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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde (PPGCS) do Centro de Ciências Biológicas e da Saúde (CCBS) da Pontifícia Universidade Católica do Paraná (PUCPR), como parte dos requisitos para a obtenção do título de Doutor em Ciências da Saúde, Área de Concentração Medicina e Áreas Afins.

Orientadora: Profa. Dra. Paula Cristina Trevilatto

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ATA DA SESSÃO PÚBLICA DE EXAME DE TESE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE EM NÍVEL DE DOUTORADO DA PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

Aos treze dias do mês de dezembro de 2010, realizou-se a sessão pública de defesa de tese **"ANÁLISE DA ASSOCIAÇÃO DE ASPECTOS CLÍNICOS E DO POLIMORFISMO *Taq* I NO GENE *VDR* COM A REABSORÇÃO RADICULAR APICAL EXTERNA (RRAE) EM INDIVÍDUOS TRATADOS ORTODONTICAMENTE"** apresentada por **MARIA LUIZA SCHMIDT SIMAS NETTA** para obtenção do título de doutora; Área de Concentração: Medicina e áreas afins.

A Banca Examinadora foi composta pelos seguintes membros:

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Presidente da Banca Examinadora

Prof. Dr. Roberto Pecoits Filho
Coordenador do PPGCS PUCPR

Rua Imaculada Conceição, 1155 Prado Velho CEP 80215 901
Tel (41) 3271 2285 Fax (41) 3271 1657 E-mail ppgcs@pucpr.br www.pucpr.br Curitiba Paraná Brasil

.....DEDICATÓRIA

Dedico este trabalho, fruto da esperança, resultado da perseverança, aos meus pais, Ivany e Luiz (*in memorian),* que nunca mediram esforços para minha formação pessoal e profissional, e para me proporcionar sempre, o melhor na vida.
Fizeram de meus sonhos e ideais, os seus.

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"*Eis que Deus é a minha salvação; confiarei e não temerei, porque o Senhor Deus é a minha força e o meu cântico; Ele se tornou a minha salvação*." *Isaias, 12:2.*

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São muitas as lembranças, imensas as saudades e infinitas as alegrias. Voces são com muito orgulho, o meu melhor exemplo de vida e determinação.
A vocês meus amados pais, deixo aqui registrada a minha eterna gratidão.
"*Filho meu, ouve o ensino de teu pai e não deixes a instrução de tua mãe." Prov.1:8*

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"*Melhor é serem dois do que um, porque tem melhor paga do seu trabalho. Porque se cairem, um levanta o companheiro; ai, porém do que estiver só; pois caindo, não haverá quem o levante. Também, se dois dormirem juntos, eles se aquentarão; mas um só como se aquentará? Se alguém quiser prevalecer contra um, os dois lhe resistirão; " Ecles. 4: 9-12*

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Que o sol aqueça o seu viver
Que chuva caia suave no seu lar
E até nos encontrarmos outra vez...
Que Deus lhe segure nas Suas mãos.”

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Rev.Elias Abraão

.....SUMÁRIO

SUMÁRIO

.....RESUMO

Resumo: A reabsorção radicular apical externa (RRAE) é uma sequela comum do tratamento ortodôntico; é considerada multifatorial, envolvendo o hospedeiro e fatores ambientais. Estudos sugerem que a RRAE apresenta um componente genético. A vitamina D é responsável pela regulação no nível de transcrição de certos genes, via interação com o receptor da vitamina D (VDR) e influencia a resposta imune e aspectos relacionados com o desenvolvimento, crescimento e homeostasia óssea. Polimorfismos (SNPs) funcionais são variações genéticas comuns, que podem ter impacto na modulação da transcrição gênica. **Objetivos:** O objetivo deste estudo foi investigar a associação de variáveis clínicas e do polimorfismo *Taq*I *VDR* (T/C) (rs731236, exon 9) com a RRAE em indivíduos tratados ortodonticamente. **Material e Métodos:** Foi selecionada uma amostra de 377 pacientes de ambos os sexos, com idade média de 14,9 (±2,96) anos e com maloclusão Classe II divisão 1. Foram realizadas radiografias periapicais dos incisivos centrais superiores com a raiz mais longa (raiz de referência) iniciais e seis meses após o início do tratamento. A amostra foi divida em 3 grupos: (1) 160 indivíduos tratados ortodonticamente com RRAE ≤1,43 mm, (2) 179 indivíduos tratados ortodonticamente com RRAE >1,43 mm, e (3) 38 indivíduos não tratados. As variáveis clínicas, como comprimento inicial da raiz de referência (IR), extração de premolar (XP), uso do aparelho *pendullun*, expansão rápida de maxila (ERM) e uso de elásticos foram analisadas nos indivíduos ortodonticamente tratados. Após a coleta e purificação do DNA, a análise do polimorfismo *Taq*I foi realizada por PCR-RFLP. Análises univariada e multivariada foram realizadas para verificar a associação de fatores clínicos e do polimorfismo genético com a RRAE; $p<0,05$ indicou significância estatística. **Resultados:** Foi observada maior proporção de RRAE nos indivíduos ortodonticamente tratados (RRAE≤1,43 mm: 0,81 mm; RRAE>1,43 mm: 2,24 mm) quando comparados com os indivíduos não tratados (RRAE: 0,05 mm). Idade ($p=0,022$), comprimento radicular inicial ($p=0,002$) e extração de pré-molares ($p=0,052$) foram associados com a RRAE nos indivíduos tratados ortodonticamente. Genótipos contendo o alelo C foram fracamente associados com proteção contra a RRAE nos indivíduos ortodonticamente tratados [CC+CT X TT (OR=0,29; IC 0,07-1.23; $p=0,091$)]. **Conclusão:** Fatores clínicos, como a idade, o comprimento inicial da raiz e a extração de pré-molares e o polimorfismo *Taq*I do VDR foram associados com a RRAE em indivíduos tratados ortodonticamente.

.....ABSTRACT

External apical root resorption (EARR) is a common complication of orthodontic treatment and is considered to be multifactorial, involving host and environmental factors. Studies have suggested that EARR has a genetic component. Vitamin D is responsible for regulation of certain genes at the transcription level, via interaction with the vitamin D receptor (VDR) and influences host immune response and aspects of bone development, growth and homeostasis. Functional single nucleotide polymorphisms (SNPs) are common genetic variations which have an impact on gene transcription modulation. **Objectives:** The aim of this study was to investigate the association of clinical variables and *Taq*I *VDR* (T/C) polymorphism (rs731236, exon 9) with EARR in patients under orthodontic treatment. **Material and Methods:** A convenient sample of 377 unrelated patients, both sexes, mean age 14.9 (±2.96) years who presented malocclusion Class II division 1 was selected for study. The periapical x-rays of the maxillary central incisors with the longer roots (reference tooth) were taken pre-treatment and six months after the beginning of the treatment. The sample was divided into 3 groups: (1) 160 individuals orthodontically treated with EARR ≤1.43 mm, (2) 179 individuals orthodontically treated with EARR >1.43 mm, and (3) 38 individuals orthodontically untreated. Clinical variable such as root initial size of the reference tooth (IR), premolar extraction (XP), use of pendulum appliance, rapid palatal expansion (RPE) and use of elastics were analyzed in individuals orthodontically treated. After DNA collection and purification, VDR T*aq*I polymorphism analysis was performed by PCR-RFLP. Univariate and multivariate analyses were performed to verify the association of clinical and genetic variables with EARR and $p<0.05$ indicated statistical significance. **Results:** It was observed a higher proportion of EARR in patients orthodontically treated (EARR≤1.43 mm: 0.81 mm; EARR>1.43 mm: 2.24 mm) when compared with individuals who never used orthodontic appliance (EARR: 0.05 mm). Age ($p=0.022$), IR ($p=0.002$) and premolar extraction ($p=0.052$) were associated with EARR in orthodontically treated patients. Genotypes containing the C allele were weakly associated with protection against EARR in patients orthodontically treated [CC+CT X TT (OR=0.29; IC 0.07-1.23; $p=0.091$)]. **Conclusion:** Clinical factors, with years, initial root length, and premolars extraction and VDR T*aq*I polymorphism were associated with EARR in individuals orthodontically treated.

……INTRODUÇÃO

1. INTRODUÇÃO

1.1 Oclusão

A oclusão normal apresenta relação harmônica maxilo-mandibular no sentido antero-posterior, dentes alinhados e pontos de contato cerrados (Capelozza Filho & Silva Filho, 1997). O mal posicionamento dentário, discrepâncias dento-esqueleticas e a má relação dos arcos dentários são características das maloclusões. (Pinto et al., 2008).

Angle (1907) considerou a relação antero-posterior maxilo-mandibular tomando como base a posição do primeiro molar superior (Moyers 1991).

Segundo Angle (1907) as maloclusões são classificadas em:

Classe I: Relação antero-posterior maxilo-mandibular normal, e a cúspide mesiovestibular do primeiro molar superior ocluindo no sulco vestibular do primeiro molar inferior.

Classe II: cúspide distovestibular do primeiro molar superior oclui no sulco mesiovestibular do primeiro molar inferior.

Classe II divisão 1: é a distoclusão onde os incisivos superiores apresentam vestibuloversão.

Classe II divisão 2: é a distoclusão onde os incisivos centrais superiores apresentam inclinação normal ou palatoversão. (Moyers, 1991)

Classe III: O molar inferior está posicionado mesialmente em relação ao molar superior, sem maiores especificações para a linha de oclusão. (Moyers, 1991; Graber & Vanarsdall Jr, 1996).

A maloclusão de Classe II divisão 1 tem como característica principal a relação distal do primeiro molar inferior em relação ao molar superior, atresia maxilar, incisivos superiores protruídos e incisivos inferiores verticalizados (Acquaro et al., 2007). Esta maloclusão que representa cerca de 50% (Silva Filho et al., 2009) a 58% (Castelo et al., 2009) do total dos casos tratados e freqüentemente produz alterações no padrão facial é a principal motivação que leva os indivíduos com esta maloclusão procurarem tratamento ortodôntico (Castelo et al., 2009).

1.2 Movimentação ortodôntica

O periodonto de sustentação é estruturalmente constituído pelo cemento, ligamento periodontal e osso alveolar (McCulloch & Melcher, 1983; Figueiredo & Parra, 2002; Meireles & Ursi, 2007). Sua função é manter a posição dentária e dissipar as forças oclusais através do osso alveolar (McCulloch & Melcher, 1983; Meireles & Ursi, 2007).

O periodonto de sustentação apresenta plasticidade que permite a movimentação dentária fisiológica e a acomodação dos movimentos que ocorrem durante a

mastigação (Figueiredo & Parra, 2002). É esta plasticidade que permite a movimentação ortodôntica (Nojima & Gonçalves, 1996). Dos três componentes do periodonto, o ligamento periodontal e o osso alveolar parecem estar mais envolvidos com a movimentação dentária, embora o cemento também participe deste processo (Interlandi, 1999). Durante a movimentação ortodôntica, as forças aplicadas alteram o fluxo sanguíneo do ligamento periodontal, modificando seu equilíbrio homeostático no lado de pressão e de tensão (Park, et al., 2011). No lado de pressão ocorre diminuição do espaço periodontal, deformação na estrutura celular (fibroblastos, cementoblastos, osteoblastos) e diminuição na oxigenação devido à compressão dos vasos sanguíneos. Concomitantemente ocorre a liberação de mediadores químicos que induzem a instalação do processo inflamatório, responsável pelo início da reabsorção do osso alveolar (Meireles & Ursi, 2007). A remodelação do cemento durante o tratamento ortodôntico, para a readaptação das fibras de Sharpey inseridas no terço apical, parece ser semelhante à que ocorre no tecido ósseo, mas, de forma pouco previsível e em menor intensidade (Interlandi, 1999) (Fig. 1).

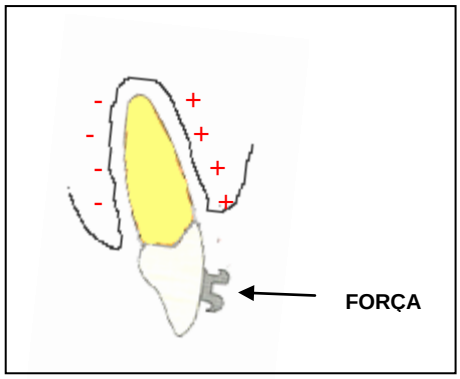

Fig. 1. Periodonto de sustentação durante a aplicação de forca ortodôntica.

Os fatores que podem interferir na movimentação ortodôntica são muito variados: o dente a ser movimentado, a história dentária (trauma, doença periodontal, cárie) (Consolaro et al., 2004), hábitos bucais deletérios, (Consolaro et al., 2004; Owman-Moll & Kurol, 2000), o tipo de maloclusão (Consolaro et al., 2004), o tipo e a amplitude de movimento (Brezniak & Wasserstein, 1993; Consolaro et al., 2004; Wu, et al., 2011), a magnitude (Zhuang, et al., 2011) e a duração da força aplicada (Brezniak & Wasserstein, 1993; Consolaro et al., 2004; Zahrowski & Jeske, 2011), a densidade e a morfologia óssea (Consolaro et al., 2004), o tamanho, o número e a forma da raiz (Levander & Malmgreen, 1988; Kjaer, 1995; Owman-Moll & Kurol, 2000; Consolaro et al., 2004; Sameshima & Sinclair, 2004), o tempo de tratamento (McNab

et al., 1999), e a associação desses itens com a condição sistêmica (McNab et al., 1999; Davidovitch et al., 1996; Owman-Moll & Kurol, 2000).

1.3 Reabsorção radicular apical externa (RRAE)

Uma seqüela pouco desejável durante o tratamento ortodôntico é a reabsorção radicular apical externa (RRAE) (Brezniak & Wasserstein, 1993, 2002; Vlaskalic et al., 1998; Killiany, 1999; Mah et al., 2000; Brezniak & Wasserstein, 2000; Jimenez-Pellegrin & Arana-Chavez, 2004). A RRAE tem sido objeto de estudo de muitos pesquisadores na busca de fatores etiológicos associados a este processo, mas ainda é considerada pobremente esclarecida (Harris et al., 1997). A maioria dos pacientes tratados ortodonticamente apresenta reabsorção em grau moderado, não comprometendo a dentição; em outros, o grau é severo, com prognóstico desfavorável (Mah & Prasad, 2004).

O método empregado para detectar a reabsorção radicular é radiográfico, por ser de fácil utilização e de diagnóstico preciso (Sameshima & Asgarifar, 2001; Brezniak et al., 2004; Mah & Prasad, 2004); no entanto, apresenta problemas, como padronização, exposição à radiação e ponto de vista limitado (Mah & Prasad, 2004). Radiografias panorâmicas e telerradiografias em norma lateral têm sido bastante utilizadas para a análise da RRAE (Harris, et al., 1997; Levander et al., 1998; AL-Qawasmi, 2003), mas o diagnóstico nestas radiografias é impreciso e questionável (Consolaro, 2004). Radiografias periapicais, especialmente com padronização de técnica, deveriam ser a alternativa de escolha, pois proporcionam um detalhamento da imagem (Linge & Linge, 1991; Davide et al., 1995; Levander et al., 1998). Apesar disso, as radiografias são um método estático e não podem precisar se o processo de reabsorção já cessou ou está ocorrendo (Andreasen et al., 1987; Owman-Moll, 1995; Jiang-Zhang, 2003; Mah & Prasad, 2004). Portanto, não é um método preditor do processo, que é identificado apenas quando um percentual de reabsorção já ocorreu. Mesmo nas radiografias periapicais as imagens das reabsorções apresentam limitações em sua interpretação, mas constituem ainda o melhor método de análise entre os acessíveis (Consolaro, 2004).

A manifestação clínica da RRAE em pacientes tratados ortodonticamente é muito variável (AL-Qawasmi et al., 2003). Sameshima & Sinclair (2001) avaliaram a possibilidade de identificar os fatores que poderiam contribuir para a RRAE. Em uma amostra de pacientes tratados com ortodontia fixa, foi constatada reabsorção radicular nos incisivos superiores e dentes com raiz anormal (pipeta, pontiaguda e dilacerada). Pacientes adultos apresentaram mais reabsorção do que crianças (Sameshima &

Sinclair, 2001). Indivíduos de origem asiática mostraram menor reabsorção que brancos ou hispânicos (Sameshima & Sinclair, 2001). Não foi observada diferença entre indivíduos do gênero masculino e feminino (Sameshima & Sinclair, 2001). A quantidade de reabsorção radicular parece estar, pelo menos em parte, na dependência de fatores genéticos (Newman, 1975; Harris et al., 1997; AL-Qawasmi et al., 2003 a,b).

O mecanismo de RRAE não está completamente esclarecido (Engstrom et al., 1988; Han et al., 2005), mas há evidências que citocinas contribuem de maneira significativa na sua etiopatogênese e progressão (AL-Qawasmi et al., 2003; Zhang et al., 2003; Lee et al., 2004). Estas citocinas promovem a reabsorção óssea (Holla et al., 2004) e são produzidas em resposta ao processo inflamatório, sendo secretadas por diferentes populações de células (Tzannetou et al., 1999; Zhang et al., 2003; Mavragani et al., 2005). As citocinas contribuem na quimiotaxia, diferenciação e ativação dos osteoclastos e seus precursores (Martin et al., 1998; Lorenzo & Raisz, 1999; Horowitz et al., 2001). Alguns estudos mostraram que as citocinas inflamatórias e prostaglandinas foram expressas quando aplicadas forças no tecido periodontal, mas as características do estresse mecânico não estão claras (Davidovich et al., 1988; Sandy et al., 1993; Teitelbaum, 2000; Alhashimi et al., 2001; Lee et al., 2004). A aplicação de força ortodôntica no ligamento periodontal induz a síntese das interleucinas (IL)-1β e IL-6, que têm importante papel na remodelação óssea durante a movimentação ortodôntica em camundongos e humanos (Uematsu et al., 1996; Alhashimi et al., 2001).

Segundo Ngan, et al. (2004), uma dificuldade para avaliar as causas de reabsorção radicular é identificar a ação dos fatores genéticos e ambientais. Harstfield et al. (2004) acreditam que a reabsorção radicular pode ocorrer não apenas em pacientes tratados ortodonticamente, mas, nestes indivíduos, pode ser multifatorial, envolvendo o hospedeiro e os fatores ambientais. Um substancial componente genético (entre 50 e 70%) tem sido sugerido para explicar a variação na reabsorção radicular apical externa (Newman, 1975; Harris et al., 1997; AL-Qawasmi et al., 2003 a,b; Ngan et al., 2004).

Apesar de estimado o componente hereditário para a reabsorção radicular (AL-Qawasmi et al., 2003), ainda não se sabe quantos e quais são os genes que contribuem para o fenótipo (Sameshima et al., 2001).

1.4 Receptor da Vitamina D (VDR)

A vitamina D, descoberta entre 1919 e 1924 (DeLuca 1988), é considerada um hormônio esteróide multifuncional, que modula a homeostasia mineral e a arquitetura esquelética normal (Nagpal et al., 2005), através de ação predominantemente no intestino (Walters et al., 2004; Collins et al., 2005). É produzida pelas células da pele, por ação dos raios ultravioletas, ou pode ser ingerida. Sua forma ativa, 1,25 dihidroxivitamina D_3 [1,25-$(OH)_2D_3$], é obtida após sua metabolização no fígado e posteriormente nos rins (Shinkio et al., 2004). A vitamina D está envolvida em uma ampla variedade de processos biológicos, como o metabolismo ósseo (Davideau et al., 2004), a modulação da resposta imune (Mathieu et al., 2004) e a regulação da proliferação e diferenciação celular (Sooy et al., 2005). Os efeitos da vitamina D são mediados via receptor intracelular de alta afinidade, o receptor da vitamina D (VDR) (Fig. 2).

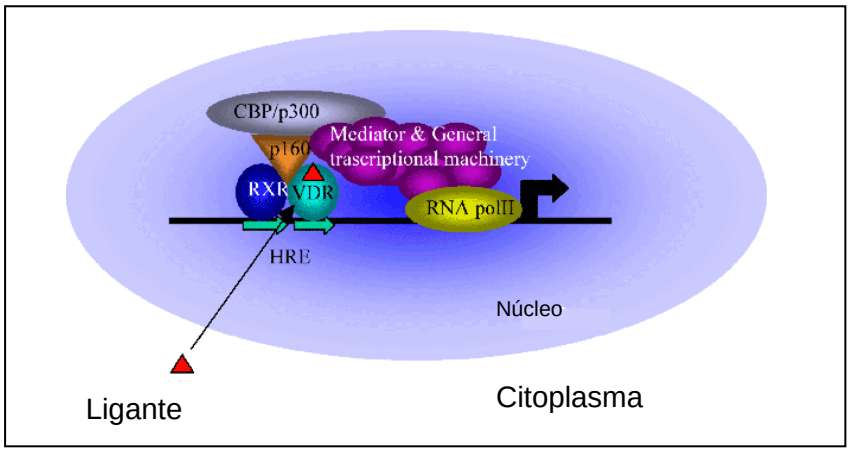

Fig. 2. Receptor da vitamina D. www.uku.fi/biokem/research/vaisanen/resdescript.shtml

O VDR é uma proteína nuclear, membro da superfamília de receptores de hormônios esteróides (Nezbedova & Brito, 2004; Shaffer & Gewirtt, 2004; Nagpal et al., 2005), amplamente expresso em muitos tipos de células, como osteoblastos (Misof et al., 2003) e diferentes células do sistema imune, como linfócitos, macrófagos e células B pancreáticas (Walters, 1992; Mathieu et al., 2004). É um fator de transcrição modulado através de um ligante (a vitamina D), formando um complexo capaz de estimular a transcrição de genes, cujo produto pode promover a diferenciação osteoclástica (Kim et al., 2005).

O gene do VDR está localizado no cromossomo 12, na região 12q13.1. É um gene considerado grande, contem 63.454 pb com 9 éxons, que possui uma extensa região promotora (Poon et al., 2004).

Polimorfismos são alterações na seqüência gênica, que geram formas variantes, denominadas alelos, cuja freqüência do mais raro é superior a 1% (Nussbaum et al. 2002).

A abundância e a grande freqüência de polimorfismos no genoma humano os transformam em alvo para explicar a variabilidade genética (Korstanje & Paigen, 2002; Thomas & Kejariwal, 2004) e sua influência no risco e progressão de algumas doenças (Morange et al., 2005; Rao et al., 2005; Sun et al., 2005).

Vários polimorfismos têm sido descritos no gene do VDR (Faraco et al., 1989; Morrison et al., 1992; Ye et al., 2000) e foram associados a diversas doenças, tais como asma (Poon et al., 2004), diabetes tipo I (Marti et al., 2004) e diversos tipos de câncer (Cheteri et al., 2004; Guy et al., 2004; Slattery et al., 2004). Alelos e genótipos específicos do gene do VDR têm sido relacionados com parâmetros de homeostasia óssea (Kim et al., 2003), com a massa e o *turnover* ósseo (Langdahl et al., 2000), e com doenças, nas quais a perda óssea é um sinal cardinal, como a osteoartrite, osteoporose (Duman et al., 2004) e a doença periodontal (Brito Junior et al., 2004).

Poon et al. (2004) estabeleceram blocos de desequilíbrio de ligação (associação de alelos de polimorfismos diferentes que são herdados em bloco) para o gene do VDR. Um polimorfismo localizado no éxon 2, reconhecido pela enzima de restrição F*ok*I (Polimorfismo de Comprimento de Fragmento de Restrição - RFLP) é considerado um marcador independente, pois não possui desequilíbrio de ligação com nenhum outro polimorfismo do VDR. Este polimorfismo mostrou-se funcional, sendo que um dos alelos (F) teve um efeito mais ativo no aumento da transcrição gênica (Jurutka et al., 2000). Foi demonstrada maior atividade do VDR quando o alelo menos funcional do F*ok*I (f) esteve associado com o alelo que determina cauda poly(A) mais curta, na região UTR não-traduzida (Whitfield et al., 2001).

A ação da vitamina D é importante para o desenvolvimento esqueletal, crescimento e homeostase óssea, mas tem sido pouco estudada no osso orofacial (Davideau et al., 2004).

A determinação dos fatores sistêmicos diretos e indiretos que influenciam a resposta do hospedeiro parece ser de grande relevância na identificação de grupos de risco ao desenvolvimento de respostas fisiopatológicas indesejáveis. Assim, a busca de marcadores genéticos que permitam a detecção de indivíduos mais prováveis de desenvolver reabsorção radicular é fundamental para a prevenção da instalação do processo destrutivo, ou na instauração de terapêutica individualizada e proservação adequada de pacientes, nos quais sinais clínicos já se manifestaram.

.....PROPOSIÇÃO

2. PROPOSIÇÃO

2.1 Objetivo geral

O objetivo do presente trabalho é investigar a associação de variáveis clínicas e alelos e genótipos específicos do polimorfismo (rs731236, *Taq*I) no gene *VDR* com a reabsorção radicular apical externa (RRAE) durante o tratamento ortodôntico.

2.2 Objetivos específicos

i) Investigar aspectos clínicos envolvidos com a RRAE em indivíduos tratados ortodonticamente.

ii) Analisar a associação entre alelos e genótipos específicos de polimorfismo (rs731236, *Taq*I) no gene *VDR* e a RRAE.

.....ARTIGO

3. ARTIGO

ASSOCIATION ANALYSIS OF CLINICAL ASPECTS AND THE *VDR* GENE POLYMORPHISM WITH EXTERNAL APICAL ROOT RESORPTION (EARR) IN INDIVIDUALS ORTHODONTICALLY TREATED

Maria Luiza S. Simas Netta Fontana[1]; Cleber Machado de Souza[1], José Fabio Bernardino[1], Felix Hoette[2]; Maura Levi Hoette[2]; Lotario Thum[3]; Terumi O. Ozawa[4]; Leopoldino Capelozza Filho[4]; Marcia Olandoski[1]; Paula Cristina Trevilatto[1]

[1] Pontifícia Universidade Católica do Paraná (PUCPR), Curitiba, PR, Brazil
[2] Private Orthodontic Clinic, Curitiba, PR, Brazil
[3] Dental Clinics of the Graduation Course in Orthodontics, Joinville, SC, Brazil
[4] Dental Clinics of the Graduation Course in Orthodontics, Bauru, SP, Brazil

Short title: Clinical and genetic aspects in EARR.

Corresponding author:

Paula Cristina Trevilatto, DDS, PhD

Center for Health and Biological Sciences (CCBS)

Pontifícia Universidade Católica do Paraná (PUCPR)

Rua Imaculada Conceição, 1155

80215-901

Curitiba, PR, Brazil

Phone/Fax: +55 (41) 3271-2618 / +55 (41) 3271-1657

email: paula.trevilatto@pucpr.br

External apical root resorption (EARR) is a common complication of orthodontic treatment and is considered to be multifactorial, involving host and environmental factors. Studies have suggested that EARR has a genetic component. Vitamin D is responsible for regulation of certain genes at the transcription level, via interaction with the vitamin D receptor (VDR) and influences host immune response and aspects of bone development, growth and homeostasis. Functional single nucleotide polymorphisms (SNPs) are common genetic variations which have an impact on gene transcription modulation. **Objectives:** The aim of this study was to investigate the association of *Taq*I *VDR* (T/C) polymorphism (rs731236, exon 9) with EARR in patients under orthodontic treatment. **Material and Methods:** A convenient sample of 377 unrelated patients, both sexes, mean age 14.9 (±2.96) years who presented malocclusion Class II division 1 was selected for study. The periapical x-rays of the maxillary central incisors with the longer roots (reference tooth) were taken pre-treatment and six months after the beginning of the treatment. The sample was divided into 3 groups: (1) 160 individuals orthodontically treated with EARR ≤1.43 mm, (2) 179 individuals orthodontically treated with EARR >1.43 mm), and (3) 38 individuals orthodontically untreated. Clinical variable such as root initial size of the reference tooth (IR), premolar extraction (XP), use of pendulum appliance, rapid palatal expansion (RPE) and use of elastics were analyzed in individuals orthodontically treated. After DNA collection and purification, VDR T*aq*I polymorphis(analysis was performed by PCR-RFLP. Univariate and multivariate analyses were performed to verify the association of clinical and genetic variables with EARR; $p<0.05$ indicated statistical significance. **Results:** It was observed a higher proportion of EARR in patients orthodontically treated (EARR≤1.43 mm: 0.81 mm; EARR>1.43 mm: 2.24 mm) when compared with individuals who never used orthodontic appliance (EARR: 0.05 mm). Age ($p=0.022$), IR ($p=0.002$) and premolar extraction ($p=0.052$) were associated with EARR in orthodontically treated patients. Genotypes containing the C allele were weakly associated with protection against EARR in patients orthodontically treated [CC+CT x TT (OR=0.29; CI 0.07-1.23; $p=0.091$)]. **Conclusion:** Clinical factors and VDR T*aq*I polymorphism were associated with EARR in individuals orthodontically treated.

INTRODUCTION

External apical root resorption (EARR) is a common consequence of orthodontic tooth movement (Brin et al., 2003; AL-Qawasmi et al, 2003a, b; Yamagushi et al., 2006; Pizzo et al., 2007; Huang, et al., 2010; Zhuang, et al., 2011). Many studies aimed to elucidate the causal relationship between orthodontic tooth movement and root resorption, but to date this issue is poorly understood and it is not possible to predict who will develop EARR (Harris et al., 1997; Ngan, et al., 2004; Mah & Prasad, 2004; Pizzo et al., 2007; Gülden et al., 2009). The clinical manifestation of EARR in orthodontic patients is highly variable (AL-Qawasmi et al., 2003). Most of the patients treated orthodontically present resorption in a moderate degree, not committing the dentition; in others, the degree is severe, with unfavorable prognostic (Mah & Prasad, 2004).

It is believed that EARR may not happen only due to orthodontic movement, but in patients under treatment, it may result from multiple variables, such as host (genetic aspects) (Hartsfield et al., 2004; Hartsfield, 2009) and environmental (mechanical forces, trauma) factors (Winter et al., 2009; Zhuang, et al., 2011). One of the difficulties to evaluate the causes of EARR is to separate the contribution made by genetic from those by environmental factors such as treatment (Ngan et al., 2004).

It was reported that genetic factors account for at least 50% of the variation in EARR (Hartsfield et al., 2004). It has been suggested a strong genetic component for EARR (Newmann, 1975; Harris et al., 1997), estimated in 70% (Harris et al., 1997), especially when monozygotic twin pairs are studied (Ngan et al., 2004). Although the efforts for the identification of the genetic component for EARR, how many and which are the genes that contribute to the phenotype of EARR are yet largely unknown (Ngan et al., 2004).

Vitamin D is responsible for both positive and negative control of certain genes at level of transcription, via interaction with the vitamin D receptor (VDR) (Sutton & MacDonald, 2003) and is important for the development skeletal, growth and bone homeostasis (Davideau, et al., 2004). The human VDR is a product of a single gene, which is located on chromosome 12q13-14 (Labuda et al., 1992). The gene is comprised of 9 exons that, together with intervening introns, span approximately 63 kb (Poon et al., 2004). Genome-wide analyses have identified over 100 polymorphisms in the VDR gene (Uitterlinden et al., 2002). Polymorphisms refer to the existence of 2 or more alleles at a given locus and, if such alleles occur at a frequency of more than 1% in a population, the locus is said to be polymorphic (Chiba-Falek & Nussbaum 2001). Single nucleotide polymorphisms (SNPs) are the most common form of DNA variation

in the human genome. Patterns of linkage disequilibrium (LD) in the VDR gene were proposed for a Canadian population (Poon et al., 2004) and 2 LD blocks are believed to represent the whole gene. Block one locates toward the 5' end and spans roughly 8.4 kb and block two locates toward the 3' end of VDR and spans approximately 5.8 kb. Near the 3' untranslated region (UTR) there is a SNP identified by the presence of a restriction site for *Taq*I enzyme (T/C) in *VDR* exon 9 (rs731236). This SNP may represent the second LD block (Poon et al., 2004). Indeed, alleles of this polymorphism are in LD with other polymorphisms in the same block and are linked and inherited together. Allelic variations in this region could be responsible for messenger RNA (mRNA) stability and differences in translational efficiency, resulting in changes in cellular expression of *VDR* (Decker & Parker 1995; Durrin et al., 1999). This polymorphism has been associated with bone mass, turnover, and mineral loss (Karkoszka et al., 1998) and diseases such as osteoarthritis (Uitterlinden et al., 1997), (Horst-Sikorska et al., 2005), and periodontal disease (Brito Junior et al., 2004). However, there is no study investigating *VDR* gene polymorphisms and their association with EARR.

The aim of this study was to investigate the association of *Taq*I *VDR* (T/C) polymorphism (rs731236, exon 9) and clinical variables with EARR in patients under orthodontic treatment.

METHODS

Study population

A sample of 377 Caucasoid unrelated patients, both sexes, mean age 14.9 years (range 8 to 21), from which 339 patients presented malocclusion Class II division 1 by edgewise or straight wire techniques, orthodontically treated and 38 patients presented malocclusion Class II division 1 but orthodontically untreated. The reason of choice for Class II division 1 is due to this type of malocclusion be the most frequent and which requires more treatment (Acquaro et al., 2007; Silva Filho et al., 2009) besides the fact that may lead to higher levels of EARR (Remmelink & Van Der Molen, 1984; Coperland & Green, 1986; Tanner et al., 1999; Brin et al., 2003; Liou & Chang, 2010). The patients were selected from the Dental Clinics of the Graduation Course in Orthodontics of Profis (Bauru-SP), from Dental Clinics of the Graduation Course in Orthodontics of Thum Institute of Research (Joinville-SC), and from two private Orthodontic Clinics (Curitiba-PR) (Table 1). Although the study sample was composed by Caucasoid, the Brazilian white population is heterogeneous. Recent articles have not recommended grouping Brazilians into ethnic groups based on color, race and

geographical origin because Brazilian individuals classified as white or black have significantly overlapping genotypes, probably due to miscegenation (Parra et al., 2003).

Subjects completed personal, medical and dental history questionnaires, and within a protocol approved by an Institutional Review Board, signed a consent form after being advised of the nature of the study (approved by the Ethical Committee in Research at PUCPR, protocol n° 546/05). The patients could not have chronic usage of anti-inflammatory drugs, HIV infection, and immunosuppressive chemotherapy history of any disease known to severely compromise immune function, current pregnancy or lactation, oral trauma, parafunctional behavior, endodontic treatment and extensive caries lesions.

The periapical x-rays of the maxillary central incisors with the longer roots (reference tooth) were taken pre-treatment and six months after the beginning of the treatment. The evaluation method consisted in measuring the root and crown lengths directly from the radiographs. The root apex, incisal edge, and cementoenamel junction (CEJ) of each tooth were demarcated on the x-rays on the light table. The longitudinal axis of each tooth was constructed from the root apex to the incisal edge following the root canal as accurately as possible. A perpendicular axis was then projected to the longitudinal axis from the mesial to the distal CEJ sides. The crown length was measured from the incisal edge to the projected CEJ, and the root length from the projected CEJ to the root apex (Fig. 1a, b). The resultant difference between those measures pre-treatment and 6 months after treatment beginning indicates the presence of EARR. A correction factor (CF) was calculated using the following formula: CF=C1/C2 (C1 is the crown length on the pre-treatment, C2 is the crown length 6 months after starting treatment). Then, EARR was calculated using the following formula: EARR=R1-(R2xCF); R1 is the root length on the pre-treatment, and R2 is the root length 6 months after treatment start). EARR was also expressed as a percentage of the original root length: EARR x 100/R1. Only teeth with complete root formation were considered for investigation. Any distortion between the pre-treatment and follow-up radiographic image was corrected using the crown length registration, assuming crown length to be unchangeable over the observation period (Linge & Linge, 1991; Mohandesan et al., 2007). The EARR was evaluated by one single examiner (M.L.S.S.N.F). The radiographs were examined over a light table and the measurements were made with a fine-tip digital caliper with accuracy up to 0.02 mm (UTUSTOOLS Professional, Electronic Digital Vernier Caliper) (Fig. 2)

A ROC curve was constructed intended to verify the cutoff point based on the sample data distribution for EARR and the value of 1.43 mm was obtained. Then, the sample was divided into 3 groups:

Group 1: 160 individuals orthodontically treated with EARR ≤ 1.43 mm

Group 2: 179 individuals orthodontically treated with EARR > 1.43 mm.

Group 3: 38 individuals orthodontically untreated.

Clinical Parameters in Orthodontically Treated Individuals

The following parameters were evaluated in patients treated orthodontically (OT): age, gender, root initial size of the reference tooth (IR), premolar extraction (XP), use of pendulum appliance, rapid palatal expansion (RPE), use of elastics (Table 2).

DNA Collection and Purification

Cells were obtained through a mouthwash with 3% glucose solution for 1 min, and scraping of the oral mucosa with a sterile spatula (Trevilatto & Line, 2000). DNA was extracted from epithelial oral cells with ammonium acetate 10 M and EDTA 1 mM (Aidar & Line, 2007).

Analysis of VDR polymorphism

VDR *Taq*I (T/C) Polymorphism (rs731236)

The following primer pair was used for polymerase chain reaction (PCR) amplification of genomic DNA samples: (F - 5' CAG AGC ATG GAC AGG GAG CAA G 3' and R - 5' GGA TGT ACG TCT GCA GTG TG 3'). Reaction conditions and cycling parameters were as follows: 1 µL of the genomic DNA were used for PCR amplification in a reaction mixture containing 22.5 µL GoTaq Green Master Mix (Promega, Madison, WI, USA) and 0.7 µL of each 25 µM primer. The reactions were performed in a Techne T-512 thermal cycler and consisted of an initial denaturation step of 95°C for 5 min, followed by 37 cycles of 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and a final extension of 72°C for 7 minutes. The restriction fragment length polymorphism (RFLP) technique was performed in a final reaction volume of 20 µL, using 1 unit *Taq*I (T↓CGA) (Invitrogen Life Technologies) and a 10-µL aliquot of PCR products, digested at 65°C overnight. The digested products were separated in 10% polyacrylamide gel electrophoresis stained by silver. The genotypes were determined by comparing the RFLP band patterns with a 1-kb-plus DNA ladder (Invitrogen Life Technologies). The RFLP is formed by a single base transition (T/C) at codon 352 in exon 9 of the *VDR* gene that creates a *Taq*I restriction site. The alleles which result from the cleavage of *Taq*I are designated 'C' (*Taq*I site present, with 2 fragments: 293 bp and 47 bp) or 'T' (*Taq*I site absent, with a fragment: 340 bp).

Statistical analysis

The results observed in the study were expressed for mean and standards deviation (quantitative variable) or frequencies/percentages (qualitative variable). To evaluate the association between two qualitative variables, the Chi-square test or Fisher's exact test were used. Comparisons between the groups in relation the quantitative variables used analysis of variance with one factor and LSD test for multiple comparisons. Adjustments of ROC curve were made for EARR and for age and measure of the initial root length, with the objective to determine cut points associated with EARR. Unpaired t-test was used to compare EARR, age, and initial root length between the groups. For the multivariate analysis, the logistic regression model and Wald's test were used. Values of $p<0.05$ indicated statistical significance. Data were organized in Excel spread sheet and analyzed with the computational program Statistica v.8.0.

RESULTS

Clinical Parameters in Orthodontically Treated Individuals

It was observed a higher proportion of EARR in patients orthodontically treated (EARR≤1.43 mm: 0.81 mm; EARR>1.43 mm: 2.24 mm) when compared with individuals who never used orthodontic appliance (EARR: 0.05 mm). The clinical impact of the use of orthodontic appliances on root resorption can be observed in figure 3.

Regarding OT individuals, no statistically significant differences (*NS*) were observed between the groups in relation to gender, use of pendulum appliance, RPE, and use of elastics. A statistically significant difference (*SD*) was found between the groups regarding age ($p=0.021$) and IR ($p=0.005$) in the univariate analysis. After the multivariate analysis age ($p=0.022$), IR ($p=0.002$) and XP ($p=0.052$) were associated with EARR (Table 2).

VDR (rs731236, TaqI) Genotyping

Considering the study SNP, the genotype distribution was not consistent with the assumption of Hardy-Weinberg equilibrium neither in the control group nor for the whole sample. No differences were found in *VDR* T*aq*I polymorphism genotype frequency ($p=0.051$) and allele distribution ($p=0.455$) between the groups (Table 4). However, when individuals orthodontically treated were analyzed versus untreated subjects, it was observed a weak protection effect of allele C against EARR [CC+CT x TT (OR=0.29; CI 0.07-1.23; $p=0.091$)].

DISCUSSION

Most clinicians are highly concerned about EARR as an undesirable side effect of orthodontic treatment. The etiology of EARR has been studied for the past few decades, but remains unclear (Gonzales el al., 2008). It is believed that no orthodontic tooth movement is possible without some proportion of EARR (Reitan & Rygh, 1994), but, in most cases, it will be minor and therefore of no clinical significance (Loenen et al., 2007). However, moderate to severe root resorption has been reported to occur with a frequency of 10 to 20% (Hollender et al., 1980; Levander & Malmgren, 1988; Brin et al., 2003).

The cause of EARR is considered to be multifactorial (Lopatiene & Dumbravaite, 2008). Several factors have been mentioned to influence EARR (Pizzo et al., 2007), both mechanical and biological (Brezniak & Wasserstein, 2002a, b). Several studies have suggested that excessive orthodontic force (Chan & Darendeliler, 2005; Harris et al., 2006), tooth intrusion, (Sameshima & Sinclair, 2001; Han et al., 2005), rapid palatal expansion (Gülden et al., 2009), as well as tooth movement against hard and highly mineralized tissue (Gülden et al., 2009) are critical factors for EARR. Regarding biological aspects, associations between EARR and age, gender (George & Evans, 2009), tooth morphology, periodontal condition (Pizzo et al., 2007), immune response (Alhashimi et al., 2004; Nishioka et al., 2006), bone metabolism (Verna et al., 2003a, b; Takada et al., 2004), and systemic and genetic factors (Al-Qawasmi et al., 2003) have been suggested.

In this study, an impact of age, root initial length of the maxillary central incisor (reference tooth), and premolar extraction was found on EARR in individuals orthodontically treated. It has been recently reported that older individuals have more EARR than the younger (Pandis et al., 2008). However, to the authors' knowledge, there are no other studies in the literature which reported the root length influencing EARR so far. It has been reported that incisors present a degree of root resorption increased (Brezniak & Wasserstein, 1993; Harris,1999; Sameshima & Sinclair, 2004) from 15% before treatment to 73% after treatment and the number of teeth with moderate and severe root resorption increases from 1% before treatment to 25% after treatment (Lupi et al., 1996; Wierzbicki et al., 2009). We also identified an association of EARR with orthodontic treatment with extraction, in accordance to other studies (Remmelink & Van Der Molen, 1984; Coperland & Green, 1986; Tanner et al., 1999; Brin et al., 2003; Mohandesan et al., 2007; Liou & Chang, 2010). However, other authors failed to find a relationship between EARR and premolar extraction (Pandis et al., 2008; Huang et al., 2010).

Regarding gender, there was no statistical difference between orthodontically treated patients with and without EARR, which has been reported by several authors (Harris et al., 1997; Sameshima & Sinclair, 2001; Mohandesan et al., 2007; Pandis et al., 2008). However, Baumrind et al. (1996) found a higher prevalence of EARR in males than in females and Kjaer (1995) observed more EARR among females than males. No statistical difference was found in relation to the use of pendulum in our study. It is worth mentioning that all the patients who made use of pendulum were orthodontically treated with the straight-wire technique and all the individuals who did not use pendulum were treated with the edgewise technique. No influence was also observed of the type of orthodontic appliance on EARR in other studies (Mohandesan et al., 2007; Lopatiene & Dumbravaite, 2008), but Mavragani et al. (2000) found greater EARR in patients treated with edgewise technique than the straight-wire. Data of this work suggest that maxillary incisors do not present susceptibility to EARR during RPE; however, other authors have found such association (Vardimon et al., 2005). It is worth mentioning that all individuals who made use of the expanse appliance type Haas in this study also used the pendulum appliance. Moreover, in the present study, no significant difference was found when Class II elastic was used, in contrast with other authors (Mavragani et al., 2000).

The x-ray is the common employed method to diagnose EARR, and is considered to be effective and predictive (Sameshima & Asgarifar, 2001; Mah & Prasad, 2004). However, it presents limitations, as standardization, radiation exposure remain, and a restricted point of view (Mah & Prasad, 2004). Moreover, radiographic method is static and cannot indicate if the process of root resorption has finished or is ongoing, just showing the result of the EARR process (Andreasen et al., 1987; Owman-Moll, 1995; Jiang & Zhang, 2003; Mah & Prasad, 2004; Makedonas, et al., 2009). So far, EARR has been measured on lateral cephalometric and panoramic radiographs (Harris, et al., 1997; AL-Qawasmi, et al., 2003). Once diagnosis by those types of x-rays is thought to be imprecise and questionable, standardized intraoral radiographs should be used instead by the fact they provide more detailed image information (Linge & Linge, 1991; Levander et al., 1998). In the present study, periapical x-rays were used to measure EARR. Even in the periapical x-rays, the image interpretation of the resorptions present limitations, but they are the most indicated method of analysis among the accessible ones (Consolaro, 2004).

Although EARR may even be detected in non-treated subjects (Lopatiene & Dumbravaite, 2008), the results of this study are consistent with the published literature showing that teeth without any forces have less resorption than teeth that have undergone orthodontic treatment (Mohandesan et al., 2007).

Our findings of a clinically significant association between the degree of EARR and orthodontic treatment suggest that individuals susceptible to EARR may be identified early in treatment (Levander et al., 1998; Levander & Malmgren, 2000). It has been shown that EARR of the upper incisors, observed during the initial months of treatment, could be a predictor of a higher risk for continuing resorption during treatment (Levander et al., 1998; Wierzbicki et al., 2009). Consequently, it has been recommended that periapical x-ray should be obtained after 6 months of the initial treatment (Levander et al., 1994; Levander et al., 1998; Levander & Malmgren, 2000).

Susceptibility to EARR is believed to have a genetic basis (Newman, 1975 Harris et al., 1997; Ngan et al., 2004) (see table 3). The genetic component controlling the occurrence and extent of EARR accounts from 50 to 70% in humans and was especially identified in maxillary central incisors (MCI) and mandibular central incisor (MLI) (Harris et al., 1997; Ngan et al. 2004). This may due to the fact those teeth are more subject anterior retraction forces in individuals with Class II division 1 malocclusion during orthodontic treatment.

Efforts to investigate host factors influencing EARR are scarce and have focused on linkage and association analysis (Table 3). Concerning linkage analysis, only one study was conducted and found an evidence for linkage of *TNFRSF11A* (RANK) with EARR (AL-Qawasmi et al., 2003b). In the case of association studies, only 2 candidate genes of the immune-inflammatory response have been investigated: *IL1A* and *IL1B*, coding for IL-1α and IL-1β pro-inflammatory mediators, respectively (Al-Qawasmi et al., 2003a; Lages et al., 2009; Gülden et al., 2009). An evidence for association was identified between EARR and alleles from *IL1A* and *IL1B* gene polymorphisms. In the case of *IL1A* (-889) polymorphism, a significant difference in the genotype distribution was found between EARR patients and the control group, with an augment of genotype TT in the group with EARR in a North-American population (Gulden et al., 2009). Concerning *IL1B* (+3954), allele C (Lages et al., 2009) and genotype CC (AL-Qawasmi et al., 2003a) were significantly associated with the EARR in a Brazilian and a North-American population, respectively (Table 3).

Vitamin D is the major regulator of calcium homeostasis and protects the organism from calcium deficiency via effects on the intestine, kidney, and bone. Vitamin D is well known as a hormone involved in mineral metabolism and bone formation, and its effect is to facilitate intestinal absorption of calcium and phosphate Vitamin, (Lips, 2006; Fleet, 2006). Numerous effects of vitamin D on bone have been demonstrated, as a synthesis of bone matrix protein such as type I collagen, alkaline phosphatase, osteocalcin and osteopontin (Gallagher & Riggs, 1990; Glenville et al., 1996). Several epidemiological studies have reported positive associations between

osteoporosis or low bone density and alveolar bone and tooth loss (Naito et al., 2007). It also inhibits lymphocyte proliferation and stimulates monocyte differentiation (Labuda et al., 1992). Thus, there is considerable scientific evidence that vitamin D has a variety of effects on immune system function that may enhance innate immunity and inhibit the development of autoimmunity (Griffin et al., 2003).

The effects of vitamin D on cells are thought to be mediated through the vitamin D receptor (VDR) that belongs to the steroid receptor super family (Sinotte et al., 2008), a transcription factor regulating gene expression in several cell types, including osteoblasts (Masuyama et al., 2006). Polymorphisms in the *VDR* gene have been associated with bone mineral density, bone turnover, and diseases in which mineral loss is a cardinal sign (Gunes et al., 2008). However, to the authors' knowledge this is the first study to investigate association of VDR polymorphisms and susceptibility to EARR. It was observed an enrichment of heterozygotes in the individuals genotyped for polymorphism VDR *Taq*I (T/C) (rs731236) in this study. The inclusion of individuals with malocclusion Class II division 1 of Angle may have favored the selection of such a genotype. It can be suggested that the craniofacial grow pattern may be related with the heterozigosity. Also, genotypes containing the C allele were observed to be weakly associated with protection against root resorption in patients orthodontically treated. Although the literature shows some controversy, it seems that C allele of polymorphism T*aq*I increases mRNA stability and VDR expression (see Uitterlinden et al., 2004). The protector effect of allele C might only be observed in individuals orthodontically treated. The mechanical forces might regulate gene expression during orthodontic treatment though a mechanism involving transducing molecules and mechanosensitive ion channels (Kung, 2005).

In summary, it was observed that clinical aspects such as age, initial root length, and premolar extraction as well as *VDR Taq*I polymorphism (rs731236) were associated with EARR in orthodontically treated individuals.

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REFERENCES

Acquaro, J. E.; Vedovello, S. A. S.; Degan, V. V.; Valdrighi, H. C.; Vedovello Filho, M.; Dona, C. M. (2007). *RGO.* 55: 281-285.

Aidar, M.; Line, S. R.(2007). A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Braz Dent J.*18: 148-152.

Alhashimi, N.; Frithiof, L.; Brudvik, P.; Bakhiet, M. (2004). CD40-CD40L expression during orthodontic tooth movement in rats. *Angle Orthod.* 74: 100-105

AL-Qawasmi, R. A.; Hartsfield, J.K.; Everett, E.T.; Flury, L.; Liu, L.; Foroud, T.M.; Macri, J.V.; Roberts, W. E. (2003a). Genetic predisposition to external apical root resorption. *Am J Orthod Dentofacial Orthop.* 123: 242-252.

AL-Qawasmi, R. A.; Hartsfield, J.K.; Everett, E.T.; Flury, L.; Liu, L.; Foroud, T.M.; Macri, J.V.; Roberts, W. E. (2003b). Genetic predisposition to external apical root resorption in orthodontic patients: Linkage of chromosome-18 marker. *Journal Dental Research.* 82: 356-360

Andreasen, F. M.; Sewerin, J.; Madel, V.; Andreasen, J.O.(1987). Radiographic assessment of simulated root resorption cavities. *Endod Dent Traumatol.* 3:21-27

Baumrind, S.; Korn, E. L.; Boyd, R. L.(1996). Apical root resorption in orthodontically treated adults. *Am J Orthod Dentofacial Orthop*. 110:311-20

Brezniak, N.; Wasserstein, A. (1993). Root resorption after orthodontic treatment: Part2. Literature review. *Am J Orthod Dentofacial Orthop.* 103:139-146

Brezniak, N.; Wasserstein, A.(2002a). Orthodontically induced inflammatory root resorption. Part I: The basic science aspects. *Angle Orthod.* 72: 175-179

Brezniak, N.; Wasserstein, A.(2002b). Orthodontically induced inflammatory root resorption. Part II: The clinical aspects. *Angle Orthod.* 72: 180-184

Brin, I.; Tulloch, C.; Koroluk, L.; Philips, C.(2003). External apical root resorption in Class II malocclusion: A retrospective review of 1-versus 2-phase treatment. *Am J Orthod Dentofacial Orthop.* 124: 151-156.

Brito Junior, R. B.; Scarel-Caminaga, R. M.; Trevilatto, P. C.; de Souza, A.P.; Barros, S. P. (2004). Polymorphisms in the vitamin D receptor gene are associated with periodontal disease. *J Periodontol.* 75: 1090-1095.

Chan, E.; Darendeliler, M. A.(2005). Physical properties of root cementum-Part 5. Volumetric analysis of root resorption craters after application of light and heavy orthodontic forces. *Am J Orthod Dentofacial Orthop.* 127: 186-195

Chiba-Falek, O.; Nussbaum, R. L.(2001). Effect of allelic variation at the NACP-Rep1 repeat upstream of the alpha-synuclein gene (SNCA) on transcription in a cell culture luciferase reporter system. *Human Molecular Genetics.* 10: 3101-3109.

Consolaro, A.(2004). Sobre alguns aspectos metodológicos de pesquisas em movimentação dentária induzida e das reabsorções dentárias: uma proposta de guia e cuidados para análise de trabalhos. *R Dental Press Ortodon Ortop Facial*. 9: 104-109

Coperland, S.; Green, L. J. (1986). Root resorption in maxillary incisors following active ortohodontic treatment. *Am J Orthod Dentofacial Orthop.*89: 91-95

Davideau, J. L.; Lezot, F.; Kato, S.; Bailleul-Forestier, I.; Berdal, A.(2004). Dental alveolar bone defects related to Vitamin D and calcium status. *J Steroid Biochem Mol Biol.* 89-90: 615-618.

Decker, C. J.; Parker, R.(1995). Diversity of cytoplasmic functions for the 3' untranslated region of eukaryotic transcripts. *Current Opinion in Cell Biology.* 7: 386-392.

Durrin, L. K.; Haile, R. W.; Ingles, S. A.; Coetzee, G. A.(1999). Vitamin D receptor 3'-untranslated region polymorphisms: lack of effect on mRNA stability. *Biochim Biophys Acta.* 1453: 311-320.

Fleet, J. C.(2006). Molecular regulation of calcium and bone metabolism through the vitamin D receptor. *J Musculoskelet Neuronal Interact.* 6: 336-337

George, A.; Evans, C. A.(2009). Detection of root resorption using dentin and bone markers. *Orthod Craniofac Res.* 12: 229-235

Gallagher, J.C.; Riggs, B. J.(1990). Action of 1,25-dihydroxyvitamin D_3 on calcium balance and bone turnover and its effect on vertebral fracture rate. *Metabolism.* 39: 30-34

Glenville, J.; Hogan, D. B.; Yendt, E.; Hanley, D. A.(1996). Prevention and management of osteporosis: Consensus statements from the Scientific Advisory Board of the Osteporosis Society of Canada.8. Vitamin D metabolities and analogs in the treatment of osteoporosis. *Canadian Medical Association J.* 155: 955-961

Gonzales, C.; Hotokeka, H.; Yoshimatsu, M.; Yozgation, J. H.; Darendeliler, M. A.; Yoshida, N.(2008). Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. *Angle Orthod.* 78: 502-509

Griffin, M. D.; Xing, N.; Kumar, R.(2003). Vitamin D and its analogs as regulators of immune activation and antigen presentation. *Ann Rev Nutri. 23: 117-145*

Gülden, N.; Eggermann, T.; Zerres, K.; Beer, M.; Meinett, A.; Deidrich, P.(2009). Interleukin-1 polymorphisms in relation to external apical root resorption (EARR). *J of Orofacial Orthop.* 3: 20-38

Gunes, S.; Sumer, A. P.; Keles, G. C.; Kara, N.; Koprulu, H.; Bagci, H.; Bek, Y.(2008). Analysis of vitamin D receptor gene polymorphisms in patients with chronic periodontitis. *Indian J Med Res.* 127: 58-64

Han, G.; Huang, S.; Von den Hoff, J.W.; Zeng, X.; Kuijpers-Jagtman, A.M.(2005). Root resorption after orthodontic intrusion and extrusion: An intraindividual study. *Angle Orthod.* 75:912-918

Harris, E. F.; Kineret, S. E.; Tolley, E. A.(1997). A heritable component for external apical root resorption in patients treated orthodontically. *Am J Orthod Dentofacial Orthop.* 111: 301-309.

Harris, R. J.(1999). Successful root coverage: a human histologic evaluation of case. *Int Periodontics Restorative Dent.* 19:439-460

Harris, D. A.; Jones, A. S.; Darendeliler, M. A.(2006). Physical properties of root cementum: part 8. Volumetric analysis of root resorption craters after application of controlled intrusive light and heavy orthodontic forces: a microcomputed tomography scan study. *Am J Orthod Dentofacial Orthop*. 130: 639-647

Hartsfield, J. K.; Everett, E. T.; Al-Qawasmi, R. A.(2004). Genetic factors in external apical root resorption and orthodontic treatment. *Crit Rev Oral Biol Med.* 15: 115-122

Hartsfield J. J. K.(2009). Pathways in external apical root resorption associated with orthodontia. *Orthod Craniofac Res.* 12: 236-242

Hollender, L.; Ronnerman, A.; Thilander, B.(1980). Root resorption, marginal bone support and clinical crown length in orthodontically treated patients. *Eur J Orthod.* 2: 197-205

Horst-Sikorska, W.; Wawrzyniak, A.; Celczynska-Bajew, L.; Marcinkowska, M., Dabrowski, S.; Kalak, R.; Slomski, R.(2005). Polymorphism of VDR gene--the most effective molecular marker of osteoporotic bone fractures risk within postmenopausal women from Wielkopolska region of Poland. *Endokrynol Pol.* 56: 233-239.

Huang, Y.; Wang, X.; Zhang, J.; Liu, C. (2010). Root shortening in patients treated with two-step and en masse space closure procedures with sliding mechanics. *Angle Orthod.* 80: 492-497.

Jiang, R. P., Zhang, D., Fu, M. K.(2003). A factors study of root resorption after orthodontic treatment. *Zhonhua Kou Qiang Yi Xue Za Zhi.* 38: 455-457

Karkoszka, H.; Chudek, J.; Strzelczyk, P.; Wiecek, A.; Schmidt-Gayk, H.; Ritz, E.; Kokot, F.(1998). Does the vitamin D receptor genotype predict bone mineral loss in haemodialysed patients? *Nephrol Dial Transplant.*13: 2077-2080

Kjaer, I.(1995). Morphological characteristics of dentitions developing excessive root resorption during orthodontic treatment. *Eur J Orthod.* 17: 25-34

Kung C.(2005). A possible unifying principle for mechanosensation. *Nature*. 4;436:647-54

Labuda, M.; Fujiwara, T. M.; Ross, M. V.; Morgan, K.; Garcia-Heras, J.; Ledbetter, D. H.; Hughes, M.R.; Glorieux, F. H.(1992). Two hereditary defects related to vitamin D metabolism map to the same region of human chromosome 12q13-14. *Journal of Bone and Mineral Research.* 7: 1447-1453.

Lages, E. M. B; Drummond, A. F.; Pretti, H.; Costa, F. O.; Lages, E. J.; Gontijo, A. I.; Miranda Cota, L.O.; Brito, R.B. Jr .(2009). Association of functional gene polymorphism IL-1beta in patients with external apical root resorption. *Am J Orthod Dentofacial Orthop.*136: 542-546

Levander, E.; Malmgren, O.(1988). Evaluation of the risk of root resorption during orthodontic treatment: a study of upper incisors. *Eur J Orthod.* 10: 30-38

Levander, E.; Malmgren, O.; Eliasson, S.(1994). Evaluation of root resorption in relation to two orthodontic treatment regimes. A clinical experimental study. *Eur J Orthod.*16: 223-228.

Levander, E.; Malmgren, O.; Stenback, K.(1998). Apical root resorption during orthodontic treatment of patients with multiple aplasia: a study of maxillary incisors. *Eur J Orthod.* 20: 427-434

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

Levander, E.; Malmgren, O.(2000). Long-term follow-up of maxillary incisors with severe apical root resorption. *Eur J Orthod. 22*: 85-92

Linge, L.; Linge, B. O.(1991). Patient characteristics and treatment variables associated with apical root resorption during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 99: 35-43

Liou, E.,J.; Chang, P.,M. (2010). Apical root resorption in orthodontic patients with en-masse maxillary anterior retraction and intrusion with miniscrews. *Am J Orthod Dentofacial Orthop.* 137: 207-212

Lips, P.(2006). Vitamin D physiology. *Prog Biophys Mol Biol.* 92: 1Loenen, M., Dermaut, L.R., Degrieck, J., De Pauw, G.A.(2007). Apical root resorption of upper incisors during the torquing stage of the tip-edge technique. *Eur J Orthod.* 29: 583-588

Loenen, M.; Dermaut, L.R.; Degrieck, J.; De Pauw, G.A.(2007). Apical root resorption of upper incisors during the torquing stage of the tip-edge technique. *Eur J Orthod.* 29: 583-588

Lopatiene, K.; Dumbravaite, A.(2008). Risk factors of root resorption after orthodontic treatment. *Stomalogija.* 10: 89-95

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

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

Makedona,s D; Odman, A.; Hansen, K. (2009). Management of root resorption in a large orthodontic clinic. *Swed Dent J.* 33: 173-180.

Masuyama, R.; Stockmans, I.; Torrekens, S.; Van Looveren, R.; Maes, C.; Carmeliet, P.; Bouillon, R.; Carmeliet, G. (2006). Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest.* 116: 3150-3159.

Mavragani, M.; Vergari, A.; Selliseth, N. J.; Boe, O. E.; Wisth, P. J.(2000). A radiographic comparison, of apical root resorption after orthodontic treatment with a standart edgewise and a straight-wire edgewise technique. *Eur J Orthod. 22*: 665-674

Meireles, J. K. S.; Weber, U. (2007). Centrex: uma proposta de sistema de forças ortodônticas para atuação no centro de resistência R Dental Press Ortodon Ortop Facial . 12: 38-47.

Mohandesan, H.; Ravanmehr, H.; Valaei, N.(2007). A radiographic analysis of external apical root resorption of maxillary incisors during active orthodontic treatment. *Eur J Orthod.* 29:134-9. Epub 2007 Jan 17.

Naito, M.; Miyaki, K.; Naito, T.; Zhang, L.; Hoshi, K.; Hara, A.; Masaki, K.; Tohyama, S.; Muramatsu, M.; Hamajima, N.; Nakayama, T.(2007). Association between vitamin D receptor gene haplotypes and chronic periodontitis among Japanese men. Int J Med Sci.22: 216-222.

Newman, W. G.(1975). Possible etiologic factors in external root resorption. *Am J Orthod.* 67:522-39.

Ngan, D. C.; Kharbanda, O. P.; Byloff, F. K.; Darendeliler, M. A.(2004). The genetic contribution to orthodontic root resorption: a retrospective twin study. *Aust Orthod J.* 20: 1-9.

Nishioka, M.; Ioi, H.; Nakata, S.; Nakasima, A.; Counts, A.(2006). Root resorption and immune system factors in the Japanese. *Angle Orthod.* 76:103-8.

Owman-Moll, P.(1995). Orthodontic tooth movement and root resorption with special reference to force magnitude and duration. A clinical and histological investigation in adolescents. *Swed Dent J Suppl*. 105:1-45. Review.

Pandis, N.; Nasika, M.; Polychronopoulou, A.; Eliades, T.(2008). External apical root resorption in patients treated with conventional and self-ligating brackets. *Am J Orthod Dentofacial Orthop*. 134:646-51

Parra, F. C.; Amado, R. C.; Lambertucci, J. R.; Rocha J.; Antunes C. M.; Pena S. D. (2003). Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci U S A*. 7:177-82.

Pinto, E. M.; Pedro Paulo da Costa Gondim, P. P. C.; Lima, N. S. (2008). Análise crítiva dos diversos métodos de avaliação e registro das más oclusões. *Rev. Dent. Press Ortodon. Ortop. Facial*. 3: 82-91.

Pizzo, G.; Licata ,M.E.; Guiglia, R.; Giuliana, G.(2007). Root resorption and orthodontic treatment. Review of the literature. *Minerva Stomatol.* 56:31-44.

Poon, A. H.; Laprise, C.; Lemire, M.; Montpetit, A.; Sinnett, D.; Schurr, E.; Hudson, T.J.(2004). Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med.* 170: 967-973.

Reitan K.; Rygh, P.(1994). Biomechanical principles and reactions. In: Orthodontics, current principles and techniques. *Graber TM, Vanarsdall RL, eds. 2nd edn. St Louis: Mosby*. 168-169.

Remmelink, H. J.; Van Der Molen, A. L. (1984). The effect of anteroposterior incisor repositioning on the root and cortical plate: following up study. *J Clin Orthod.* 18: 41-49.

Sameshima G. T.; Asgarifar K. O.(2001). Assessment of root resorption and root shape: periapical vs panoramic films. *Angle Orthod.* 71:185-9.

Sameshima, G. T.; Sinclair, P. M.(2001). Predicting and preventing root resorption: Part I. Diagnostic factors. *Am J Orthod Dentofacial Orthop*. 119:505-10.

Sameshima, G. T.; Asgarifar, K. O.(2003). Assessment of root resorption and root shape: Periapical vs Panoramic films. *Angle Orthod.* 71: 185-189

Sameshima, G. T.; Sinclair, P. M. (2004). Characteristics of patients with severe root resorption. *Orthod Craniofac Res.* 7: 108-114.

Silva Filho, O. G.; Ferrari Junior, F. M.; Ozawa, T. O. (2009). Dimensoes dos arcos dentários na ma oclusão de Classe II divisão 1 com deficiência mandibular. *Rev Dent Press Ortodon Ortop Facial.* 14: 120-130.

Sinotte, M.; Rousseau, F.; Ayotte, P.; Dewailly, E.; Berube, S.; Brisson, J.(2008). Vitamin D receptor polymorphisms (*Fok*I, *Bsm*I) and breast cancer risk:

association replication in two case-control studies within French Canadian population. *Endocrine – Related Cancer.* 15: 975-983

Sutton, A. L.; MacDonald, P. N.(2003) Vitamin D: more than a "bone-a-fide" hormone. *Mol Endocrinol.* 17:777-91. Epub 2003 Mar 13.

Takada K.; Kajiya, H.; Fukushima, H.; Okamoto, F.; Motokawa, W.; Okabe, K. (2004). Calcitonin in human odontoclasts regulates root resorption activity via protein kinase A. *J Bone Miner Metab*. 22:12-8.

Tanner, T.; Ciger, S.; Sencift, Y. (1999). Evaluation of apical root resorption following extaction therapy in subjects with Class II and Class I malocclusions. *Eur J Orthod.* 21: 491-496.

Trevilatto, P. C.; Line, S. R.(2000). Use of buccal epithelial cells for PCR amplification of large DNA fragments. *The Journal of Forensic Odonto-Stomatology.* 18: 6-9.

Uitterlinden, A. G.; Burger, H.; Huang, Q.; Oddung, E.; Duijin, C.M.; Hofman, A.; Birkenhager, J.C.; van Leeuwen, J. P.; Pols, H.A.(1997). Vitamin D receptor genotype is associated with radiographic osteoarthristis at the knee. *J Clin Invest.* 100:259-263.

Uitterlinden, A.G.; Fang, Y.; Bergink, A.P.; van Meurs, J.B.; van Leeuwen, H.P.; Pols, H.A.(2002). The role of vitamin D receptor gene polymorphisms in bone biology. *Molecular and Cellular Endocrinology* .197: 15-21.

Uitterlinden, A.G.; Fang, Y.; Van Meurs, J.B.; Pols, H.A.; Van Leeuwen, J.P. (2004). Genetics and biology of vitamin D receptor polymorphisms. *Gene.*1;338:143-56. Review.

Vardimon, A. D.; Weinreb, M.(2005). Maxillary incisor root resorption after rapid palatal expansion in Felis catus. *Eur J Oral Sci*. 113: 41-46

Verna, C.; Dalstra, M.; Melsen, B.(2003a) Bone turnover rate in rats does not influence root resorption induced by orthodontic treatment. *Eur J Orthod.*25: 359-363

Verna, C.; Melsen, B.(2003b). Tissue reaction to orthodontic tooth movement in different bone turnover conditions. *Orthod Craniofac Res.* 6: 155-163

Yamagushi, M.; Aishara, N.; Kojima, T.; Kasai, K.(2006). RankL increase in compressed periodontal ligament cells front root resorption. *J Dent Res.* 85: 751-756

Wierzbicki, T.; El-Bialy, T.; Aldaghreer, S.; Li., G.; Doschak, M.(2009). Analysis of Orthodontically induced root resorption using micro-computed tomography (Micro-CT). *Angle Orthod.* 79:91-96

Winter, B. U.; Stenvik, A.; Vandevska-Radunovic, V.(2009). Dynamics of orthodontic root resorption and repair in human premolars: a light microscopy study. *Eur J Orthod*. 31:346-51. Epub 2009 May 22.

Zhuang, L.; Bai, Y.; Meng, X. (2011). Three-dimensional morphology of root and alveolar trabecular boné during tooth movement using micro-computed tomography. *Angle Orthod*. 81: 420-425

Table 1. Baseline clinical characteristics of the study population.

	Group 1 n=160, (%)	**Group 2** n=179, (%)	**Group 3** n=38 (%)	***p* value**
Gender, n (%) *				
Female	86 (53.7)	99 (55.3)	23 (60.5)	0.695
Male	74 (46.3)	80 (44.7)	15 (39.5)	
Age, years **				
mean ± SD (range)	14.50±3.01 (8-21)[a]	15.33±2.64 (9.9-20)[a]	16.46±1.93 (11-19)[a]	<0.001

Group 1: individuals orthodontically treated with EARR ≤1.43 mm; **Group 2**: individuals orthodontically treated with EARR >1.43 mm; **Group 3**: individuals orthodontically untreated.
* Chi-square; ** ANOVA, mean ± standard deviation. Equal letters mean statically significant differences.

Table 2. Clinical variables influencing EARR in individuals orthodontically treated.

Variable	n	EARR		*p*-value* (univariate) n=339	*p*-value** (multivariate) n=339	OR	CI 95%
		≤ 1,43 n=160 (47.2%)	> 1,43 n=179 (52.8%)				
Age (years) [a]							
≤ 14	147	80 (54.4)	67 (45,6)				
> 14	192	80 (41.7)	112 (58.3)	**0.021**	**0.022**	1.69	1.08 – 2.66
Gender							
Female	185	86 (46.5)	99 (53.5)				
Male	154	74 (48.1)	80 (51.9)	0.827			
Initial Root (mm) [b]							
< 30	258	133 (51.6)	125 (48.4)				
≥ 30	81	27 (33.3)	54 (66.7)	**0.005**	**0.002**	2.34	1.36 – 4.03
XP							
No	291	143 (49.1)	148 (50.9)				
Yes	48	17 (35.4)	31 (64.6)	0.087	**0.052**	194	0.99 – 3.78
Elastics							
No	276	133 (48.2)	143 (51.8)				
Yes	63	42 (42.9)	36 (57.1)	0.486			
RPE							
No	285	130 (45.6)	155 (54.4)				
Yes	54	30 (55.6)	24 (44.4)	0.185			
Pendulum							
No	252	113 (44.8)	139 (55.2)				
Yes	87	47 (54)	40 (46)	0.171			
Genotype TT	58	33 (56.9)	25 (43.1)				
TC + CC	274	124 (45.3)	150 (54.7)	0.114	0.104	1.64	0.90 – 2.97

* Exact Fisher's test, $p<0.05$

** Regression Logistic Model, Wald's test, $p<0.05$ (variable were included when $p<0.20$ in univariate analysis).

[a] Cutoff point (14 years) suggested by ROC curve (0.574, $p=0.017$).

[b] Cutoff point (30 mm) suggested by ROC curve (0.620, $p<0.001$).

Table 3. A summary of studies in humans investigating genetic aspects on external apical root resorption (EARR).

Authors (year)	Type of study	Sample (n)	Mean age (yr-old)	Population	X-ray	Malocclusion	Results
Newman (1975)	Descriptive	47 individuals (case: 41; control: 6)	14.5	North-American	Pe[1]	50% Class I 27% Class II d1 6.8% Class II d2 9.1% Class III	Familial aggregation suggested
Harris et al. (1997)	Twin study	103 twin pairs	boys:14.5 girls:13.3	North-American	P[2] C[3]	30% Class I 63% Class II 9% Class III	~70% heritability to EARR of MCI[6] and LM[7]
AL-Qawasmi et al. (2003 a)	Family-based association study	35 families (118 individuals: 73 siblings and 45 parents)	12.1	North-American	P C	Class I Class II Class III	*IL1A* (-889) polymorphism not associated. *IL1B* (+3954) genotype CC [OR 5.6 CI 95% 1.9-21.2 p=0.0003] associated with EARR>2mm in MCI, MdCI[9], LM
AL-Qawasmi et al. (2003 b)	Linkage analysis	38 families (124 individuals; 79 siblings and 45 parents)	12.3	North-American	P C	Class I Class II Class III	Evidence for linkage of D18S64 microsatellite marker [lightly linked to *TNFRSF11A* (*RANK*)] with EARR>2mm [LOD=2.5; p=0.02] in MCI
Ngan et al. (2004)	Twin study	26 twin pairs (16 MZ[4] 10 DZ[5])	13.5MZ 2.9DZ	Australian Austrian	P	Class I Class II Class III	49% heritability to EARR in MCI and 66% in LM
Lages et al. (2009)	Population-based association study	61 Individuals (case: 23; control: 38)	?	Brazilian	Pe	54% Class I 39% Class II 6% Class III	Allele C of *IL1B* (+3954) is associated with EARR [OR=4.0 CI 95% p<0.05] in MCI and MLI[10]
Gulden et al. (2009)	Population-based association study	94 individuals (case: 45; control: 49)	≥12	North-American	P		*IL1B* (+3954) polymorphism not associated with EARR. Genotype TT of *IL1A* (-889) polymorphism is associated with EARR (p<0.032)

Pe[1] periapical X-ray; P[2]: panoramic X-ray; C[3]: Cephalometric X-ray; MZ[4]: monozygotic; DZ[5]: dizygotic

MCI[6]: maxillary central incisor; LM[8]: lower molar; MdCI[9]: mandibular central incisor; MLI[10]: maxillary lateral incisor.

Table 4. Genotype and allele distribution of the VDR T*aq*I polymorphism between the groups.

Genotypes	**Group 1**, n=157 (%)	**Group 2**, n=175 (%)	**Group 3**, n=35 (%)	***p*-value***
TT	33 (21.02)	25 (14.29)	2 (5.71)	
TC	117(74.52)	139 (79.43)	33 (94.29)	0.051
CC	7 (4.46)	11 (6.29)	0 (0.00)	

Alleles	**Group 1,** n=314 (%)	**Group 2**, n=350 (%)	**Group 3**, n=70 (%)	***p*-value***
T	183 (58.28)	164 (46.85)	35 (50.00)	0.455
C	131 (50.00)	138 (39.42)	33 (47.14)	

Group 1: EARR ≤1.43 mm; **Group 2**: EARR >1.43 mm; **Group 3**: non orthodontically treated.
*Chi-square, $p<0.05$.

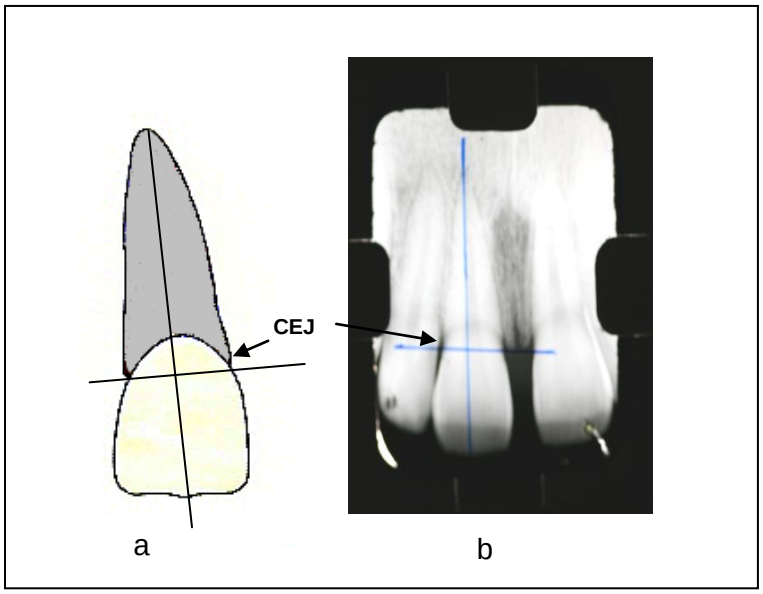

Fig. 1. (a) Anatomical references to measure EARR: cementoenamel junction (CEJ). (b) References of measurements in x-ray.

Fig. 2. Measurements with Calipter in x-ray.

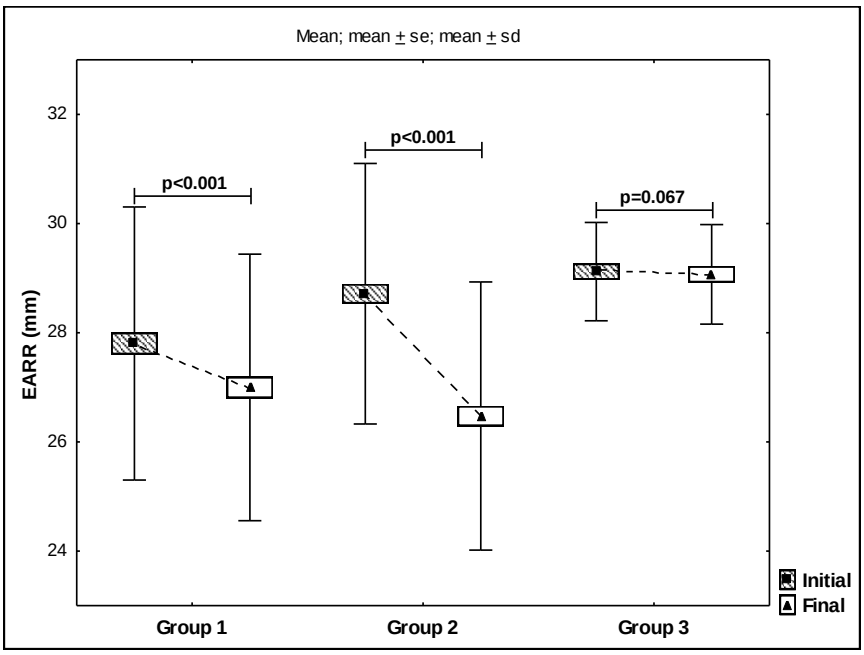

Fig. 3. Comparison of the EARR values (initial and final root length) among the groups (Paired t-test). **Group 1:** individuals orthodontically treated with EARR ≤1.43mm; **Group 2:** individuals orthodontically treated with EARR >1.43 mm; **Group 3:** individuals orthodontically untreated.

.....CONCLUSÃO

4. CONCLUSÃO

i) Os seguintes parâmetros clínicos estiveram associados à reabsorção radicular apical externa: pacientes com maior idade, dentes com raízes mais longas e extração de pré-molares.

ii) Genótipos contendo o alelo C do polimorfismo T*aq*I (C/T) no gene *VDR* (rs731236) foram fracamente associados com proteção contra a RRAE em indivíduos ortodonticamente tratados.

REFERÊNCIAS

6. REFERÊNCIAS

Acquaro, J. E.; Vedovello, S. A. S.; Degan, V. V.; Valdrighi, H. C.; Vedovello Filho, M.; Dona, C.M. (2007). *RGO.* 55: 281-285.

Aidar, M.; Line, S. R. (2007) A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Brazilian dental journal.*

Alhashimi, N.; Frithiof, L.; Brudvik, M. (2001). Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. *Am J Orthod Dentofacial Orthop.* 19: 307-312.

Alhashimi, N.; Frithiof, L.; Brudvik, P.; Bakhiet, M. (2004). CD40-CD40L expression during orthodontic tooth movement in rats. *Angle Orthod.* 74: 100-105

AL-Qawasmi, R. A.; Hartsfield, J. K.; Everett, E.T.; Flury, L.; Liu, L.; Foroud, T.M.; Macri, J. V.; Roberts, W. E. (2003a). Genetic predisposition to external apical root resorption. *Am J Orthod Dentofacial Orthop.* 123: 242-252.

AL-Qawasmi, R. A.; Hartsfield, J.K.; Everett, E.T.; Flury, L.; Liu, L.; Foroud, T.M.; Macri, J.V.; Roberts, W.E. (2003b). Genetic predisposition to external apical root resorption in orthodontic patients: Linkage of chromosome-18 marker. *Journal Dental Research.* 82: 356-360

Andreasen, F.M.; Sewerin, J.; Madel, V.; Andreasen, J.O.(1987). Radiographic assessment of simulated root resorption cavities. *Endod Dent Traumatol.* 3:21-27

Angle, E.H. Malocclusion of the Teeth.(1907). 7th ed.Philadelphia. SSWhite Dental.

Bastos Lages, E.M.; Drummond, A.F.; Pretti, H.; Costa, F.O.; Lages, E.J.; Gontijo, A.I.; Miranda Cota; L.O., Brito Junior, R. B. (2009). Association of functional gene polymorphism IL-1beta in patients with external apical root resorption. *Am J Orthod Dentofacial Orthop.*136: 542-546

Baumrind, S.; Korn, E.L.; Boyd, R.L.(1996). Apical root resorption in orthodontically treated adults. *Am J Orthod Dentofacial Orthop*. 110:311-20

Brezniak, N.; Wasserstein, A. (1993). Root resorption after orthodontic treatment: Part2. Literature review. *Am J Orthod Dentofacial Orthop.* 103:139-146

Brezniak, N.; Wasserstein, A.(2002a). Orthodontically induced inflammatory root resorption. Part I: The basic science aspects. *Angle Orthod.* 72: 175-179

Brezniak, N.; Wasserstein, A.(2002b). Orthodontically induced inflammatory root resorption. Part II: The clinical aspects. *Angle Orthod.* 72: 180-184

Brezniak, N.; Goren, S.; Zoizner, R.; Dinbar, A.; Arad, A.; Wasserstein, A.; Heller, M (2004). A comparison of three methods to accurately measure root length. *Angle Orthod.* 74: 786-791.

Brin, I.; Tulloch, C.; Koroluk, L.; Philips, C.(2003). External apical root resorption in Class II malocclusion: A retrospective review of 1-versus 2-phase treatment. *Am J Orthod Dentofacial Orthop.* 124: 151-156.

Brito Junior; R.B., Scarel-Caminaga, R.M.; Trevilatto, P.C.. (2004). Polymorphisms in the vitamin D receptor gene are associated with periodontal disease. *J Periodontol.* 75: 1090-1095.

Capelozza Filho, L.; Silva Filho, O.G.(1997) Expansão Rápida da Maxila: Considerações Gerais e Aplicação Clínica. Parte I. *Rev Dental Press de Ortodontia e Ortopedia Maxilar.* 2: 88-102.

Castelo, K.M.S.; Bramante, F.S.; Pinzan-Vercelino, C.R.M (2009). Caracteristicas estruturais da má-oclusão de Classe II, divisão 1. *Ortodontia*. 42 135-140.

Chan, E.; Darendeliler, M.A.(2005). Physical properties of root cementum-Part 5. Volumetric analysis of root resorption craters after application of light and heavy orthodontic forces. *Am J Orthod Dentofacial Orthop.* 127: 186-195

Cheteri, M. B.; Stanford, J. l.; Friedrichsen, M. A. P.; Iwasaki, L.; Langlois, M. C.; Ziding, F.; Ostrander, E. A. (2004). Vitamin D receptor gene polymorphism and prostate cancer risk. *The Prostate.* 59: 409-418.

Chiba-Falek, O.; Nussbaum, R.L.(2001). Effect of allelic variation at the NACP-Rep1 repeat upstream of the alpha-synuclein gene (SNCA) on transcription in a cell culture luciferase reporter system. *Human Molecular Genetics.* 10: 3101-3109.

Collins, M.T.; Lindsay, J.R.; Jain, A.; Kelly, M.H.; Cutler, C.M.; Weinstein, L.S.; Liu, J.; Fedarko, N.S.; Winer, K.K. (2005). Fibroblast growth factor-23 is regulated by 1 alpha, 25-dihydroxyvitamin D. *J Bone Miner Res*. 20: 1944-50

Consolaro, A.(2004). Sobre alguns aspectos metodológicos de pesquisas em movimentação dentária induzida e das reabsorções dentárias: uma proposta de guia e cuidados para análise de trabalhos. *R Dental Press Ortodon Ortop Facial*. 9: 104-109

Davideau, J.L.; Lezot, F.; Kato, S.; Bailleul-Forestier, I.; Berdal, A. (2004). Dental alveolar bone defects related to Vitamin D and calcium status. *J Steroid Biochem Mol Biol.* 89-90: 615-618.

Davidovitch Z; Nicolay O.F.; Ngan P. W.; Shanfeld, J.L. (1988). Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. *Dent Clin North Am*. 32:411-35.

Davidovitch, Z.; Godwin, S.; Young-Guk, P.; Taverne, A. A. R.; Dobeck, J. M.; De Sanctis, G. T. (1996). The etiology of root resorption. In: McNamaraJA, Trotman CA, eds. *Orthodontic Treatment: Management ofUnfavorable Sequelae.* Ann

Arbor, Mich: Center of Human Growth and Development, University of Michigan; 93–117.

Decker, C. J; Parker, R. (1995). Diversity of cytoplasmic functions for the 3' untranslated region of eukaryotic transcripts. *Current Opinion in Cell Biology.* 7: 386-392. DeLuca, H.F. (1988) The vitamin D story: a collaborative effort of basic science and clinical medicine. *Faseb J* 2: 224-236.

DeLuca, H. (1988) The vitamin D story: a collaborative effort of basic science and clinical medicine. *Faseb J* 2: 224-236.

Duman, B. S.; Tanakol, R.; Erensoy, N.; Ozturk, M.; Yilmazer, S. (2004). Vitamin D receptor alleles, bone mineral density and turnover in postmenopausal osteoporotic and healthy women. *Med Princ Pract.* 13: 260-266.

Durrin, L.K.; Haile, R.W.; Ingles, S.A.; Coetzee, G. A. (1999). Vitamin D receptor 3'-untranslated region polymorphisms: lack of effect on mRNA stability. *Biochim Biophys Acta.* 1453: 311-320.

Engström, C.; Granström, G.; Thilander, B. (1988). Effect of orthodontic force on periodontal tissue metabolism. A histologic and biochemical study in normal and hypocalcemic young rats. *Am J Orthod Dentofacial Orthop.* 93: 486-495.

Faraco, J.H.; Morrison, N.A.; Baker, A.; Shine, J.; Frossard, P.M. (1989) ApaI dimorphism at the human vitamin D receptor gene locus. *Nucleic Acids Res* 17: 2150.

Figueiredo, M.C; Parra S.L.N. Aspectos normais da membrana periodontal e osso alveolar. 2002.http://www.odontologia.com.br/artigos.

Fleet, J.C.(2006). Molecular regulation of calcium and bone metabolism through the vitamin D receptor. *J Musculoskelet Neuronal Interact.* 6: 336-337

George, A.; Evans, C.A. (2009). Detection of root resorption using dentin and bone markers. *Orthod Craniofac Res.* 12: 229-235

Gallagher, J.C.; Riggs, B.J. (1990). Action of 1,25-dihydroxyvitamin D_3 on calcium balance and bone turnover and its effect on vertebral fracture rate. *Metabolism.* 39: 30-34

Glenville, J.; Hogan, D.B.; Yendt, E.; Hanley, D.A. (1996). Prevention and management of osteporosis: Consensus statements from the Scientific Advisory Board of the Osteporosis Society of Canada.8. Vitamin D metabolities and analogs in the treatment of osteoporosis. *Canadian Medical Association J.* 155: 955-961

Gonzales, C.; Hotokeka, H.; Yoshimatsu, M.; Yozgation, J.H.; Darendeliler, M. A.; Yoshida, N. (2008). Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. *Angle Orthod.* 78: 502-509

Graber, T.M.; Vanarsdall, R.L. (1996). Princípios e Técnicas Atuais. 2ed. Ed Guanabara Koogan S.A.

Griffin, M.D., Xing, N., Kumar, R.(2003). Vitamin D and its analogs as regulators of immune activation and antigen presentation. *Ann Rev Nutri. 23: 117-145*

Gülden, N.; Eggermann, T.; Zerres, K.; Beer, M., Meinett, A.; Deidrich, P; (2009). Interleukin-1 polymorphisms in relation to external apical root resorption (EARR). *J of Orofacial Orthop.* 3: 20-38

Gunes, S.; Sumer, A. P.; Keles, G.C.; Kara, N.; Koprulu, H.; Bagci, H.; Bek, Y. (2008). Analysis of vitamin D receptor gene polymorphisms in patients with chronic periodontitis. *Indian J Med Res.* 127: 58-64

Guy, M.; Lowe, L.C.; Bretherton-Watt, D.; Mansi, J.L.; Peckitt, C.; Bliss, J., Wilson, R.G.; Thomas, V.; Colston, K.W. (2004) Vitamin D receptor gene polymorphisms and breast cancer risk. *Clin Cancer Res* 10: 5472-5481.

Han, G.; Huang, S.; Von den Hoff, J.W.; Zeng, X.; Kuijpers-Jagtman, A.M. (2005). Root resorption after orthodontic intrusion and extrusion: An intraindividual study. *Angle Orthod.* 75:912-918

Harris, E.F.; Kineret, S.E.; Tolley, E.A.(1997). A heritable component for external apical root resorption in patients treated orthodontically. *Am J Orthod Dentofacial Orthop.* 111: 301-309.

Harris, R.J. (1999). Successful root coverage: a human histologic evaluation of case. *Int Periodontics Restorative Dent.* 19:439-460

Harris, D.A.; Jones, A. S.; Darendeliler, M.A. (2006). Physical properties of root cementum: part 8. Volumetric analysis of root resorption craters after application of controlled intrusive light and heavy orthodontic forces: a microcomputed tomography scan study. *Am J Orthod Dentofacial Orthop*. 130: 639-647

Hartsfield,J.K.; Everett, E.T.; Al-Qawasmi, R.A.(2004). Genetic factors in external apical root resorption and orthodontic treatment. *Crit Rev Oral Biol Med.* 15: 115-122

Hartsfield Jr, J.K.(2009). Pathways in external apical root resorption associated with orthodontia. *Orthod Craniofac Res.* 12: 236-242

Holla L.I.; Fassmann A.; Stejskalová A.; Znojil V.; Vaněk J.; Vacha, J. (2004). Analysis of the interleukin-6 gene promoter polymorphisms in Czech patients with chronic periodontitis. J Periodontol. 2004 Jan;75(1):30-6.

Hollender, L.; Ronnerman, A.; Thilander, B. (1980). Root resorption, marginal bone support and clinical crown length in orthodontically treated patients. *Eur J Orthod.* 2: 197-205

Horowitz, M.C.; Xi, Y.; Wilson, K.; Kacena, M.A. (2001). Control of osteoclastogenesis and bone resorption by members of the TNF family of receptors and ligants. *Cytokine and Growth Factor Review.* 12: 9-18.

Horst-Sikorska, W.; Wawrzyniak, A.; Celczynska-Bajew, L.; Marcinkowska, M.; Dabrowski, S.; Kalak, R.; Slomski, R. (2005). Polymorphism of VDR gene--the most effective molecular marker of osteoporotic bone fractures risk within postmenopausal women from Wielkopolska region of Poland. *Endokrynol Pol.* 56: 233-239.

Interlandi, S.(1999). Ortodontia. Bases para a iniciação. 4ªed. Artes Médicas. São Paulo.

Jiang, R.P.; Zhang, D.; Fu, M.K. (2003). A factors study of root resorption after orthodontic treatment. *Zhonhua Kou Qiang Yi Xue Za Zhi.* 38: 455-457

Jimenez-Pellegrin, P.C.; Arana-Chavez, V.E. (2004). Root resorption in human mandibular first premolars after rotation as detected by scanning electron microscopy. *Am J Orthod Dentofacial Orthop.* 126: 178-184

Jurutka, P. W.; Remus, L. B. S.; Whitfield, G. K.; Thompson, P. D.; Hsieh, J. C.; Zitzer, H.; Tavakkolic, P.; Galligan, M. A.; Dang, H. T.; Haussler, C. A. (2000). The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. *Mol Endocrinol.* 14: 401-420.

Karkoszka, H.; Chudek, J.; Strzelczyk, P.; Wiecek, A.; Schmidt-Gayk, H.; Ritz, E.; Kokot, F.(1998). Does the vitamin D receptor genotype predict bone mineral loss in haemodialysed patients? *Nephrol Dial Transplant.*13: 2077-2080

Killiany, D.M. (1999). Root resorption caused by orthodontic treatment: an evidence-based review of literature. *Semin Orthod.* 5:128-33.

Kim, J. G.; Kwon, J. H.; Kim, S. H.; Choi, Y. M.; Moon, S. Y.; Lee, J. Y. (2003). Association between vitamin D receptor gene haplotypes and bone mass in postmenoáusal Korean women. *Am J of Obstetrics & Gynecology.* 189: 1234-1240.

Kim, S.; Shevde, N.K; Pike, J.W. (2005) 1,25-Dihydroxyvitamin D3 stimulates cyclic vitamin D receptor/retinoid X receptor DNA-binding, co-activator recruitment, and histone acetylation in intact osteoblasts. *J Bone Miner Res* 20: 305-317.

Kjaer, I.(1995). Morphological characteristics of dentitions developing excessive root resorption during orthodontic treatment. *Eur J Orthod.* 17: 25-34

Korstanje, R.; Paigen, B. (2002) From QTL to gene: the harvest begins. *Nat Genet* 31: 235-236.

Kung, C. (2005). A possible unifying principle for mechnosensation. *Nature.* 436: 647-654.

Labuda, M.; Fujiwara, T.M.; Ross, M.V.; Morgan, K.; Garcia-Heras, J.; Ledbetter, D.H.; Hughes, M.R.; Glorieux, F.H.(1992). Two hereditary defects related to vitamin D metabolism map to the same region of human chromosome 12q13-14. *Journal of Bone and Mineral Research.* 7: 1447-1453.

Lages, E.M.B; Drummond, A.F.; Pretti, H.; Costa, F.O.; Lages, E.J.; Gontijo, A.I.; Miranda Cota, L.O.; Brito, R.B. Jr .(2009). Association of functional gene polymorphism IL-1beta in patients with external apical root resorption. *Am J Orthod Dentofacial Orthop.*136: 542-546

Langdahl, B.L.; Cartens, M.; Stenkjaer, L.; Eriksen, E. F. (2000). Polymorphisms in the vitamin D receptor gene and bone mass, bone turnover and osteoporotis fractures. *Eur J Clin Invest.* 30: 608-617.

Lee, K-J.; Park, Y-C.; Yu, H-S.; Choi, S-H.; Yoo, Y-J. (2004) Effects of continuous and interrupted orthodontic force on interleukin-1β and prostaglandin E_2 prodction in gingival crevicular fluid. *Am J Orthod Dentofacial Orthop.* 125: 168-177.

Levander, E.; Malmgren, O. (1988). Evaluation of the risk of root resorption during orthodontic treatment: a study of upper incisors. *Eur J Orthod.* 10: 30-38

Levander, E.; Malmgren, O.; Stenback, K.(1988). Apical root resorption during orthodontic treatment of patients with multiple aplasia: a study of maxillary incisors. *Eur J Orthod.* 20: 427-434

Levander, E.; Bajka, R.; Malmgren, O. (1988). Early radiographic diagnosis of apical root resorption during orthodontic treatment: a study of maxillary incisors. *Eur J Orthod.* 20: 57-63

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

Levander, E.; Malmgren, O. (2000). Long-term follow-up of maxillary incisors with severe apical root resorption. *Eur J Orthod. 22*: 85-92

Linge, L.; Linge, B.O.(1991). Patient characteristics and treatment variables associated with apical root resorption during orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 99: 35-43

Lips, P.(2006). Vitamin D physiology. *Prog Biophys Mol Biol.* 92: 1

Loenen, M.; Dermaut, L.R.; Degrieck, J.; De Pauw, G. A. (2007). Apical root resorption of upper incisors during the torquing stage of the tip-edge technique. *Eur J Orthod.* 29: 583-588

Lopatiene, K., Dumbravaite, A. (2008). Risk factors of root resorption after orthodontic treatment. *Stomalogija.* 10: 89-95

Lorenzo, J.A.; Raisz, L.G. (1999). Cytokine and prostaglnadins.In: Seibel, M.J.; Robbins, S.; Bilezikian, J.P. (eds) Principles of bone and cartilage metabolisms. Academic Press, San Diego, pp 97-109.

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

Mah, J.; Carvalho, R. S.; Bumann, A. (2000). Current status of root resorption. In: Davidovitch, Z.; Mah, J. (eds) Biological mechanisms of tooth eruption and craniofacial adaptation. Harvard Society for the Advancement of orthodontics, Boston, M. A. 195-200.

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

Makedonas, D.; Odman, A.; Hansen, K. (2009). Management of root resorption in a large orthodontic clinic. *Swed Dent J.* 33: 173-180.

Marti, G.; Audi, L.; Esteban, C.; Oyarzabal, M.; Chueca, M.; Gussinye, M.; Yeste, D.; Fernandez-Cancio, M.; Andaluz, P.; Carrascosa, A. (2004) [Association of vitamin D receptor gene polymorphism with type 1 diabetes mellitus in two Spanish populations]. *Med Clin (Barc)* 123: 286-290.

Martin, T.J.; Romas, E.; Gillespie, M.T. (1998). Interleukins in the control of osteoclast differentiation. *Crit Rev Eukaryot Gene Expr*. 8:107-23. Review

Masuyama, R.; Stockmans, I.; Torrekens, S.; Van Looveren R.; Maes C, Carmeliet P, Bouillon R, Carmeliet G. (2006). Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest.* 116: 3150-3159.

Mathieu, C.; van Etten, E.; Decallonne, B., Guilietti, A.; Gysemans, C.; Bouillon, R. Overbergh. L. (2004) Vitamin D and 1,25-dihydroxyvitamin D3 as modulators in the immune system. *J Steroid Biochem Mol Biol* 89-90: 449-452.

Mavragani, M.; Vergari, A.; Selliseth, N.J.; Boe, O.E.; Wisth, P.J. (2000). A radiographic comparison, of apical root resorption after orthodontic treatment with a standart edgewise and a straight-wire edgewise technique. *Eur J Orthod. 22*: 665-674

Mavragani, M.; Brudvik, P.; Selvig K.A. (2005). Orthodontically induced root and alveolar bone resorption: inhibitory effect of systemic doxycycline administration in rats. *Eur J Orthod.* 27:215-25

McCulloch, C. A.; Melcher, A. H. (1983). Cell migration in the periodontal ligament of mice. *J Periodontal Res*. 18: 339-52.

McNab, S.; Battistutta, D.; Taverne, A.; Symons, A.L. (1999). External apical root resorption of posterior teeth in asthmatics after orthodontic treatment. *Am J Orthod Dentofacial Orthop.* 116: 545-51.

Meireles, J. K. S.; Ursi, W. (2007). Centrex: uma proposta de sistema de forças ortodônticas para atuação no centro de resistência. *Rev. Dent. Press Ortodon. Ortop. Facial*. 12: 38-47

Misof, B.M.; Roschger, P.; Tesch, W.; Baldock, P.A.; Valenta, A.; Messmer, P.; Eisman, J.A.; Boskey, A.L.; Gardiner, E.M.; Fratzil, P.; Klaushofer, K. (2003). Targeted overexpression of vitamin D receptor in osteoblasts increases calcium concentration without affecting structural properties of bone mineral crystals. *Calcif Tissue Int*. 73: 251-7.

Mohandesan, H.; Ravanmehr, H.; Valaei, N. (2007). A radiographic analysis of external apical root resorption of maxillary incisors during active orthodontic treatment. *Eur J Orthod.* 29:134-9. Epub 2007 Jan 17.

Morange, P.E.; Saut, N.; Alessi, M.C.; Frere, C.; Hawe, E.; Yudkin, J.S.; Tremoli, E.; Margaglione, M.; Di Minno, G.; Hamsten, A., Humphries, S.E. & Juhan-Vague, I. (2005) Interaction between the C-260T polymorphism of the CD14 gene and the plasma IL-6 concentration on the risk of myocardial infarction: the HIFMECH study. *Atherosclerosis* 179: 317-323.

Morrison, N. A.; Yeoman, R.; Kelly, P.J.; Eisman, J.A. (1992) Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin. *Proc Natl Acad Sci U S A* 89: 6665-6669

Moyers, R.E. (1991). Ortodontia. 4ed. Ed Guanabara Koogan S.A.

Nagpal, S.; Na, S.; Rathnachalam, R. (2005) Non-Calcemic Actions of Vitamin D Receptor Ligands. *Endocr Rev*.

Naito, M.; Miyaki, K.; Naito, T.; Zhang, L.; Hoshi, K.; Hara, A.; Masaki, K.; Tohyama, S.; Muramatsu, M.; Hamajima, N.; Nakayama, T.(2007). Association between vitamin D receptor gene haplotypes and chronic periodontitis among Japanese men. *Int J Med Sci.*22: 216-222.

Newman, W.G.(1975). Possible etiologic factors in external root resorption. *Am J Orthod.* 67:522-39.

Nezbedova, P.; Brito, J. (2004) 1alpha,25-dihydroxyvitamin D3 inducible transcription factor and its role in the vitamin D action. *Endocr Regul* 38: 29-38.

Ngan, D. C.; Kharbanda, O. P.; Byloff, F. K.; Darendeliler, M. A. (2004). The genetic contribution to orthodontic root resorption: a retrospective twin study. *Aust Orthod J.* 20: 1-9.

Nishioka, M.; Ioi, H.; Nakata, S.; Nakasima, A.; Counts, A. (2006). Root resorption and immune system factors in the Japanese. *Angle Orthod.* 76:103-8.

Nojima, L. I.; Gonçalves, M. C. (1990). Mudanças tissulares decorrentes do movimento ortodôntico. http://www.odontologia.com.br/artigos.

Nussbaum, R.L.; McInnes, R. R.; Willard, H. F. (2002). *Genetics in medicine* (Rio de Janeiro, RJ: Guanabara Koogan), pp. 69-82.

Owman-Moll, P.(1995). Orthodontic tooth movement and root resorption with special reference to force magnitude and duration. A clinical and histological investigation in adolescents. *Swed Dent J Suppl*. 105:1-45. Review.

Owman-Moll, P.; Kurol, J.(2000). Root resorption after orthodontic treatment in high- and low-risk patients: analysis of allergy as a possible predisposing factor. *Eur J Orthod.* 22: 657-63.

Pandis, N.; Nasika, M.; Polychronopoulou, A.; Eliades, T. (2008). External apical root resorption in patients treated with conventional and self-ligating brackets. *Am J Orthod Dentofacial Orthop*. 134:646-51

Park, H.J.; Baek, K.H.; Lee, H.L.; Kwon, A.; Hwang, H.R.; Qadir, A.S.; Woo, K.M.; Ryoo, H.M.; Baek, J.H. (2011). Hypoxia inducible factor-1α directly induces the expression of receptor activator of nuclear factor-kB ligand in periodontal ligament fibroblasts. Mol Cells. Apr 20

Parra, F.C.; Amado, R.C.; Lambertucci, J.R.; Rocha, J.; Antunes, C.M.; Pena, S.D. (2003) Color and genomic ancestry in Brazilians. *Proceedings of the National Academy of Sciences of the United States of America* 100: 177-182.

Pinto, E.M.; Gondim, P.P.C.; Lima, N.S. (2008). Análise critica dos diversos métodos de avaliação e registro das más oclusões. *Rev Dent Press Ortodon Ortop Facial*. 13: 82-91.

Pizzo, G.; Licata ,M.E.; Guiglia, R.; Giuliana, G.(2007). Root resorption and orthodontic treatment. Review of the literature. *Minerva Stomatol.* 56:31-44.

Poon, A.H.; Laprise, C.; Lemire, M.; Montpetit, A.; Sinnett, D.; Schurr, E.; Hudson, T.J. (2004). Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med.* 170: 967-973.

Rao, S.; Austin, H.; Davidoff, M. N.; Zafari, A. M. (2005) Endothelial nitric oxide synthase intron 4 polymorphism is a marker for coronary artery disease in African-American and Caucasian men. *Ethn Dis* 15: 191-197.

Reitan, K.; Rygh, P. (1994). Biomechanical principles and reactions. In: Orthodontics, current principles and techniques. *Graber TM, Vanarsdall RL, eds. 2nd edn. St Louis: Mosby*. 168-169.

Sameshima, G.T.; Sinclair, P.M.(2001). Predicting and preventing root resorption: Part I. Diagnostic factors. *Am J Orthod Dentofacial Orthop*. 119:505-10.

Sameshima, G.T.; Asgarifar, K.O. (2003). Assessment of root resorption and root shape: Periapical vs Panoramic films. *Angle Orthod.* 71: 185-189

Sameshima, G.T.; Sinclair, P.M. (2004). Characteristics of patients with severe root resorption. *Orthod Craniofac Res*. 7:108-14.

Sandy, J.R.; Farndale, R.W.; Meikle, M.C. (1993). Recent advances in understanding mechanically induced bone remodeling and their relevance to orthodontic theory and practice. *Am J Orthod Dentofacial Orthop.* 103: 212-22.

Shaffer, P.L.; Gewirtt, D.T. (2004). Vitamin D receptor – DNA interactions. *Vitam Horm*. 68: 257-73.

Shinkyo, R.; Sakaki, T.; Kamakura, M.; Ohta, M.; Inouye, K. (2004) Metabolism of vitamin D by human microsomal CYP2R1. *Biochem Biophys Res Commun* 324: 451-457.

Silva Filho, O.G.; Ferrari Junior, F.M.; Ozawa, T.O. (2009). Dimensões dos arcos dentários na má oclusão Classe II, divisão 1, com deficiência mandibular. *Rev. Dent Press Ortodon Ortop Facial.* 14: 120-130;

Sinotte, M.; Rousseau, F.; Ayotte, P.; Dewailly, E.; Berube, S.; Brisson, J. (2008). Vitamin D receptor polymorphisms (*Fok*I, *Bsm*I) and breast cancer risk: association replication in two case-control studies within French Canadian population. *Endocrine – Related Cancer.* 15: 975-983

Slattery, M. L.; Murtaugh, M.; Caan, B.; Ma, K.N.; Wolff, R.; Samowitz, W. (2004) Associations between BMI, energy intake, energy expenditure, VDR genotype and colon and rectal cancers (United States). *Cancer Causes Control* 15: 863-872.

Sooy, K.; Sabbagh, Y.; Demay, M. B. (2005) Osteoblasts lacking the vitamin D receptor display enhanced osteogenic potential in vitro. *J Cell Biochem* 94: 81-87.

Sun, J. L.; Meng, H.X.; Cao, C.F.; Tachi, Y.; Shinohara, M.; Ueda, M.; Imai, H.; Ohura, K. (2002) Relationship between vitamin D receptor gene polymorphism and periodontitis. *J Periodontal Res* 37: 263-267.

Sun, J.; Wiklund, F.; Zheng, S.L.; Chang, B.; Balter, K.; Li, L.; Johansson, J.E.; Li, G.; Adami, H.O.; Liu, W.; Tolin, A.; Turner, A.R.; Meyers, D.A.; Isaacs, W.B.; Xu, J.; Gronberg, H. (2005) Sequence variants in Toll-like receptor gene cluster (TLR6-TLR1-TLR10) and prostate cancer risk. *J Natl Cancer Inst* 97: 525-532.

Sutton, A.L.; MacDonald, P. N. (2003) Vitamin D: more than a "bone-a-fide" hormone. *Mol Endocrinol.* 17:777-91. Epub 2003 Mar 13.

Takada, K.; Kajiya, H.; Fukushima, H.; Okamoto, F.; Motokawa, W.; Okabe, K. (2004). Calcitonin in human odontoclasts regulates root resorption activity via protein kinase A. *J Bone Miner Metab*. 22:12-8.

Teitelbaum, S.L. (2000). Bone resorption by osteoclasts.*Science.* 1;289(5484):1504-8. Review

Thomas, P.D.; Kejariwal, A. (2004) Coding single-nucleotide polymorphisms associated with complex vs. Mendelian disease: evolutionary evidence for differences in molecular effects. *Proc Natl Acad Sci U S A* 101: 15398-15403.

Trevilatto, P.C.; Line, S. R. (2000). Use of buccal epithelial cells for PCR amplification of large DNA fragments. *The Journal of Forensic Odonto-Stomatology.* 18: 6-9.

Tzannetou, S.; Efstratiadis, S.; Nicolay, O.; Grbic, J.; Lamster, I. (1999). Interleukin-1beta and beta-glucuronidase in gingival crevicular fluid from molars during rapid palatal expansion. *Am J Orthod Dentofacial Orthop.* 115: 686-96.

Uematsu, S.; Mogi, M.; Deguchi, T. (1996). Interleukin (IL)-1β, IL-6, Tumor Necorsis Factor-α, Epidermal Growth Factor, and β_2-Microglobulin Levels Are Alevated in Gingival Crevicular Fluid during Human Orthodontic Tooth Movement. *J Dent Res.* 75: 562-567.

Uitterlinden, A. G.; Burger, H.; Huang, Q.; Oddung, E.; Duijin, C.M.; Hofman, A.; Birkenhager, J.C.; van Leeuwen, J. P.; Pols, H. A. (1997). Vitamin D receptor genotype is associated with radiographic osteoarthristis at the knee. *J Clin Invest.* 100:259-263.

Uitterlinden, A. G.; Fang, Y.; Bergink, A. P.; van Meurs, J. B.; van Leeuwen, H. P.; Pols, H. A.(2002). The role of vitamin D receptor gene polymorphisms in bone biology. *Molecular and Cellular Endocrinology* .197: 15-21.

Vardimon, A. D.; Weinreb, M. (2005). Maxillary incisor root resorption after rapid palatal expansion in Felis catus. *Eur J Oral Sci*. 113: 41-46

Verna, C.; Melsen, B. (2003). Tissue reaction to orthodontic tooth movement in different bone turnover conditions. *Orthod Craniofac Res.* 6: 155-163

Vlaskalic, V.; Boyd, R. L.; Baumrind, S. (1998). Etiology and sequelae of root resorption. *Semin Orthod.*;4:124-31.

Yamagushi, M.; Aishara, N.; Kojima, T.; Kasai, K. (2006). RankL increase in compressed periodontal ligament cells front root resorption. *J Dent Res.* 85: 751-756

Walters, M.R. (1992). Newly identified actions of the vitamin D endocrine system. *Endocrine Reviews.* 13: 719-764.

Walters, J.R.; Barley, N. F.; Khanji, M.; Rhodes-Kendler, O. (2004) Duodenal expression of the epithelial calcium transporter gene TRPV6: is there evidence for Vitamin D-dependence in humans? *J Steroid Biochem Mol Biol* 89-90: 317-319.

Whitfield, G. K.; Remus, L. S.; Jurutka, P. W.; Zitzer, H.; Oza, A. K.; Dang, H. T.; Haussler, C. A.; Galligan, M. A.; Thatcher, M. L.; Encinas Dominguez, C.; Haussler, M. R. (2001). Functionally relevant polymorphism in the human nuclear vitamin D receptor gene. *Mol Cell Endocrinol.* 25: 145-159.

Wierzbicki, T.; El-Bialy, T.; Aldaghreer, S.; Li., G.; Doschak, M. (2009). Analysis of Orthodontically induced root resorption using micro-computed tomography (Micro-CT). *Angle Orthod.* 79:91-96

Winter, B.U.; Stenvik, A.; Vandevska-Radunovic, V. (2009). Dynamics of orthodontic root resorption and repair in human premolars: a light microscopy study. *Eur J Orthod*. 31:346-51. Epub 2009 May 22.

Wu, A.T.; Turk, T.; Colak, C.; Elekdaq-Turk, S.; Jones, A.S.; Petocz, P.; Darendeliler, M.A. (2011). Physical properties of root cementum: Part 18. The extent of root resorption after the application of light and heavy controlled rotation orthodontic forces for 4 weeks: A microcomputed tomography study. *Am J Orthod Dentofacial Orthop.* 139: 495-503.

Ye, W. Z.; Reis, A. F.; Velho, G. (2000) Identification of a novel Tru9 I polymorphism in the human vitamin D receptor gene. *J Hum Genet* 45: 56-57.

Zahrowski, J; Jeske, A. (2011). Apical root resorption is associated with comprehensive orthodontic treatment but not clearly dependent on prior tooth characterisitcs or orthodontic techniques. *J Am Dent Assoc*. 142: 66-68.

Zhang, D.; Goetz, W.; Braumann, B; Bourauel, C.; Jaeger, A. (2003). Effect of soluble receptors to interleukin-1 and tumor necrosis factor alpha on experimentally induced root resorption in rats. *J Periodontal Res.* 38: 324-332

Zhuang, L.; Bai, Y.; Meng, X. (2011). Three-dimensional morphology of root and alveolar trabecular boné during tooth movement using micro-computed tomography. *Angle Orthod*. 81: 420-425