Similar to the IRR, the self-limiting osteoclastic activity takes place followed by the healing of cementum and PDL reattachment. Once the stimulation withdraws, resorbed cavity heals eventually. If the injury only affects cementum, then complete recovery occurs however, if it extends to dentin then the root surface is partially restored (Andreasen, 1985).

External inflammatory resorption is a consequence of prolonging stimulation to the root surface which progressive towards dentinal tubules and pulp. Sometimes it causes pulp necrosis. However, younger teeth are mostly affected by this process. It might also be observed due to the excessive pressure. If unnoticed, then complete resorption of the root might occur (Andreasen, 1985; Darcey and Qualtrough, 2013) (Figure 2.2).

External cervical resorption usually observed in the cervical area of the root just underneath the epithelial attachment (Yu *et al.*, 2011). In this phenomenon, the tooth remains vital except it affects the pulp (Frank and Torabinejad, 1998). It might extend both apically and coronally surrounding the pulp. Though the exact aetiology

of this process is poorly understood, it probably occurs due to trauma, internal bleaching procedure and periodontal problem (Heithersay, 2004).

External replacement resorption is also rarely found similar to the internal replacement resorption and the aetiology is still vague (Hammarström *et al.*, 1989). In this process root surface is replaced with bone which is otherwise known as ankylosis. Bony trabecula used to develop in the PDL area and fuse the surface of the root. No radiolucency appears in the radiographs. However, if this condition is developed in an early stage of life then the tooth may lose within three to seven years. Whereas, in adult cases the tooth might survive until twenty years (Andreasen, 1985; Darcey and Qualtrough, 2013). External resorption is also classified in different ways based on the aetiology (Feiglin, 1986; Fuss *et al.*, 2003; Patel and Ford, 2007). According to the aetiological factor, the most common types of resorptions are root resorption due to pulpal infection, periodontal infection, tumour or impacted tooth pressure, ankylosis and orthodontic pressure.

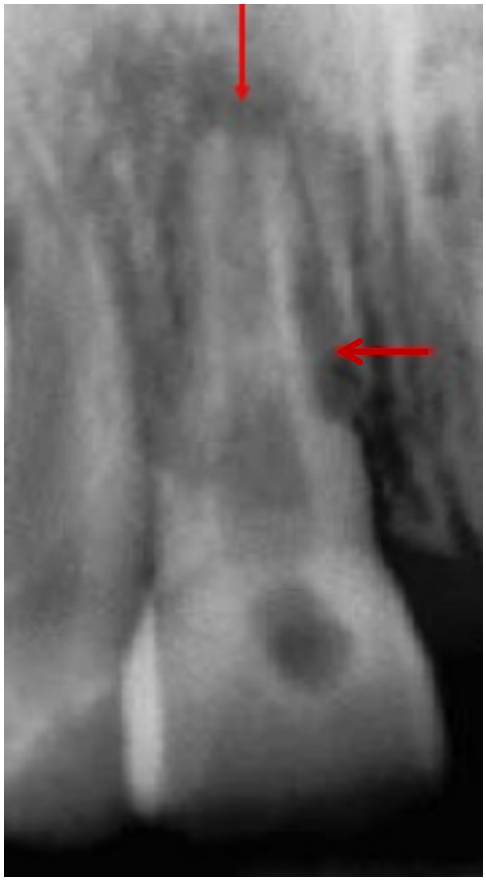

Figure 2.2: External root resorption

Figure shows radiographic identification of ERR which are observed at the distal and the apical surface of the root (arrows)

(Photos adapted from Fuss *et al.,* 2003)

Pulpal infection is one of the most important incentive factors of root resorption. Any trauma or caries initiate an inflammatory progression in the pulp tissue. Pre-cementum and pre-dentine are affected by the injury and leads towards the dentinal tubules which might stimulate the inflammatory process with osteoclastic activity. Consequently, this osteoclastic activity leads to internal or external resorption (Fuss *et al.*, 2003; Trope, 1998). In the radiographic examination, this condition could be observed as a radiolucent area in the external surface of the dentine or internal root canal in the dentinal wall.

Root resorption may occur due to periodontal infection. Due to the presence of gingivitis, the inflammatory process in marginal gingiva occurs in response to the determined challenge of bacterial plaque. Periodontitis develops when the inflammatory process leads to the apical margin of epithelial attachment and loss of collagen attachment occurs (Darcey and Qualtrough, 2013). Bacteria from the periodontal sulcus penetrate the dentinal tubules and epithelial attachment, exit apical to the epithelial attachment without penetrating the pulpal surface. Therefore, hard tissue colonization takes place in the damaged area of the root surface and penetrates dentin resulting in resorption inside the root (Tronstad, 1988). Initially, the resorption process does not involve the pulp area due to the pre-dentine which acts as a protective layer. However, with time, the resorption spreads irregularly and penetrates the root canal (Wedenberg and Zetterqvist, 1987). In radiographs, the lesion appears as a radiolucent area in dentin, expanding in apical and coronal direction (Fuss *et al.*, 2003).

Root resorption might also be found due to the pressure of any underlying tumours or impacted tooth. Most of the resorption occurs in deciduous teeth during the eruption of permanent teeth, however this is considered as a natural process. In

permanent dentition, the resorption process widely appears in second molars due to impacted third molars or in lateral incisors due to permanent canines (Fuss *et al.*, 2003). The phenomenon occurs due to the follicular space encroachment in the periodontium of the adjacent tooth (Barbaglio *et al.*, 2015; Brudvik and Rygh, 1995). Other than the impacted tooth, slowly expansion lesions such as ameloblastoma, cysts, giant cell tumour also cause root resorption compared to the rapidly expansion lesions (Darcey and Qualtrough, 2013; Tronstad, 1988). In radiographs, the radiolucent area is observed just near to the stimulation and if the lesion involves any tooth roots, resorption of that tooth could be determined with the radiographs.

Dento-alveolar ankylosis used to occur when injury or trauma of the tooth is more severe that cementum could not heal the injury, therefore bone started to form around the root surface without an intermediate attachment apparatus. In this situation, osteoclasts form directly in contact with mineralized dentin in the exposed root surface resulting in root resorption which takes place spontaneously. In ankylotic root resorption, no radiolucency is observed in the radiographs as resorption lacuna filled with the bone. In addition, periodontal space is also missing (Fuss *et al.*, 2003).

Another most common ERR is orthodontic treatment-induced resorption. A total of 19-34.5% resorption has been reported in all patients who are undergoing orthodontic treatment, mostly in incisors of both arches (Pereira *et al.*, 2014b). Resorption in the apex area of the tooth is observed in orthodontic treatment due to the continuous pressure of the root during tooth movement. Simultaneous pressure resorbed the apical third of the root which causes shortening of the tooth root. However, the tooth remains vital unless it disturbs the apical blood supply. In radiographs, no radiolucency is found in the root or the bone; however, resorption is

situated in the apical third of the tooth root. This common condition is known as external apical root resorption (EARR).

In recent years, most of the studies on root resorption have been focused on the orthodontically induced root resorption or EARR and becoming a current trend of research as this condition observed in almost all orthodontic treatment. Researchers are still trying to scrutinize the exact aetiology of EARR and factors associated with this specific condition.

2.3 External apical root resorption

The hard tissues such as enamel, dentin and cementum of the teeth usually do not endure resorption. The resorption of the permanent teeth usually is the outcome of trauma, inflammation of the periodontal tissues, pulp or pressure in the periodontal ligaments due to orthodontic tooth movement (Gunraj, 1999). On the external surface of the root, each tooth is encircled by the PDL, which acts as a barrier between the cementum and alveolar bone. Therefore, it is believed that the cells of PDL are responsible for osteoclasis-osteogenesis, fibroclasis-fibrogenesis and cementoclasis-cementogenesis on the root surface (Melcher, 1976).

EARR is one of the most common clinical complications of orthodontic treatment during orthodontic tooth movement. The phenomenon is the permanent shortening of the apex of the tooth root which could be seen on routine dental radiographs (Hartsfield *et al.*, 2004). An extensive review of EARR signified that many factors participate in the incidence of EARR such as types of tooth movement,

gender, orthodontic force magnitude, types of force and the duration of orthodontic tooth movement (Brezniak and Wasserstein, 2002).

EARR associated with orthodontic treatment or orthodontic tooth movement is classified as an inflammatory factor because of the inflammation of periodontium in the apical area which is the sequel of tooth structure destruction by clastic activity (Freitas *et al.*, 2013). Though EARR might appear in any or all teeth after orthodontic treatment, it is most often found in maxillary incisors (Maués *et al.*, 2015). EARR in maxillary central incisor can be found about 1 to 2 millimetres (mm) from the original tooth length in many orthodontic patients without any effects on function. Around 1 of 20 patients enduring orthodontic treatment, maximum 5 mm resorption can occur which compromising the longevity of the tooth (Brezniak and Wasserstein, 2002). During orthodontic treatment, PDL is exposed to the mechanical forces of tension and compression. Injuries in PDL and hyalinization of tissue formation occur due to the heavy forces applied during orthodontic treatment for a longer duration. The process of EARR is related to the remodelling of the PDL as a result of its injury and necrosis (Brudvik and Rygh, 1993; Brudvik and Rygh, 1994a; Brudvik and Rygh, 1994b).

2.4 Cellular and molecular pathways of EARR

During the orthodontic treatment, EARR involves particular molecular pathways that orchestrate non-physiologic cellular stimulation. EARR is a pathological side effect due to the undesirable activity of clastic cells around the root surface area (Aminoshariae *et al.*, 2016; Brezniak *et al.*, 2004). Many cells are responsible for the process of EARR, specifically, the odontoclasts. Functional and morphologic characteristics of odontoclasts cells are very similar to the osteoclasts (Wang and McCauley, 2011).

A current study explained the critical influence of macrophages in the process of root resorption (He *et al.*, 2015b). Two distinct types of macrophages, classically activated macrophage (M1) and alternatively activated macrophage (M2) which are known as 'killer' and 'healer' macrophages, respectively (Novak and Koh, 2013). Increase in the number of M1: M2 ratio is expected to be linked with root resorption. Mechanism of root resorption described that M1 macrophage enhances the inflammatory process by secreting proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and nitric oxide (NO). On the other hand, M2 macrophages secrete interleukin-10 (IL-10) and Arginase 1 which act as inhibitory effects of inflammation (He *et al.*, 2015a; Hunter *et al.*, 2010). Type 1 T helper (Th1) cells such as interferon gamma (γ) activate the M1 macrophages, while M2 macrophages are activated by T helper cell type 2 (Th2) such as IL-4 or IL-13 (Davies *et al.*, 2013; He *et al.*, 2015a). During orthodontic treatment, root resorption observed concomitantly due to an increase of the M1 macrophages with the upregulation of TNF-α and interferon γ. However, while the orthodontic forces are retracted, the number of M2 macrophages increases with the upregulation of IL-4 and anti-inflammatory IL-10.