Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2019;9:3s:27-31.

Evaluation of Role of Periodontal Pathogens In Endodontic Periodontal Diseases

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ABSTRACT

Background: The penetration of bacteria through dentinal tubules may lead to endo- perio lesions. The present study aimed to correlate periodontal pathogens in endodontic periodontal diseases.

Materials & Methods: The present study was conducted on 40 patients of both genders and participants were obtained from department of endodontics and periodontology with history of endod-perio lesion in same teeth. PCR 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 *T. forsythia* were 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). 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 & A. actinomycetemcomitans* (r=0.524, P<0.05) and *T. forsythia & A. actinomycetemcomitans*(r=0.427, P<0.05). **Conclusion:** There was correlation between targeted bacterial species levels from concurrent endodontic- periodontal diseases. Thus, this can be suggested that dentinal tubules may be the pathway for spread of bacteria.

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

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 towards the dentinal tubules helps in closing while the protective covering of dentinal cementum may be mechanically eroded, removed, abraded and shattered with various processes such as clinical, pathological and erosive processes.[1] In periodontal diseases, scaling and root planning is the mainstay of treatment in which infected cementum is removed. It has been postulated that bacteria of the oral biofilm attached to the root surface can enter to the adjacent dentinal tubules.

The other useful pathways considered to be the route of communication in endodontic-periodontal

Received: Mar. 5, 2019**: Accepted**: Apr. 21, 2019
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pISSN 2278-0076
eISSN 2321-9270

niches are apical foramen, lateral canals, accessory canals, and dentinal tubules.[2] While cementum may represent a barrier for bacterial migration through dentinal tubules, movement of bacteria from the outer surface of the root to the root canal system may reinforce the reservoir effect of the dentinal tubules. Factors such as bacterial size, adhesive properties, and motility may affect the degree of permeability to the dentinal tubules. This in turns depends upon different areas of the tooth and the age of the patient.[3]

The correlation of infective organisms in endodontic- periodontal niches has been studied in the past by various methods. The vital pulp tissue can immunologically respond to bacteria and bacterial bioproducts and preserve its vitality and the type of infection. It has shown that bacteria may travel through dentinal tubules in both directions to coloniz both endodontic-periodontal tissues.[4] Considering this, the present study aimed to correlate periodontal pathogens in endodontic periodontal diseases.

MATERIAL AND METHODS

The present study was conducted in the department of Periodontology and Endodontics. It comprised of 40 patients of both genders. Patients within age group 20- 50 years, patients with single rooted tooth having endodontic or periodontal pathology, patients with periodontal pockets < 6 mm were included in the study. 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. The study protocol was approved from institutional ethical committee. All patients were informed regarding the study in local language and informed written consent was obtained.

Patient data such as name, age, gender etc. was 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, 2-3 paper points were inserted into periodontal pockets and left in situ for 1 minute each. For collection of samples from tooth with single root canal strict aseptic procedure was followed. 2-3 sterile paper points were inserted into the root canal and left for 1 minute. The paper points were then transferred to cryotubes containing 1 ml of TE buffer. Samples were immediately frozen and kept at -80°Celcius.

Extraction of DNA

Frozen endodontic and periodontal specimens were resuspended 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 (PCR) was used for the identification of periodontal pathogens for *T. 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 *P. 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 *A. actinomycetemcomitans, Aa* forward 5'-AAA CCC ATC TCT GAG TTC 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.

Statistical analysis

Data thus obtained were tabulated and entered in excel sheet. SPSS package (21.0 version, Inc.; Chicago, IL) was used for all statistical analysis. Student T test was used to check endo- perio pathogens correlation. P value less than 0.05 was considered significant.

RESULTS

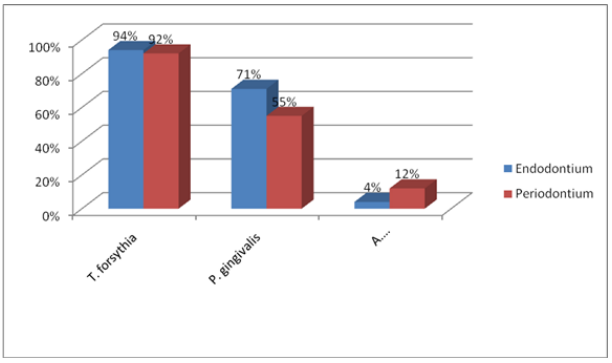
Table I shows that out of 40 patients, males were 18 and females were 22. The mean age of male was

Table I: Distribution of patients

Parameters	Male	Female
Number	18	22
Mean age (years)	42.5	41.3

Table II: Detected species in endodontium and periodontium

Detected species	Endodontium	Periodontium	P value
T. forsythia	94%	92%	0.021
P. gingivalis	71%	55%	
A. actinomycetemcomitans	4%	12%	



Graph 1: Showing count of microorganisms.

Table III: Comparison of bacterial quantity of the same tooth using absolute quantitation real time PCR method.

Species	P value	Average difference in copy number	Std. 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

42.5 years and female was 41.3 years. Table II, Graph I 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). Table III show that there was non- significant difference in the quantity of targeted pathogens between the endodontium and periodontium of the same tooth. Table IV shows that 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* & *A. actinomycetemcomitans* (r=0.524, P<0.05) and *T. forsythia* & *A. actinomycetemcomitans* (r=0.427, P<0.05).

DISCUSSION

It was Simring and Goldberg who first established actual relationship between periodontal and pulpal disease in 1964. It is their work the term "perio-endo" lesion has been used to describe lesions due to inflammatory products found in varying degrees in both the periodontium and the pulpal tissues.[5]

It has found that there are embryonic, anatomic and functional inter-relationships between the pulp and periodontium. Perio-endo lesions may pose varied pathogenesis which requires careful evaluation of disease processes in order to give final diagnosis.[6] The management of each endodontic-periodontal disease type varies from patients to patient. Primary periodontal disease with secondary endodontic involvement and true combined endodontic-periodontal diseases require both endodontic and periodontal therapies. The prognosis of these cases depends on the severity of periodontal disease and the response to periodontal treatment.[7] The present study was aimed to correlate periodontal pathogens in endodontic periodontal diseases.

In present study out of 40 patients, 18 were males and females were 22. The mean age of male was 42.5 years and female was 41.3 years. Specimens of *T. forsythia* were isolated from 94% endodontium and 92% periodontium, *P. gingivalis* from 71% endodontium and 55% periodontium, *A. actinomycetemcomitans* from 12% endodontium and 58% periodontium.

Lačević et al[8] conducted a study to investigated the correlation between Tannerella forsythia, Porphyromonas gingivalis, Fusobacterium nucleatum, and Aggregatibacter 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 present study we found that there was non-significant 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 & A. actinomycetemcomitans* and *T. forsythia & A. actinomycetemcomitans.*

Abbot et al[9] stated that that presence in bacteria the 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 main route of transmission of bacteria is apical foramen. 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 favourable for endo- perio lesions.[10]In relation to apical foramen, there are lateral canals also which may be the route of transmission of bacteria. These ramifications are now currently termed as 'accessory canals'. The term accessory canal is now used to describe any ramification that connects the root canal system to the periodontal ligament.[11]

Due to presence of multiple accessory canals in multi-rooted teeth such as premolars and molar, the occurrence of endo- perio lesions are quite common. Presence of dentinal tubules is also one of the routes especially in case of denuded cementum. The presence of coronal and cervical dentinal tubules may represent a viable pathway that allows spreading and maintaining of dual sites infection. It is seen that endodontic infections are sustaining periodontal lesions and periodontal lesions are most likely a bacterial source for endodontic infections.[12] 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

The topic of endo- perio lesions is well studied. There was correlation between targeted bacterial species levels from concurrent endodontic-periodontal diseases. Thus this can be suggested that dentinal tubules may be the pathway for spread of bacteria.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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