Revista ARACÊ
ISSN: 2358-2472

ASSOCIATION OF VITAMIN D RECEPTOR GENE POLYMORPHISM WITH EXTERNAL APICAL ROOT RESORPTION IN ORTHODONTICALLY TREATED PATIENTS

https://doi.org/10.56238/arev6n3-204

Submitted on: 15/10/2024 **Publication date:** 15/11/2024

Gabriela Paschoalini Romagni[1], Mayara Dolphin Sentone Rossato[2] and Queen Célia Poli[3]

ABSTRACT

The vitamin D receptor influences host immune responses and aspects of bone development, growth, and homeostasis. The aim of this study was to investigate the association of the polymorphism of the vitamin D receptor gene TaqI with external apical root resorption during orthodontic treatment. Methods: Our sample consisted of 50 patients aged between 12 and 40 years, who received fixed total orthodontic treatment during the years 1999 and 2000. External apical root resorption (RAAR) of the maxillary incisors was evaluated on periapical radiographs taken before and after 6 months of treatment. After DNA collection and purification, the analysis of the TaqI polymorphism of the vitamin D receptor was performed by polymerase chain reaction followed by restriction fragment length polymorphism. Univariate and multivariate analyses were performed to verify the association of clinical and genetic variables with external apical root resorption ($P<0.05$). There was a correlation between the VDR and RRAE variables ($r= 0.306$ and $p= 0.030$). The genotype resulting from Taq1 cleavage and allele frequencies differed between the groups with and without RAAR. Individuals containing the T allele were more likely to develop RAAR when compared to individuals carrying the tt genotype (OR= 7.5; 95% CI= 1.8 – 31.4; $p= 0.0094$). The TaqI polymorphism of the vitamin D receptor gene was associated with external apical root resorption in orthodontically treated patients.

Keywords: External Apical Root Resorption. Genetic polymorphism. VDR. Orthodontics.

[1] Student of the Graduate Program in Dentistry
UNIDERP
Email: gabriela-odontologia@hotmail.com
ORCID: 0000-0002-4207-0037
[2] Student of the Graduate Program in Dentistry
UNIDERP
Email: mayara_sentone@hotmail.com
ORCID: 0000-0003-1566-7716
[3] Professor of the Graduate Program in Dentistry
UNIDERP
E-mail: regina.frederico@unopar.br
ORCID: 0000-0003-4631-4606

Revista ARACÊ
ISSN: 2358-2472

INTRODUCTION

Orthodontics is the specialty of dentistry that is characterized by the movement of the teeth to a correct position in the dental arches. By applying force to the tooth, a narrowing of the periodontal ligament is created on the pressure side and an identical compressive action on its limits, that is, the root and bone surface. As cementum is more resistant to resorption when compared to more vulnerable bone, the forces applied usually induce bone resorption, the tooth moves and the periodontal membrane returns to its original dimension. However, resorption of cementum and dentin can also occur (BREZNIAK and WASSERSTEIN, 1993; CAPELOZZA FILHO and SILVA FILHO, 1998). RAAR (external periapical root resorption) mainly affects the maxillary central incisors, and can occur in 2 to 5% of the population (TAITHONGCHAI et al., 1996; KILLIANY, 1999; HARTSFIELD JR et al., 2004).

RAAR is a condition associated with both physiological and pathological processes, resulting in a loss of dentin, cementum, or bone (NE et al., 1999). It is considered a normal, essential and physiological process when it occurs in the deciduous teeth to allow their exfoliation and facilitate the eruption of the permanent successor (BREZNIAK and WASSERSTEIN, 1993).

Genetic, physiological and anatomical factors may be involved in the etiopathogenesis of RRAE (CAPELOZZA FILHO & SILVA FILHO, 1998). According to GRABER & VANARSDALL (1996), the etiology of root resorption during orthodontic treatment is complex and some factors alone or in association can contribute to the development, such as the patient's age, type of orthodontic appliance, duration of force, direction of tooth movement and tooth vulnerability.

There are three types of external root resorption: surface resorption, inflammatory resorption, and replacement resorption. Root resorption associated with corrective orthodontic treatment, due to the application of orthodontic forces, can be of the superficial type or of the transient or progressive inflammatory type, however, resorption by replacement is rarely observed after orthodontic treatment (LINGE and LINGE, 1991, BREZNIAK and WASSERSTEIN, 1993; SHAFER et al., 1987).

Consolaro (2005) established criteria for the classification of pathological tooth resorptions as: affected tooth surface (internal, external and internal-external), phase of process evolution (active, paralyzed and repaired), affected dental region (coronary, cervical, lateral and apical root) and extent of root involvement (single and multiple),

Revista ARACÊ
ISSN: 2358-2472

dimension of the cause of the process (local, systemic and idiopathic) and mechanism of occurrence of the process (inflammatory and by substitution). MALMGREN et al. (1982) classified apical root resorptions associated with orthodontic tooth movement into four grades.

While some degrees of root resorption occur in almost all patients (DESHIELDS, 1969), 93% of treated adolescents demonstrated some form of root resorption (KUROL, OWMAN-MOLL, LUDGREN, 1996). Approximately 15% (12-17% of orthodontically treated patients) showed moderate to severe apical root resorption (LINGE and LINGE, 1991) and 10-20% of cases reported severe resorption of >3 mm (LEVANDER and MALMGREN, 1988; LEVANDER et al., 1994). However, in most patients, root resorption is minor and insignificant (LEVANDER and MALMGREN, 1988).

For CONSOLARO (2005), the predisposition to RAAR associated with orthodontic treatment is related to root morphology (shape, length and angle between crown and root) and bone morphology (height, thickness and shape of the alveolar crest).

NEWMAN (1975) was the first researcher to relate heredity traits with root resorption. The following factors were investigated: genetic influences on family members, systemic causes, type of malocclusion, previous medical and dental history, and were related to root resorption. This author considered genetic potential as the cause of greater resorption of teeth with short roots after orthodontic treatment, although the pattern of inheritance was not clear.

AL-QAWASMI et al. (2003a) evaluated the association between polymorphisms in the IL-1 β genes and RAAR resulting from orthodontic treatment. These genes were investigated because they encode cytokines known to be involved in bone resorption accompanied by orthodontic tooth movement. Thirty-five American families were examined. Oral mucosal cells were collected to isolate and analyze the DNA. The RAAR in the maxillary central incisors, mandibular central incisors, and in the mesial and distal roots of the mandibular first molars were analyzed separately and together. There was high (statistically significant) evidence of association between the IL-1 polymorphism *β* and the RRAE manifestation. In addition, according to these authors, these findings are consistent with the interpretation of RAAR as a complex condition influenced by many factors, with the IL-1 gene *contributing β* an important predisposition to this problem.

The vitamin D receptor is a protein belonging to the superfamily of transcriptional regulatory factors of trans action, composed of steroid and thyroid hormone receptors

Revista ARACÊ
ISSN: 2358-2472

(BAKER et al., 1988; HANNAH & NORMAN., 1994). To exert its effects, vitamin D is converted into its active form, to 1,25 dihydroxyvitamin D3 [1,25(OH)2D3] or calcitriol. The action of calcitriol, in turn, occurs through a specific nuclear receptor, the VDR (HAUSSLER et al., 1998; ERBEN, 2001), which regulates gene transcription by binding to the responsive elements of vitamin D in the promoter region of the target genes (HANNAH & NORMAN., 1994; HAUSSLER et al., 1998; JONES et al., 1998; OZISIK et al., 2001; ERBEN, 2001).

METHODOLOGY

The sample was submitted to the UNOPAR Ethics Committee, and a favorable opinion was obtained. A cross-sectional study was carried out with 50 study participants between 12 and 40 years of age, who received fixed total orthodontic treatment during the years 1999 and 2000.

DNA was obtained from epithelial cells of the oral mucosa by mouthwash with 3% glucose for one minute. This solution was centrifuged for 2 min. At 2000 rpm, the discarded supernatant and cells were resuspended in 500 µL of the TNE extraction solution (AIDAR and LINE, 2007).

DNA extraction was performed using the DNA extraction kit following the manufacturers' instructions. The extracted DNAs were stored properly for later analysis of the polymorphism.

For polymerase chain reaction (PCR) amplification of the VDR gene, the following primers described by HENNING et al. (1999) were used:

a) Foward 5'- CAG AGC ATG GAC AGG GAG CAA G-3'
b) Reverse 5'- GGA TGT ACG TCT GCA GTG TG-3'

The PCR mixture consisted of a total volume of 50µl, containing approximately 500ng of genomic DNA, 1µM of each primer, 200µM of dNTPs and two units of Taq DNA polymerase. The solutions were incubated in a Multi-Gene Thermocycler. The amplification conditions were one cycle at 95ºC for two minutes of initial denaturation, 35 cycles of amplification consisting of each cycle for 45s at 95ºC (denaturation), one minute at 63ºC (primer pairing) and one minute at 72ºC (polymerization), and one cycle at 72ºC for seven minutes for the final extension. The amplified fragment is 340bp.

After amplification, 5 µl of the PCR product was analyzed by agarose gel electrophoresis (1.0%). Subsequently, the gel was stained with ethidium bromide and the newly synthesized fragments were visualized under ultraviolet light. The size of the product

Revista ARACÊ
ISSN: 2358-2472

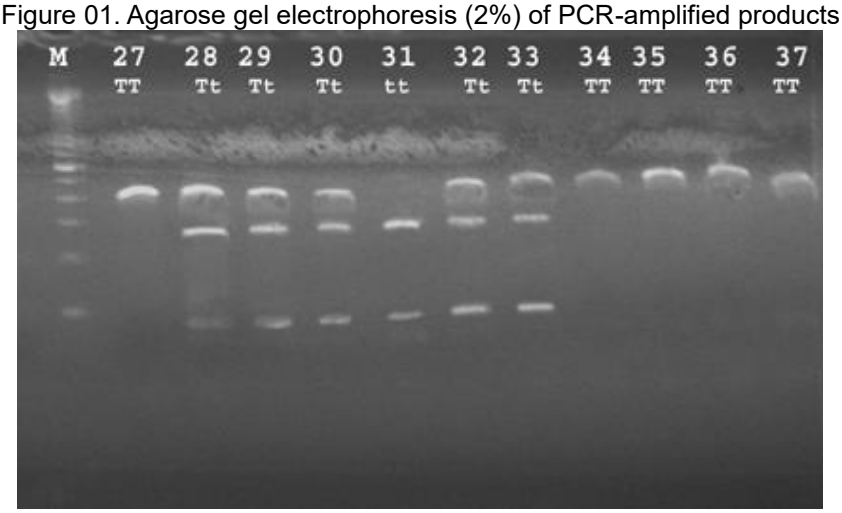
amplified by PCR was estimated from the electrophoretic migration of the product relative to the 100 bp marker DNA Ladder (Invitrogen).

For the RFLP (Restriction Fragment Length Polymorphism) technique, 12µl of the product amplified by PCR were added to 13µl of solution, containing 2.5µl of NE 10x buffer, 0.4µl of TaqI (10,000U/ml) and 10.1 µl of sterile deionized water. The solution was incubated at 65ºC overnight.

RFLP was created by the transition of a single base (T/C) at codon 352 of exon 9 of the VDR gene upon exposure to the restriction enzyme Taq1. The alleles were designated "t" (presence of the site for Taq1, forming a fragment of 260 bp and another with 80 bp) and "T" (absence of site for Taq1, maintaining the fragment of 340 bp).

The digestion products were separated by electrophoresis in 1.0% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. Figure 01.

Figure 01. Agarose gel electrophoresis (2%) of PCR-amplified products



RESULTS

In the present study, 50 individuals between 12 and 40 years of age who were orthodontically treated between 1999 and 2000 were evaluated, in which 60% of the sample corresponded to the female gender and 40% to the male gender. Regarding age, 54% were between 12 and 18 years old, 22% between 19 and 22 years old, and 24% between 23 and 40 years old (Table 01)

Similar proportions (32%) of dominant homozygotes (TT) and recessive homozygotes (TT) were found (Figure 02). The frequency of heterozygous individuals (Tt) was 36% (Table 02)

Revista ARACÊ
ISSN: 2358-2472

It is noteworthy that there was a correlation between the VDR and RRAE variables (r= 0.306 and p= 0.030).

The genotype resulting from Taq1 cleavage and allele frequencies differed between the groups with and without RAAR. Individuals containing the T allele were more likely to develop RAAR when compared to individuals carrying the tt genotype (OR= 7.5; 95% CI= 1.8 – 31.4; p= 0.0094).

Table 01. Characteristics of individuals treated orthodontically in terms of gender and age (N=50).

VARIABLES	N	%
GENDER Male Female	 20 30	 40 60
AGE 12-18 19-22 23-40	 27 11 12	 54 22 24

Table 02. Genotypic frequency of VDR gene polymorphism in orthodontically treated individuals (N=50).

Genotypic Frequency	N	%
TT dominant homozygous Homozygous Recessive tt Heterozigoto Tt	16 16 18	32 32 36

DISCUSSION

The VDR gene is expressed in a large number of tissues, but the highest concentrations are in the intestine, where vitamin D exerts its main function: to increase the absorption of calcium and phosphorus (HAUSSLER et al., 1998; JONES et al., 1998; ERBEN, 2001). Although osteoblasts (NARBAITZ et al., 1983) and osteoclast precursor cells (ROODMAN, 1996) also express the VDR gene, the direct action of vitamin D on bone metabolism cannot yet be confirmed.

The findings of the present study are consistent with the interpretation of RAAR as a complex condition influenced by many factors, and the polymorphism in the VDR gene predisposes the individual to this problem. Thus, the results of this study are consistent with the literature showing that teeth without strength have less resorption than teeth that have undergone orthodontic treatment (Mohandesan et al., 2007).

Revista ARACÊ
ISSN: 2358-2472

It has been shown that upper incisor earr, observed during the initial months of treatment, may be a predictor of increased risk of continued resorption during treatment. (Wierzbicki et al., 2009; Levander et al., 1998). Consequently, it has been recommended that periapical radiographs should be obtained after the first 6 months of treatment (Levander et al., 2000).

Other authors have evaluated the association between polymorphisms in the IL-1 β genes and RAAR resulting from orthodontic treatment (AL-QAWASMI et al.; 2003a). These genes were investigated because they encode cytokines known to be involved in bone resorption accompanied by orthodontic tooth movement. Thirty-five American families were examined. Oral mucosal cells were collected to isolate and analyze the DNA. The RAAR in the maxillary central incisors, mandibular central incisors, and in the mesial and distal roots of the mandibular first molars were analyzed separately and together. There was high (statistically significant) evidence of association between the IL-1 polymorphism *β* and the RRAE manifestation. In addition, according to these authors, these findings are consistent with the interpretation of RAAR as a complex condition influenced by many factors, with the IL-1 gene *contributing β* an important predisposition to this problem.

Our findings of a clinically significant association between LARR and orthodontic treatment suggest that patients susceptible to RASR can be identified early at the beginning of treatment, however few studies investigating the relationship between gene polymorphism and LARR have been described in the literature, evidencing the need for additional studies.

CONCLUSION

It was possible to conclude with the present study that TST individuals are 7.5 times more likely to develop RAAR compared to recessive homozygous (tt) and heterozygous (Tt) individuals.

ACKNOWLEDGMENTS

To patients treated orthodontically.

Revista ARACÊ
ISSN: 2358-2472

REFERENCES

1. Aidar, M., & Line, S. R. P. (2007). A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J, 18(2), 148-152.

2. Al-Qawasmi, R. A., Hartsfield, J. K. Jr, Everett, E. T., Flury, L., Liu, L., Foroud, T. M., et al. (2003a). Genetic predisposition to external apical root resorption. Am J Orthod Dentofacial Orthop, 123, 242-252.

3. Al-Qawasmi, R. A., Hartsfield, J. K. Jr, Everett, E. T., Flury, L., Liu, L., Foroud, T. M., et al. (2003b). Genetic predisposition to external apical root resorption in orthodontic patients: linkage of chromosome-18 marker. J Dent Res, 82, 356-360.

4. Al-Qawasmi, R. A., Hartsfield, J. K. Jr, Everett, E. T., Weaver, M. R., Foroud, T. M., Faust, D. M., & Roberts, W. E. (2006). Root resorption associated with orthodontic force in inbred mice: genetic contributions. Eur J Orthod, 28(1), 13-19.

5. Baker, A. R., McDonnell, D. P., Hughes, M., Crisp, T. M., Mangelsdorf, D. J., Haussler, M. R., Pike, J. W., Shine, J., & O'Malley, B. W. (1988). Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proceedings of the National Academy of Sciences of U.S.A., 25(10), 3294-3498.

6. Bender, I. B., Byers, M. R., & Mori, K. (1997). Periapical replacement resorption of permanent, vital, endodontically treated incisors after orthodontic movement: report of two cases. J Endod, 23(12), 768-773.

7. Brezniak, N., & Wasserstein, A. (1993). Root resorption after orthodontic treatment. Part 2. Literature review. Am J Orthod Dentofacial Orthop, 103, 138-146.

8. Capelozza Filho, L., & Silva Filho, O. (1998). Reabsorção radicular na clínica ortodôntica: atitudes para uma conduta preventiva. R Dental Press Ortodon Ortop Facial, 3(1).

9. Consolaro, A. (2005). Reabsorções dentárias nas especialidades clínicas (2ª ed.). Maringá: Dental Press International.

10. DeShields, R. W. (1969). A study of root resorption in treated Class II, Division I malocclusions. Angle Orthod, 39(4), 231-245.

11. Erben, R. G. (2001). Vitamin D analogs and bone. Journal of Muskuloskeletal and Neuronal Interaction, 2(1), 59-69.

12. Graber, T. M., Vanarsdall Jr, R. L., & Barbosa, J. A. (1996). Ortodontia, princípios e técnicas atuais (2ª ed.). Rio de Janeiro: Guanabara Koogan.

13. Gomez Alonso, C., Naves Diaz, M. L., Díazo-Corte, C., Fernandez Martin, J. L., & Cannata Andia, J. B. (1998). Vitamin D receptor (VDR) gene polymorphisms: effect on bone mass, bone loss and parathyroid regulation. Nephrology Dialysis Transplantation, 13(3), 73-77.

Revista ARACÊ
ISSN: 2358-2472

14. Hannah, S. S., & Norman, A. W. (1994). 1,25 (OH)2 vitamin D3 regulated expression of eukaryotic genome. Nutrition Reviews, 52(11), 376-382.

15. Hartsfield, J. K., Everett, E. T., & Al-Qawasmi, R. A. (2004). Genetic factors in external apical root resorption and orthodontic treatment. Critical Reviews in Oral Biology and Medicine, 15, 115-122.

16. Haussler, M. R., Whitfield, G. K., Haussler, C. A., Hsieh, J. C., Thompson, P. D., Selznick, S. H., Encians Dominguez, C., & Jurutka, P. W. (1998). The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. Journal of Bone and Mineral Research, 13(3), 325-349.

17. Henning, B. J. W., Parkhill, J. M., Chapple, I. L. C., Heasman, P. A., & Taylor, J. J. (1999). Association of a vitamin D receptor gene polymorphism with localized early-onset periodontal diseases. J Periodontol, 70, 1032-1038.

18. Jones, G., Strugnell, S. A., & Deluca, H. F. (1998). Current understanding of the molecular actions of vitamin D. Physiological Reviews, 78(4), 1193-1291.

19. Killiany, D. M. (1999). Root resorption caused by orthodontic treatment: an evidence-based review of literature. Semin Orthod, 5(2), 128-133.

20. Kurol, J., Owman-Moll, P., & Lundgren, D. (1996). Time-related root resorption after application of a controlled continuous orthodontic force. Am J Orthod Dentofacial Orthop, 110(3), 303-310.

21. 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.

22. 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(3), 223-228.

23. 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.

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

25. 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.

26. Malmgren, O., Goldson, L., Hill, C., Orwin, A., Petrini, L., & Lundberg, M. (1982). Root resorption after orthodontic treatment of traumatized teeth. Am J Orthod, 82(6), 487-491.

Revista ARACÊ
ISSN: 2358-2472

27. 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, 134-139.

28. Narbaitz, R., Stumpf, W. E., Sar, M., Huang, S., & Deluca, H. F. (1983). Autoradiographic localization of target cells for 1,25 dihydroxyvitamin D3 in bones from fetal rats. Calcified Tissue International, 35(2), 177-182.

29. Ne, R. F., Witherspoon, D. E., & Gutmann, J. L. (1999). Tooth resorption. Quintessence Int, 30, 9-25.

30. Newman, W. G. (1975). Possible etiologic factors in external root resorption. Am J Orthod, 67, 522-539.

31. Ozisik, G., Mergen, H., Ozata, M., Uyanik, C., Caglayan, S., Turan, M., Bolu, E., Ilgin, S., Oner, R., Ozdemir, I. C., Oner, C. (2001). Vitamin D-receptor gene polymorphisms and vertebral bone density in men with idiopathic hypogonadotrophic hypogonadism. Medical Science Monitor, 7(2), 233-237.

32. Roodman, G. D. (1989). Advances in bone biology: the osteoclast. Endocrine Reviews, 17(4), 308-332.

33. Taithongchai, R., Sookkorn, K., & Killiany, D. M. (1996). Facial and dentoalveolar structure and the prediction of apical root shortening. Am J Orthod Dentofacial Orthop, 110, 296-302.

34. Shafer, W. G., Hine, M. K., & Levy, B. M. (1987). Tratado de Patologia Bucal. Rio de Janeiro: Interamericana.

35. 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.