

Association of specific microorganisms with endodontic signs and symptoms. A comparative study

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ABSTRACT

Aim: The study aimed to evaluate the association of root canal microorganisms red complex and *E. faecalis* with endodontic clinical signs and symptoms using polymerase chain reaction. **Materials and Methods:** Bacterial samples were obtained using sterile paper points from the teeth of 100 subjects divided into two groups; Group I: 50 individuals with primary tooth infections and Group II: 50 individuals with failed endodontic treatment having the secondary infection. DNA extracted from samples was analyzed for endodontic pathogens by using species-specific primers. **Results:** The pain was noticed in 66% (33 of 50 subjects) in primary infection and 60% (30 of 50) in a secondary infection. A statistically significant association between pain and *E. faecalis* bacteria observed both in primary infection and secondary infection ($P < 0.05$). Tenderness on percussion was associated with 40% cases in Group I and 70% cases in Group II. The red complex accounted for 94% of cases associated with tenderness on percussion in primary infection while 86% of cases associated with secondary infection with a statistically significant association ($P < 0.05$). **Conclusion:** Prevalence of red complex bacteria and *E. faecalis* suggested the association of studied bacteria with symptomatic infected pulp and periradicular diseases.

Keywords: Bacteria, pulp, red complex

Introduction

The presence of bacteria in the root canal leads to the development of periapical periodontitis. Several studies have shown an association between painful exacerbation of periapical lesions and the presence in the root canal of specific bacteria.^[1] Based on root canal infection and severity there may be the presence of different microorganisms like Gram-negative anaerobes such

as- *Prevotella*, *Veilonella*, *Porphyromonas*; Gram-positive anaerobes such as- *Peptostreptococcus*, *Actinomyces*; Gram-negative facultative such as- *Eikenella*, *Hemophilus*, and Gram-positive facultative such as- *Lactobacillus*, *Actinobacillus*, *Propionibacterium*, etc.^[2]

An infected root canal comprises of a unique microenvironment housing selective microflora. These organisms grow in planktonic forms or as aggregates as well as in biofilms. The microbial composition in the root canal system is an interesting area of research nowadays. Due to advancements in recent years, novel technologies such as- immunological assays and molecular methods like- polymerase chain reaction.^[3]

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While many different bacterial species can colonize the dental root canals, it has been shown that there is a correlation between the presence of specific bacteria and some endodontic symptoms and signs. Knowledge regarding the nature of endodontic microbiota depends upon the recognition of those microorganisms present in the root canal system of teeth with necrotic pulp and failed endodontic treatment.^[4-6]

The present study aimed to evaluate the correlation between clinical signs and the presence of root canal microorganisms (red complex bacteria and *E. faecalis*) in an infected root canal.

Materials and Methods

One hundred symptomatic individuals of age group 18 to 65 years undergoing root canal treatment were selected and divided into two groups: Group A: 50 individuals with primary infections, Group B: 50 individuals requiring retreatment with a failed root canal. Inclusion criteria for case selection included- necrotic pulp or periapical periodontitis and clear or purulent discharge. Written informed consent was obtained from all the individuals before the sampling procedure. The study design was approved by the institutional ethical committee (Reg No. IEC/62/BRS/2019).

The following clinical features were recorded for each patient so that they could be correlated with the microbial findings: Pain, tenderness to percussion, swelling, caries, pus discharge, and mobility. In primary infection, the clinical condition of pulp was evaluated by thermal stimuli and vitality tests.

Samples collection was made by inserting a sterile paper point until the complete working length for 1 min. The paper points were then immediately placed into the sterile transport medium and were stored at -70°C for polymerase chain reaction (PCR). The root canal samples were shaken in a vortex mixer for 60 s. After vortexing, 50 μL of the sample was plated onto selective culture media. Then the paper points were removed. Samples were centrifuged at 2000 g for 5 min. Pellets obtained were suspended in 100 μL of HCl buffer (pH 8.5). Microbial DNA extraction and purification of the samples was performed using GenElute Bacterial Genomic DNA Kit (Sigma–Aldrich, St. Louis, MO, USA) according to the manufacturer’s instructions. The DNA content was analyzed using a spectrophotometer.

The PCR program cycle was as under- 94°C for 4 min; 30 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and finally, 72°C for 10 min. Visualization of bands was done under UV illumination using 1% agarose gel electrophoresis employing ethidium bromide.

The data collected from each sample was typed in Microsoft Excel Worksheet 2007 and was analyzed using SPSS 12.0 (SPSS Inc., Chicago, IL).

Results

In the Group, I the clinical characteristics studied were as pain 33 (66%) and tenderness on percussion 40 (80%), but in Group II teeth associated with pain were 30 (60%) and teeth associated with tenderness on percussion were 35 (70%) [Tables 1 and 2].

The pain was noticed in 66%, (33 of 50 subjects) in primary infection and 60% (30 of 50) in a secondary infection. A statistically significant association between pain and *E. faecalis* bacteria was observed both in primary infection and secondary infection ($P < 0.05$) by Fisher’s exact test [Table 1]. Tenderness on percussion was associated with 40% cases in Group I and 70% cases in Group II. The red complex accounted for 94% of cases associated with tenderness on percussion in primary infection while 86% of cases associated with secondary infection with the statistically significant association ($P < 0.05$) as evaluated by Fisher’s exact test [Table 2].

Discussion

The dental root canal is close to some of the most heavily bacterially contaminated sites in the body. It is extremely likely that a diverse range of species reach the root canal and surrounding dentin. Some early studies failed to report the presence of obligate anaerobes. This can be attributed to the methods employed. Even now, it is salutary to consider that molecular biology has revealed oral species that are currently “uncultivable”. There remains the possibility that studies relying on microbial culture may have overlooked such uncultivable species. Very few investigations have examined the flora of microenvironments of the endodontium. Initially, the presence of microorganisms in the endodontium goes unrecognized. This is because problems associated with a change in the canal’s environment only appear when pulpal inflammation is widespread and has reached an advanced state whose outcome is complete pulpal necrosis.^[7,8]

A wide range of species has been reported in endodontic samples. Nevertheless, it is obvious that certain species are more frequently isolated than others and that most infected root canals have a mixed flora.

E. faecalis is found to be the most prevalent microorganism in root canals with persistent periapical lesions. This microorganism can

Table 1: Table demonstrating association of red complex and *E. faecalis* with pain in primary and secondary infections

	Pain	Total %	<i>E. faecalis</i>		Red complex	
			Present	Absent	Present	Absent
Group I						
	Present	33 (66%)	20 (71%)	13 (59%)	11 (61%)	22 (69%)
	Absent	17 (34%)	8 (29%)	9 (41%)	7 (39%)	10 (31%)
	Total	50	28	22	18	32
Group II						
	Present	30 (60%)	25 (71%)	5 (33%)	10 (56%)	20 (63%)
	Absent	20 (40%)	10 (29%)	10 (66%)	8 (44%)	12 (38%)
	Total	50	35	15	18	32

Table 2: Table demonstrating association of red complex and *E. faecalis* with tenderness on percussion in primary and secondary infections

	Tenderness on percussion	Total %	Red complex		<i>E. faecalis</i>	
			Present	Absent	Present	Absent
Group I	Present	40 (80%)	30 (94%)	10 (56%)	17 (68%)	23 (92%)
	Absent	10 (20%)	2 (6%)	8 (44%)	8 (32%)	2 (8%)
	Total	50	32	18	25	25
Group II	Present	35 (70%)	30 (86%)	5 (33%)	15 (60%)	20 (80%)
	Absent	15 (30%)	5 (14%)	10 (66%)	10 (40%)	5 (20%)
	Total	50	35	15	25	25

even survive in an environment with scant available nutrients and in which commensality with other bacteria is minimal. Studies by Pirani *et al.*^[9] have demonstrated that *E. faecalis* can penetrate dentinal tubules, sometimes to a deep extent, which can enable this species to escape the effects of intracanal antimicrobial procedures. Besides, *E. faecalis* can form biofilms in root canals, and this ability can be important for bacterial resistance and persistence after endodontic procedures. *E. faecalis* is also resistant to calcium hydroxide, a commonly used intracanal medicament, and such ability to resist high pH values seems to be related to a functioning proton pump, which drives protons into the cell to acidify the cytoplasm.^[10-12]

Although *E. faecalis* is found in most cases of infected canals, its role, if any, in the pathogenesis of the periapical diseases associated with necrotic pulp or endodontic failure, remains to be elucidated. In our study, there was a significant association between pain and the presence of *E. faecalis* in both primary and secondary infections.^[4]

Rocas *et al.*^[13] have also suggested that *E. faecalis* was more frequently detected in asymptomatic cases than in symptomatic ones using nested polymerase chain reaction analysis in which they detected *E. faecalis* in 11.5% of the asymptomatic teeth and 3.7% of symptomatic cases.

Selcuk M. Ozbek *et al.*^[14] investigated the presence of “red complex” in acute periradicular abscesses by using real-time PCR method. At least 1 member of the red complex was found in 84% of the cases. In general, *T. denticola*, *P. gingivalis*, and *T. forsythia* were detected in 65.6%, 43.7%, and 40.6% of the cases, respectively. The red complex was detected in 15.6% of samples taken from acute periradicular abscesses.

“Red complex” is directly associated with the severity of the periodontal disease, but limited information is available on the role of red complex in the endodontic lesion. Therefore, we have taken the red complex as an experimental group.^[15]

Isabela N Rocas *et al.*^[16] assessed the occurrence of the red complex in root canal infections through the use of a sensitive technique- the 16S rDNA-directed PCR. Samples were obtained from 50 necrotic pulps with periradicular pathosis. Ten cases were diagnosed as acute periradicular abscesses. DNA was extracted

from the samples and analyzed with a PCR-based identification assay. It was concluded that at least 1 member of the red complex was found in 33 of 50 cases. *T. denticola*, *P. gingivalis*, and *B. forsythus* were detected in 44%, 30%, and 26% of the cases, respectively. The red complex was found in 4 of 50 cases. No particular signs or symptoms were associated with the presence of these bacterial species. However, because the bacterial species from the red complex are recognized oral pathogens, their occurrence in root canal infections suggests that they may play a role in the pathogenesis of periradicular diseases.

In the present study, *E. faecalis* was found to be significantly associated with pain in primary and secondary infections. The present study was not in accordance with the study conducted by R. V. Vineet *et al.*^[4] that postulated the significant association of *S. mitis* with pain in primary root canal infections.

Kemthong Mitrakul conducted a study to evaluate the presence of Lactobacilli and Enterococcus faecalis levels in root canal infections of primary teeth and analyzed the association between clinical signs and symptoms with the presence of these bacterias. It was concluded that the bacterial levels of total bacteria, Lactobacillus and *E. faecalis* in the pulp necrosis group were higher than the irreversible pulpitis group. Levels of Lactobacillus and *E. faecalis* were associated with a history of pain, pathologic finding of lamina dura, and furcation involvement. Gingival swelling was correlated with Lactobacillus quantities.^[17]

Paulo Nelson-Filho *et al.* compared the Gram-negative pathogens identified in the root canals of primary teeth with irreversible inflammatory pulpitis and in teeth showing apical periodontitis. It was concluded that a higher number of Gram-negative bacteria were found in teeth with apical periodontitis compared to teeth with irreversible inflammatory pulpitis.^[18]

Isabela N. Rocas *et al.* Samples were taken from the root canals of teeth with asymptomatic apical periodontitis (n = 73) and by aspiration of purulent exudate from acute abscesses (n = 55). Bacterial DNA was detected in all cases. In abscesses, the most prevalent taxa were *Fusobacterium nucleatum* (60%), *Porphyromonas endodontalis* (53%), *Parvimonas Micra* (51%), and *Streptococcus* species (45%). The most frequently detected taxa in asymptomatic teeth were *P. endodontalis* (63%), *Dialister invisus* (58%), *Olsenella uli* (56%), and *F. nucleatum* (51%).

However, semiquantitative data demonstrated that *P. endodontalis*, *Prevotella baroniae*, *Treponema denticola*, and *Streptococcus* species were significantly more frequent at levels $>10^5$ in abscesses than in asymptomatic cases ($P < 0.05$).^[19]

Pradeep Shetty *et al.* evaluated and compared the anaerobic microflora (obligate and facultative) of the root canals of nonvital teeth with periapical lesions, in diabetic (Type II) and nondiabetic patients. Obligate anaerobes were significantly more associated with cases of Type II diabetes mellitus, suggesting the role of these microorganisms in modulating the inflammatory and immunologic responses as well as its level in root canal infections, which can influence the pain threshold of the patient.^[20]

Foschi *et al.*^[21] performed a study to analyze the role of *T. denticola* as a monoinfection as well as a part of “red complex” infection in the causation of endodontic infections into 8 weeks old severe combined immunodeficiency mice. Study results demonstrated periapical bone resorption in *T. denticola* monoinfection when compared to the red complex.

T. denticola was detected in 15/60 cases with periapical lesions of which five were tender on percussion. The red complex was detected in 10% (6/60) samples taken from acute periradicular abscess samples. These observations are suggestive of the role of “red complex” in the pathogenesis of acute periradicular abscesses. These findings have been corroborated by Sanghavi *et al.*^[22] using the multiplex PCR technique.

In continuously changing environmental conditions bacteria form multiple complexes to survive, and the relationships between bacterial species and hosts can provide a better understanding of disease processes. Synergistic bacterial interactions allow different species to coexist in habitats in which interacting bacteria do not exist alone and increase the possibility of survival. Bacterial synergism may be another significant factor in the development of symptoms and signs of endodontic origin and the presence of some pathogenic species in the root canal could provide an environment for the formation of acute periradicular inflammation.^[23,24]

Implications for primary care

Endodontic disease refers to an infection of the root canal space and is a significant cause of dental morbidity worldwide. Endodontic treatment or root canal treatment serves to eliminate the microbial biofilm and prevents reinfection of the root canal space. The primary objective of endodontic therapy is to create a biologically acceptable environment within the root canal system that allows for the healing and maintenance of the health of the periradicular tissue. Bacteria are one of the main causes of pulp problems, and they have different methods of penetrating and invading the endodontic space such as through carious lesions, traumatic pulp exposures, and fractures. The types of bacteria found to range from facultative anaerobes to aerobes, up to the most resistant species able to survive in nutrient-free

environments; the bacterial species *Enterococcus faecalis* belongs to this last group. *Enterococcus faecalis* is considered one of the main causes of recurring apical periodontal lesions following endodontic treatment, with persistent lesions occurring even after retreatment.^[25,26]

Conclusion

The high prevalence of red complex bacteria in the present study suggests that these bacteria can be correlated with the etiopathogenesis of periradicular diseases. Identification of such microorganisms help in treating the endodontic infections at an early stage.

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Conflicts of interest

There are no conflicts of interest.

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