

# Evaluation of role of periodontal pathogens in endodontic periodontal diseases

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# Abstract

**Aim:** This study aimed to correlate periodontal pathogens in endodontic periodontal diseases. **Methodology:** This study was conducted on 40 patients of both genders. All the participants were obtained from department of endodontics and periodontology with history of endo-perio lesion in same teeth. Polymerase chain reaction was performed and correlation was established. **Results:** This study included 18 males and 22 females. The mean age of male was 42.5 years and female was 41.3 years. Specimens of *Tannerella forsythia* were isolated from 94% endodontium and 92% periodontium, *Porphyromonas gingivalis* from 71% endodontium and 55% periodontium, *Aggregatibacter actinomycetemcomitans* from 12% endodontium and 58% periodontium. The difference was significant (P < 0.05). Bacteria in endodontic-periodontal infection confirmed statistically significant correlation between absolute quantitation of *T. forsythia* and *P. gingivalis* (r = 0.412, P < 0.05), *P. gingivalis* and *A. actinomycetemcomitans* (r = 0.524, P < 0.05), and *T. forsythia* and *A. actinomycetemcomitans* (r = 0.427, P < 0.05). **Conclusion:** There was correlation between targeted bacterial species levels from concurrent endodontic-periodontal diseases. Thus, it can be suggested that dentinal tubules may be the pathway for spread of bacteria.

Keywords: Endodontic-periodontal diseases, P. gingivalis, T. forsythia

# Introduction

The penetration of bacteria through dentinal tubules is considered to be the matter of controversy and infection of endodontic-periodontal route niche is not well understood. Cementum facing toward the dentinal tubules helps in closing while the protective covering of dentinal cementum may be mechanically eroded, removed, and abraded with various processes such as clinical, pathological, and erosive processes.<sup>[1]</sup>

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In periodontal diseases, scaling and root planing are the mainstay of treatment in which infected cementum is removed.

Apical foramen, lateral canals, accessory canals, and dentinal tubules are other possible pathways considered to be the route of communication in endodontic-periodontal niches.<sup>[2]</sup> Movement of bacteria from the outer root surface to the root canal system may reinforce the reservoir effect of the dentinal tubules, whereas cementum acts as a barrier for bacterial migration through dentinal tubules. Factors such as bacterial size, adhesive properties, and motility may affect the degree of permeability to the dentinal tubules. This, in turn, depends upon different areas of the tooth and the age of the patient.<sup>[3]</sup>

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The correlation of infective organisms in endodontic-periodontal niches has been studied in the past by various methods. The bacteria and bacterial bioproducts and the type of infection can stimulate immunological response of vital pulp. It has shown that bacteria may travel through dentinal tubules in both directions to colonize both endodontic-periodontal tissues.<sup>[4]</sup> Considering this, this study aimed to correlate periodontal pathogens in endodontic periodontal diseases.

### **Materials and Methods**

This study was conducted in the Department of Periodontology and Endodontics. Ethical approval was obtained form institutional ethical committe on 6<sup>th</sup> Feb 2018. All patients were informed regarding the study in local language and informed written consent was obtained. The study comprised of 40 patients of both genders with age range of 20–50 years, with single rooted tooth having endodontic or periodontal pathology, patients with periodontal pockets <6 mm. Patients with presence of fistula, radiographic proof of endodontic-periodontal communication and patients with aggressive periodontitis, patients with history of antibiotic use in last 3 months were excluded. Patient data, such as name, age, gender, etc., were recorded in case history performa. All patients were subjected to intraoral periapical radiographs to reach the diagnosis of endodontic-periodontal lesions.

#### **Collection of specimens**

For the collection of periodontal samples, two to three paper points were inserted into periodontal pockets and left *in situ* for 1 min each. For collection of samples from tooth with single root canal strict aseptic procedure was followed. Two to three sterile paper points were inserted into the root canal and left for 1 min. The paper points were then transferred to cryotubes containing 1 mL of TE buffer. Samples were immediately frozen and kept at  $-80^{\circ}$ C.

#### **Extraction of DNA**

Frozen endodontic and periodontal specimens were re-suspended and thawed at room temperature before the DNA extraction. This procedure was performed using Qiamp DNA (Qiagen, Hilden, Germany) mini kit. DNA concentration and purity was assessed using UV mini spectrophotometer 1240.

#### Primers description and amplification

Polymerase chain reaction (PET plus; MIP Pharma, Germany) was used for the identification of periodontal pathogens for *Tannerella forsythia*, *Tf* forward 5'-GCG TAT GTA ACC TGC CCG CA-3' *Tf* reverse 5'-TGC TTC AGT GTC AGT TAT ACC T-3' with amplicon length 641 base pairs, for *Porphyromonas gingivalis Pg* forward 5'-AGG CAG CTT GCC ATA CTG CG-3' *Pg* reverse 5'-ACT GTT AGC AAC TAC CGA TGT-3' with amplicon length 404 bp, and for *Aggregatibacter actinomycetemcomitans, Aa* forward 5'-AAA CCC ATC TCT GAG TTC TTC AG-3' Aa reverse 5'-ATG CCA ACT TGA CGT TAA AT-3' with amplicon length 557 bp.

Exact number of bacterial DNA copies was assessed using absolute quantitation method. Strains were obtained from the American type culture collection (ATCC; Manassas, VA): *T. forsythia* ATCC 43037, *P. gingivalis* ATCC 33277, and *A. actinomycetemcomitans* ATCC 29523. Quantitative and qualitative bacterial microflora evaluations were done using PET plus (MIP Pharma, Germany) diagnostic tests.

Data thus obtained were tabulated and statistically evaluated with SPSS package (21.0 version, Inc.; Chicago, IL) using Student *t* at *P* value <0.05 was considered significant.

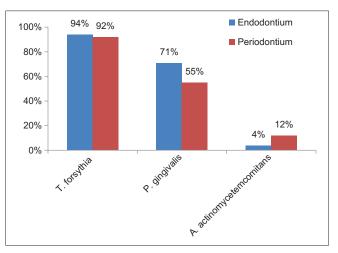
#### Results

Table 1 shows that out of 40 patients, males were 18 and females were 22. The mean age of male was 42.5 years and female was 41.3 years. Table 2 and Graph 1 shows that specimens of *T. forsythia* was isolated from 94% endodontium and 92% periodontium, *P. gingivalis* from 71% endodontium and 55% periodontium, *A. actinomycetemcomitans* from 12% endodontium and 58% periodontium. The difference was significant (P < 0.05). There was nonsignificant difference in the quantity of targeted pathogens between the endodontium and periodontium for the same tooth [Table 3]. Table 4 shows that bacteria in endodontic-periodontal infection confirmed

Table 1: Distribution of patients				
Parameters	Male	Female		
Number	18	22		
Mean age (years)	42.5	41.3		

Table 2: Detected species in endodontium and					
periodontium					
Detected species	Endodontium (%)	Periodontium (%)	Р		
T. forsythia	94	92	0.021		
P. gingivalis	71	55			
A. actinomycetemcomitans	4	12			

P>0.05, test: t-test



Graph 1: Detected species in endodontium and periodontium

Table 3: Comparison of bacterial quantity of the same tooth using absolute quantitation real time Polymerase chain reaction method

Species	Р	Average difference in copy number	Standard error
T. forsythia	0.612	-5286.3	9502.1
P. gingivalis	0.719	205251.6	620713.25
A. actinomycetemcomitans	0.325	-172.60	174.8

Table 4: Pearson correlation between bacterial species of same tooth

Pearson correlation	r	Р
T. forsythia and P. gingivalis	0.412	0.01
P. gingivalis and A. actinomycetemcomitans	0.524	0.001
T. forsythia and A. actinomycetemcomitans	0.427	0.02

statistically significant correlation between absolute quantitation of *T. forsythia* and *P. gingivalis* (r = 0.412, P < 0.05), *P. gingivalis* and *A. actinomycetemcomitans* (r = 0.524, P < 0.05) and *T. forsythia* and *A. actinomycetemcomitans* (r = 0.427, P < 0.05).

#### Discussion

It was Simring and Goldberg who first established relationship between periodontal and pulpal disease in 1964. The term "perio-endo" lesion has been used to describe lesions due to inflammatory products found in some way in both the periodontium and the pulpal tissues.<sup>[5]</sup>

It has found that there are embryonic, anatomic and functional interrelationships between the pulp and periodontium. The management of each endodontic-periodontal disease type varies from patients to patient.<sup>[6]</sup> Most periodontal disease with secondary endodontic involvement requires both endodontic and periodontal therapies. This study was aimed to correlate periodontal pathogens in endodontic periodontal diseases.

Lačević *et al.*<sup>[7]</sup> conducted a study to investigated the correlation between *T. forsythia*, *P. gingivalis*, *Fusobacterium nucleatum*, and *A. actinomycetemcomitans* at dual sites in concurrent endodontic-periodontal diseases. They found no statistical difference in the number of detected endodontic-periodontal pathogens between the endodontium and periodontium. The Pearson test detected significant correlation (P < 0.001) between targeted bacteria; *T. forsythia*, *F. nucleatum*, and *P. gingivalis* from endodontic-periodontal lesions. Synergistic component observed separately in endodontic biofilm was found only between *T. forsythia* and *F. nucleatum* (r = 0.380, P = 0.03) while in periodontal biofilm *T. forsythia*, *F. nucleatum* and *P. gingivalis* gave high synergism result (P < 0.0001). Correlation analysis showed that *T. forsythia* in primary endodontic infection and in periodontal lesion was significantly decreased with the increase of patients age (r = -0.308, P = 0.017).

In this study, we found that there was nonsignificant difference in the quantity of targeted pathogens between the endodontium and periodontium of the same tooth. We observed that bacteria in endodontic-periodontal infection confirmed statistically significant correlation between absolute quantitation of *T. forsythia* and *P. gingivalis*, *P. gingivalis*, and *A. actinomycetemcomitans* and *T. forsythia* and *A. actinomycetemcomitans*.

Abbot *et al.*<sup>[8]</sup> stated that presence of bacteria in root canal may lead to periodontal disease via apical foramen. These bacteria may migrate from these canals or from dentinal tubules and may serve as a risk factor in periodontitis progression.

The pulp and periodontal tissues are derived from highly vascular mesenchymal tissues of the tooth germ. The vascular supply between these tissues is through the apical foramen and lateral canals throughout the development of the tooth. The apical foramen is the principal and most direct route of communication between the periodontium and the pulp. Hence, it is one of the routes considered favorable for endo-perio lesions.<sup>[9]</sup> Combined lesions have more complex microflora than in teeth with pathosis confined to the periapical region.<sup>[10]</sup>

Due to presence of multiple accessory canals in multirooted teeth such as premolars and molar, the occurrence of endo-perio lesions are quite common. The presence of coronal and cervical dentinal tubules may represent a viable pathway that allows spreading and maintaining of dual sites infection. Immediate performance of both endo-perio treatments have less treatment duration and better patient compliance.<sup>[10]</sup> Implementation of oral preventive measures and early diagnosis and treatment with primary care help to improve prognosis. Early primary care helps to save the teeth. It has been observed that persistent periodontal disease resolves only after definitive periodontal therapy with successful endodontic therapy.<sup>[11]</sup>

The shortcoming of present study is less number of patients. The selection of large number of cases of endo-perio lesions could have been proved beneficial in establishing role of pathogens in causing lesions.

#### Conclusion

There was correlation between targeted bacterial species levels from concurrent endodontic-periodontal diseases. It can be suggested that dentinal tubules may be the pathway for spread of bacteria.

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Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

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