



Assessment of Correlation between Clinical Parameters and Pulp Canal Pathogens in Endodontic Pathologies: A Microbiological Study

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ABSTRACT

Introduction: Success and failure of root canal therapy is largely dependent on the clinician and the patient. Once the tooth is restored into its functional and esthetic place, the root canal therapy is considered to be complete. It is a well-known fact that root canal space acts as a rich reservoir for microorganisms. Past studies show that root canal harbors an array of different microorganisms. *Streptococcus mitis* and *Enterococcus faecalis* have been found to be the most prevalent microorganisms isolated from the infected pulp canal space. Hence, we evaluated the association of endodontic signs and symptoms with root canal pathogens.

Materials and methods: A total of 120 patients were selected for the study and divided broadly into two groups: Group I containing patients with primary infection and group II containing patients in which retreatment was required. Both groups contained 60 patients each. Clinical parameters recorded for the study are pain, tenderness on percussion, swelling, periapical radiolucency on radiographic analysis, caries, sinus formation,

and tooth mobility. After assessment, opening, and initial filing, sterile paper points were inserted into the root canal space for collection of microbiological sample, which was further sent to laboratory for cultural analysis. All the results were analyzed by Statistical Package for the Social Sciences (SPSS) software. Student's t-test and Fisher's exact test were used to evaluate the level of significance.

Results: While comparing the mean age of the patients in the two groups, no significant association was seen. Statistically significant results were obtained while comparing the association between pain and *S. mitis*. Positive correlation was seen on comparing the association between tenderness on percussion and *E. faecalis*. Also, a positive association was seen between periapical radiolucency and *S. mitis*.

Conclusion: Strong positive correlation exists between *S. mitis* and *E. faecalis* and endodontic signs and symptoms.

Clinical significance: Sound knowledge regarding the association of microbes of root canal and specific endodontic signs and symptoms could help therapeutic management of root canal infections effectively.

Keywords: Endodontic, Microorganisms, Root canal.

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INTRODUCTION

Despite the clinical and oral factors, a clinician expects complete success of the root canal therapy. However, success of the treatment depends largely on the clinician performing the treatment and on the patient that how the treated area is taken care. When the tooth is completely restored and can perform the functions in occlusion and in

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rest, the endodontic therapy is considered to be complete.¹ Analysis of the prognosis of the endodontic therapy relies mainly on two factors: The dentist and the patient (tooth). A dentist considers endodontic therapy to be complete when the tooth is symptom-free, both clinically and radiographically. Correct diagnosis of the lesion largely affects the prognosis of the lesion. Endodontic therapy is considered to be finally complete when the tooth is restored, both in terms of esthetics and occlusal function.² It is well documented that pulp canal space serves as a rich and unique niche of microorganisms.³ From the last few years, the topic of research is the composition of this microbial flora, which is shown to be different in primary endodontic therapy patients and in retreatment cases.⁴ Results of various studies prove that pulp canal harbors an array of microorganisms. However, these vary considerably depending on the type and severity of root canal infection. *Streptococcus mitis* and *Enterococcus faecalis* have been found to be the most prevalent microorganisms isolated from the infected pulp canal space. Hence, we evaluated the association of endodontic symptoms with root canal pathogens.

MATERIALS AND METHODS

The present study was conducted on the patients reporting to the Department of Conservative Dentistry of a dental institution with the chief complaint of irreversible pulpitis. A total of 120 patients were selected for the study and divided broadly into two groups, as shown in Table 1. Group I included patients in whom endodontic therapy was indicated due to irreversible pulpitis, and group II included patients with failed endodontic treatment in the past and are advised reendodontic therapy. Patients were preinformed about the study procedure and written consent was obtained from them. All the patients were between 25 and 50 years of age. Only those patients were included in which radiographic evidence of root canal pathology or failed root canal therapy was confirmed. Exclusion criteria included:

- Patients with history of any systemic illness.
- Patients with any known drug allergy.
- Patients with history of any trauma in the same tooth region in past 1 year.
- Female patients with pregnancy.
- Patients with history of any immune-compromised disease.

Table 1: Division of patients into groups

Groups	No. of patients	Parameter
A	60	With primary pulpal infection
B	60	With secondary pulpal infection (failed endodontic therapy)

- Patients with periodontal problems in the offending tooth.

Pain in the tooth, tenderness on percussion, swelling, caries, sinus (if present), and mobility of the offending tooth were the clinical parameters recorded in the patients and these parameters were compared to microbial findings. Periapical pathologies were examined with the help of radiographs and size of periapical pathology was taken as the largest dimension of the lesion. Maintenance of the aseptic environment was done throughout the sampling procedure. Rubber dam was used to isolate the tooth. Initial step was the removal of carious lesion and coronal restorations, if present. Sterile instruments were used for making access preparation in the isolated tooth. Gates Glidden drills and files were used for removing filling materials from the canals of the failed root canal treated tooth. Normal saline was used for rinsing the canal space for removal of any remaining filling material and dentinal debris. Radiographs were used for determination of the working length. Gentle filing was done into the root canal with a sterile endodontic instrument. Sterile paper points were placed into the root canals up to full length, followed by immediate placement into the transporting media which had sterile transporting liquid. The samples were transported into the microbiological section for cultural growth. The samples were transferred into vortex mixer for shaking of specimen followed by placement of samples into the cultural plates containing selective media. For the cultural growth of *S. mitis* and *E. faecalis*, Mitis-Salivarius agar was used. All the samples were incubated in the cultural plates 37°C for 5 days. Assessment of the growth of the microorganisms on the cultural plates was done by evaluating the colony morphologies and by using Gram-staining. All the results were analyzed by Statistical Package for the Social Sciences (SPSS) software. Student's t-test and Fisher's exact test were used to evaluate the level of significance.

RESULTS

In the present study, the age of the study population varied from 25 to 50 years, with 35 to 33 years being the average of age of patients with primary infection and secondary infection respectively. No significant result was obtained on comparing the mean age of the patients in the two groups (p-value = 0.586) as shown in Table 2. After assessing the clinical signs and symptoms, the following data were obtained: Pain (80/120), tenderness to percussion (101/120), swelling (72/120), sinus track formation

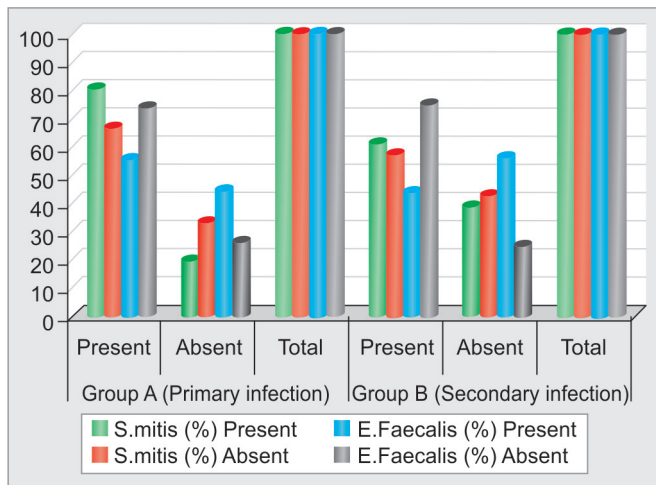
Table 2: Mean age of patients into groups

Groups	No. of patients	Mean age (years)	p-value
A	60	35	0.586 NS
B	60	33	

NS: Nonsignificance

Table 3: Association of *S. mitis* and *E. faecalis* with pain in primary and secondary infections

Groups	Pain	Total	<i>S. mitis</i> (%)		<i>E. faecalis</i> (%)	
			Present	Absent	Present	Absent
A (primary infection)	Present	45 (75%)	29 (80.4%)	16 (66.7%)	10 (55.6%)	31 (73.8%)
	Absent	15 (25%)	7 (19.6%)	8 (33.3%)	8 (44.4%)	11 (26.2%)
	Total	60 (100%)	36 (100%)	24 (100%)	18 (100%)	42 (100%)
B (secondary infection)	Present	35 (58.4%)	11 (61.1%)	24 (57.1%)	14 (43.7%)	21 (75%)
	Absent	25 (41.6%)	7 (38.9%)	18 (42.9%)	18 (56.3%)	7 (25%)
	Total	60 (100%)	18 (100%)	42 (%)	32 (100%)	28 (100%)



Graph 1: Association of *S. mitis* and *E. faecalis* with pain in primary and secondary infections

(88/120), tooth mobility (32/120), and periapical radiolucency was seen in all the 120 cases. While comparing the association between pain and *S. mitis* by Fisher’s exact test, statistically significant correlation was observed, as shown in Table 3 and Graph 1 (p-value = 0.004). In all the cases of primary infection, tenderness was noted on percussion. *E. faecalis* association with tenderness on percussion was found to be statistically significant, as shown in Table 4 (p-value = 0.001). In case of periapical radiolucency, association of *S. mitis* was found to be statistically significant, as shown in Table 5 and Graph 2 (p-value = 0.002).

DISCUSSION

Persistence of periapical lesions and infections leads to failure of endodontic therapy.⁵ Literature quotes numerous studies that show that up to 35% of the root

canal-filled teeth with periapical lesions have high prevalence of microorganisms.⁶⁻⁸ A distinct variable microbial flora as compared to primary infections has been observed in various studies.^{8,9} However, there are great chances that uncultivated bacterial species can go underestimated and undetectable by totally depending on the cultural methods alone.¹⁰ With the advancements in the technology of the molecular level, detection of uncultivated microorganisms of known species formed in the infected pulp canals has become possible and approachable.¹¹ For further understanding of the etiopathogenesis of root canal and periapical lesions, a better knowledge and insight of the complete microbial flora of the pulp canals treated with pulp canal therapy is required.¹² Hence, we tried to assess the association of endodontic pain with pulp canal pathogens.

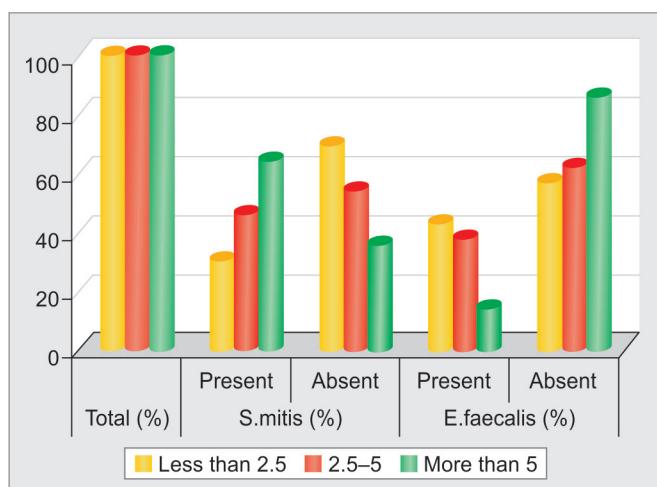
From the results we observed a significant correlation of *S. mitis* with clinical pain in cases of primary infection (Table 3). Our results are in correlation with the observations of Guimaraes et al¹³ and Vineet et al¹⁴ who found a positive association of pain with *Streptococcus* species. Along with being a normal inhabitant of the oral cavity, it also possess “adhesins” proteins that aid in its attachment to the tooth surfaces. They contain certain inflammation modulating components in their cell wall which enhances the pain reaction.¹⁵ We also observed a positive correlation between tenderness on percussion with the presence of *E. faecalis* (p-value = 0.002), as shown in Table 4. Our results were in correlation with the results of previous studies.¹⁴ However, with the help of polymerase chain reaction technique, Rôças et al¹⁶ found higher prevalence of *E. faecalis* in asymptomatic cases as compared to symptomatic cases. *S. mitis* was found to be predominant

Table 4: Association of *S. mitis* and *E. faecalis* with tenderness in primary and secondary infections

Groups	Pain	Total	<i>S. mitis</i> (%)		<i>E. faecalis</i> (%)	
			Present	Absent	Present	Absent
A (primary infection)	Present	60 (100%)	34 (100%)	26 (100%)	14 (100%)	46 (100%)
	Absent	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Total	60 (100%)	34 (100%)	26 (100%)	14 (100%)	46 (100%)
B (secondary infection)	Present	41 (68.3%)	14 (73.6%)	27 (65.8%)	21 (77.7%)	20 (60.6%)
	Absent	19 (31.7%)	5 (26.4%)	14 (34.2%)	6 (22.2%)	13 (39.4%)
	Total	60 (100%)	19 (100%)	41 (100%)	27 (100%)	33 (100%)

Table 5: Association of *S. mitis* and *E. faecalis* and size of primary lesion in primary and secondary infections

Lesion size (mm)	Total (%)	<i>S. mitis</i> (%)		<i>E. faecalis</i> (%)	
		Present	Absent	Present	Absent
Less than 2.5	56 (100%)	17 (30.4%)	39 (69.6%)	24 (42.9%)	32 (57.1%)
2.5–5	50 (100%)	23 (46%)	27 (54%)	19 (38%)	31 (62%)
More than 5	14 (100%)	9 (64.3%)	5 (35.7%)	2 (14.2%)	12 (85.8%)

**Graph 2:** Association of *S. mitis* and *E. faecalis* and size of primary lesion in primary and secondary infections

species in the periapical lesion of larger size (Table 5, Graph 2). Similar results were seen by Vineet et al,¹⁴ who also observed high number of same microorganism in larger lesion. This can be hypothesized in this way that larger lesions have high prevalence of large number of microorganisms.¹⁷ Attachment of microorganisms to the tooth surface in the infected pulp canals might play a vital role in causing apical periodontitis. This is supported by detection of *Streptococcus* species, which were found to invade the dentinal tubules by releasing various proteins that help them to adapt and survive various harsh conditions.¹⁸ Therefore, the mechanism by which *Streptococcus* species survive in infected pulp spaces can be explained based on the environmental-induced intrinsic regulatory changes.⁶ Vineet et al¹⁴ assessed the association of *S. mitis* and *E. faecalis* with specific endodontic signs and symptoms. From the results they concluded that positive correlation of *S. mitis* with pain and periapical lesions, and *E. faecalis* association with tenderness on percussion suggests a positive association between endodontic symptoms with specific pulp canal bacterial species. Anderson et al¹⁹ conducted a study to combine cultural methods of bacterial identification with culture-independent cloning methods to analyze the microbial flora of pulp canal-filled teeth with periapical pathologies. They observed that *E. faecalis* was the only detectable species in the samples using culture methods. With the combination of the culture-dependent and independent approaches,

new candidate for endodontic pathogens along with high diversity of the microbial flora was revealed in the root-filled teeth with periapical pathologies. They concluded that combination of different methods of bacterial detection increases the sensitivity of microbial detection. Kutllovci et al²⁰ identified the presence of different cultivating microorganisms causing dental lesions in symptomatic cases of deciduous and permanent teeth. From the results they concluded that dental infections in primary teeth are polymicrobial in nature with predominantly anaerobic bacteria. Narayanan and Vaishnavi²¹ in 2010 reviewed the data available in PubMed from 1995 to 2010 on microbiology associated with root canals. They compiled all the data and observed that in root canal retreatment cases with persistence of infection in pulpal spaces, the most common microorganisms to be recovered were *E. faecalis* and yeast (*Candida albicans*). *E. faecalis* are Gram-positive facultative anaerobes. They are a part of normal inhabitant flora of intestinal track and might also be seen as normal flora in gingival sulcus. Their survival is largely dependent on their number. Large quantity of bacteria are difficult to remove while when present in small quantities, they can be easily eradicated. Root canal infection is not a random event. In response to surrounding environmental conditions, different types of bacteria develop. Unfilled or improperly filled root canal offers a great diversity of environmental nutrition to the microorganisms in contrast to very limited amount of nutrition available for microorganisms in properly filled root canals. Hence, a better knowledge of the type and quality of bacteria and other microorganisms is required for the success of root canal therapy.

CONCLUSION

From the above results, it can be concluded that a strong positive correlation exists between *S. mitis* and *E. faecalis* and symptomatic patients requiring endodontic therapy. Therefore, it requires future research to study in-depth the root canal pathogens for improving the prognosis of root canal therapy. Despite the newer methods available for chemomechanical preparation of root canal, more emphasis should be laid on the sterilization part of the pulp canals.

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