

# Types of External Root Resorption of Replanted Teeth: Analysis of the Clinical Aspects and of Interleukin-4 Gene Polymorphisms Involvement

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## Abstract

**Introduction:** The absence or presence of root resorption on the surface of a replanted tooth indicates an immune-inflammatory reaction. Recent research even suggests the participation of host predominant immunologic profile on types of resorptions detected on the root surface. Because interleukin 4 (IL-4) is an important anti-inflammatory cytokine, this study aimed to investigate the association of clinical variables and polymorphisms in *IL4* with types of resorption of replanted teeth after 1 year of follow-up. **Methods:** One hundred twenty-seven avulsed teeth that were replanted were selected. Periapical radiographs were taken after replantation and for 1 year to detect the types of root resorption. Real-time polymerase chain reaction was used to genotype *IL4* polymorphisms. The  $\chi^2$  and Z tests were performed to verify the association of clinical and genetic variables with the outcomes of replanted teeth ( $P < .05$ ). **Results:** An association was observed of extra-alveolar time, storage medium, and development of the root ( $P < .05$ ), but not of *IL4* polymorphisms, with the outcomes of replanted teeth ( $P > .05$ ). **Conclusions:** Extraoral time, storage medium, and development of the root, but not *IL4* polymorphisms, may influence the types of resorption of avulsed and replanted teeth in the first year after trauma. (*J Endod* 2017; ■:1–5)

## Key Words

Avulsion, cytokines, inflammation, polymorphisms, replantation, root resorption

Dental avulsion is a very serious trauma, and the most appropriate approach is dental replantation (1). However, root resorption presents a major challenge. It mainly occurs on total or partial loss of the periodontal

ligament, compromising the cementoblast layer in addition to the immune-inflammatory response of the patient (2, 3). It can result in tooth loss, compromising the patient aesthetically, functionally, and psychologically.

Clinical aspects of replantation, such as the extra-alveolar time of the avulsed tooth, time elapsed between trauma and first treatment, storage medium before dental replantation, intracanal medication, intensity of the inflammatory reaction triggered by the avulsion, and the presence of bacteria in the socket, will determine the impact on the root surface (1). Thus, the predominantly immune-inflammatory host response may have a fundamental importance in the maintenance of tissue health or disease. In previous studies, it was demonstrated that an atopic patient who has a predominantly T-helper 2 (Th2) immunologic profile, which means responds predominantly with anti-inflammatory cytokines such as interleukin 4 (IL-4), had a better outcome 1 year after replantation (3–5). This condition may also create an advantage in losing fewer teeth because of replacement resorption after 5 years of follow-up (6).

Because cytokines are encoded by DNA and regulate the immune response, it is important to analyze the genetic characteristics of the patients to determine their contribution to the development of root resorption. Single nucleotide polymorphisms (SNPs) are the most common forms of variation in DNA. They may affect the quantity or function of the expressed protein, resulting in changes in both innate and adaptive immune responses, by determining the susceptibility or protection to a given disease (7). For this reason, this study focused on the polymorphic variations of *IL4* gene (8), in high linkage disequilibrium (LD), to genotype all the “target” SNPs (tag SNP), an approach that captures all the variability information in *IL4* gene (9).

Therefore, this research aimed to verify a possible association of tag SNPs in *IL4* and clinical characteristics with the types of external root resorption of replanted teeth in 1 year of follow-up.

## Significance

This is the first study in literature demonstrating the influence of the management of avulsed teeth and *IL4* polymorphisms on different types of root resorption in replanted teeth. It may contribute to research molecular biomarkers and supporting materials and techniques for preventing resorption.

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## Materials and Methods

### Population Study

This study included a sample of 127 avulsed and replanted teeth. The patients had their teeth replanted with different ranges of extra-alveolar time, and they were placed in various storage media until replantation. Patients were taken to receive endodontic treatment when necessary and were followed up in the same dental clinics during 1 year. Flexible splint placed in emergency care was maintained for 2 weeks, and then a temporary filling with calcium hydroxide was placed inside the root canal for at least 1 month. The final obturation was performed when the conditions of the tooth seemed stable.

Next to avulsion and replantation, sensibility tests give no response, indicating a transient lack of pulpal response. Therefore, teeth with opened apices were followed up every 15 days, and at least 2 signs and symptoms were necessary to establish the diagnosis of their necrotic pulp: symptomatic, negative response for cold pulp test, signs of apical periodontitis, or discontinuity of root development.

All the objectives and procedures followed in this study were explained to the patients or guardians, and they signed an informed consent. This research was approved by the local Research Ethics Committee, number 02320084000-10.

### Inclusion and Exclusion Criteria

This study included patients of both genders who received endodontic treatment, when necessary, in the same dental clinics because of the replantation caused by avulsion and were evaluated clinically and radiographically during 1 year. Only nonsmoking patients and patients without periodontal disease or any other soft or hard lesion in the mouth were included. Only those teeth that were extracted because of severe external root resorption were included. Immunocompromised patients and those taking continuous medication were excluded.

### Radiographic Assessment of Root Resorption

The patients were evaluated clinically and radiographically every 15 days during the endodontic treatment and every 3 months during the follow-up. Radiographic examination compared the initial radiographs taken during emergency care soon after replantation with those taken after 1 year of follow-up. Radiography was performed with the aid of radiographic positioners JON (São Paulo, SP, Brazil) with sensitivity E/F and size 2 Kodak Insight periapical films (Eastman Kodak Co, Rochester, NY). The images were analyzed in a scattering-box light with the aid of a  $\times 4$  magnifier and dark card stock. Two calibrated PhD endodontists independently evaluated the teeth clinically and radiographically, with very high intraexaminer agreement for both examiners (kappa scores, 0.90% and 0.88%) and very good interexaminer agreement (0.88%). Radiographic signs such as presence or absence of external root resorption, complete presence, total, or partial interruption of lamina dura, ankylosis, and bone loss were evaluated.

Inflammatory resorption was defined as bowl-shaped radiolucencies in the external root resorption area, with adjacent bone loss. Replacement resorption was defined as the presence of bone and loss of periodontal ligament in the resorption area.

### Clinical Variables

The following were the clinical variables evaluated in relation to absence, type of the present external root resorption, and extraction because of severe root resorption:

- age range of the patient: younger than or up to 17 years,
- development of the root: open or closed apices,
- extra-alveolar time: less or more than 1 hour, and

- storage medium: favorable (socket for up to 20 minutes or milk for up to 6 hours) or unfavorable (dry, saliva, saline solution, water).

### Collection and DNA Purification

To collect oral mucosa cells, the selected subjects were rinsed for 1 minute with 3% glucose solution. A sterile wooden spatula was used to scrape the buccal mucosa, and the tip of the spatula was then agitated in the rinsed solution. The buccal epithelial cells were pelleted by centrifugation at 2000 rpm for 10 minutes. The supernatant was discarded, and the pellet was suspended in 1300  $\mu\text{L}$  extraction buffer (10 mmol/L Tris-HCl [pH 7.8], 5 mmol/L EDTA, 0.5% sodium dodecyl-sulfate [Biotec, São Paulo, SP, Brazil]). Then, 10  $\mu\text{L}$  proteinase K (Invitrogen, Waltham, MA) (20 mg/mL) was added to the solution and incubated overnight at 65°C. DNA was purified by the addition of 10 mol/L ammonium acetate, precipitated with isopropanol, ethanol 70%, and suspended in 50  $\mu\text{L}$  10 mmol/L Tris (pH 7.6) and 1 mmol/L EDTA (10).

### Analysis of the Polymorphisms in the *IL4* Gene

Tag SNP markers capturing all the information of *IL4* gene were selected according to the data available on the website of International HapMap Project, phase III/Rel#2 (<http://www.hapmap.org>, 2014) (11).

The selection of tag SNPs used some criteria such as 5% population minimum allele frequency and 80% cutoff ( $r^2 < 0.8$ ) to define LD to investigate *IL4* gene. The CEU (Caucasian) was selected as the basic population. After applying the above criteria, 2 tag SNPs capturing all of the information of *IL4* gene were selected by HapMap, reference SNP rs2227284 and rs2243268.

The selected tag SNPs were genotyped by real-time polymerase chain reaction (PCR) by using the Applied Biosystems 7500 (7500 Real-Time PCR System) and the TaqMan Technology Genotyping Master Mix (Applied Biosystems, Foster City, CA).

### Statistical Analysis

Estimation of Hardy-Weinberg equilibrium and evaluation of LD in the study population were performed by using 4.2 Haploview software. To evaluate the outcome of replanted teeth, the clinical and demographic variables, and the *IL4* polymorphisms, this study used the  $\chi^2$  test and the Z test for differences between 2 proportions when the sample size was small at a probability level of  $P < .05$ .

## Results

### Demographic and Clinical Characteristics

Of the 127 teeth, 79 (62.2%) remained without resorption, 18 (14.2%) presented inflammatory external root resorption, 10 (7.9%) presented replacement root resorption, and 20 teeth (15.7%) were extracted because of very severe inflammatory external root resorption. No tooth was extracted because of replacement root resorption after 1 year of replantation (Table 1).

Even though there was not a significant difference between the outcome of the replanted teeth and age range ( $P > .05$ ), the Z test demonstrated a significant difference between the several outcomes. The patients older than 17 years had more chances to keep healthy teeth after replantation; of the 32 teeth of patients older than 17 years, 25 (78.1%) remained without root resorption (Table 1).

Of the 127 patients, 97 (76.4%) had teeth with complete root formation, and 30 (23.6%) presented open apices. Of the 30 teeth with open apices, 16 (53.3%) remained without resorption. Five of these 16 (31.3%) did not need to receive any endodontic treatment because of spontaneous pulp revascularization and continuity

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