Case Study Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Apical Periodontitis as an Indicator for the Presence of *Enterococcus Faecalis* in Endodontic Retreatment Cases

Nayak Moksha and Shenoy Vidhya*

KVG Dental College and hospital, Sullia, Dakshina Kannada, Karnataka, India- 574327
E. mail address: shenoyvidhya93@gmail.com, Mobile number: 9480033357

ABSTRACT

To determine the association of tenderness to percussion with the presence of *Enterococcus faecalis* in endodontic retreatment cases with apical periodontitis.

One hundred and thirty subjects aged 25 to 65 years requiring endodontic retreatment were selected for this study. The endodontic retreatment was initiated following removal of the root filling material using ProTaper retreatment files and Hedstrom file (H file) without the use of solvent. The canals were sampled as per Moller's criteria using sterile paper points and transported in tris-EDTA buffer (TE buffer) for conventional PCR analysis of *Enterococcus faecalis (E. faecalis)*. Evaluation of the organism was performed using semiquantitative conventional PCR. Results were charted and statistical analysis was performed using Chi square test.

Enterococcus faecalis was isolated from a total of 98 teeth (75.38%) of the 130 endodontic retreatment cases examined. Tenderness to percussion was associated with 89 root filled teeth with apical periodontitis of which *E. faecalis* was isolated from 75 teeth (57.7%). Fourteen teeth requiring endodontic retreatment which were associated with apical periodontitis did not yield *E. faecalis*.

The clinical symptom of tenderness to percussion a finding of apical periodontitis could be an indicator for the presence of *E. faecalis* in teeth with failed endodontic treatment with apical periodontitis.

KEYWORDS: *Enterococcus faecalis*, Retreatment, Apical periodontitis, Tenderness to percussion

*CORRESPONDING AUTHOR:

Vidhya Shenoy

Post graduate student*

KVG Dental College and hospital, Sullia, Dakshina Kannada

Karnataka, India- 574327

E. mail address: shenoyvidhya93@gmail.com, Mobile number: 9480033357

INTRODUCTION

Persistent intraradicular bacteria are the major cause of failure of endodontic treatment.[1] The microbial flora detected in previously root filled teeth with apical periodontitis can be characterized as monoinfection with predominantly Gram positive microorganisms with approximately equal proportions of facultative and obligate anaerobes.[1,2] *Enterococcus faecalis*, a non spore forming, Gram positive facultative anaerobe is the most prevalent species in root canals of teeth with failed endodontic treatment and its occurrence ranges from 24% to 77%. It is an opportunistic pathogen in persistent apical periodontitis.[2-7]

Apical periodontitis is an inflammatory disorder of periradicular tissues caused by persistent microbial infection within the root canal system of the affected tooth.[8,9] Teeth with apical periodontitis are associated with clinical symptoms such as spontaneous pain, marked tenderness to percussion, and other associated symptoms such as increased mobility, a feeling of pressure building up in the periapical region.[10] Various authors have reported significantly high prevalence of apical periodontitis in root filled teeth ranging from 24% to 54%.[11,12]

Tenderness to percussion determines the presence of pathosis in the periapical tissues, indicating inflammation in the periodontal ligament.[13] Periapical inflammation is a direct effect of bacterial infection of the root canal system.[10] Gram positive bacteria are seen to be associated in teeth with tenderness to percussion.[14] It has been demonstrated that there is a correlation between the presence of certain bacterial species and clinical features of periradicular diseases.[15] Since *E. faecalis* is the most common organism isolated from root filled teeth with apical periodontitis, this study aimed to determine whether the presence of tenderness to percussion is an indicator for the growth of *E. faecalis* in endodontic retreatment cases with apical periodontitis.

MATERIALS AND METHODOLOGY

Patient selection

One hundred and thirty subjects requiring endodontic retreatment referred to Department of Conservative Dentistry and Endodontics, K.V.G Dental College and Hospital (Sullia, Dakshina Kannada, Karnataka) were selected for the study based on the inclusion and exclusion criteria. The ethical clearance for the study protocol was obtained from Institutional Ethics Committee. The subjects were informed of the study protocol, and written consent was obtained before the sampling procedure was performed. Both male and female subjects aged 25–65 years were included in the study. Patients

requiring retreatment of endodontically treated teeth with a diagnosis of apical periodontitis, root-filled teeth with radiographic evidence of periradicular disease with a periapical index score of 3 or > 3, periapical lesion size of > 3mm and the termini of the root canal fillings at least 2 mm short of the radiographic apex were included in the study. Pregnant and lactating mothers, subjects who have used any antibiotics during past 3 months, subjects with any systemic diseases, teeth that cannot be isolated with a rubber dam, calcified and tortuous canals and teeth with failures such as fractured instrument, resorption, perforation, ledge, overfillings and transportation were excluded from the study.

Sampling procedure

The presence of tenderness to percussion was noted for each patient so that it could be correlated with the microbial finding. Aseptic techniques were used throughout the root canal sampling procedure. The tooth was isolated with a rubber dam. All coronal restorations and carious lesions were completely removed. The tooth and surrounding field was disinfected according to Moller's protocol. All instruments used for access cavity preparation were sterile. The canal filling material was removed with the use of ProTaper retreatment files and endodontic files without the use of any solvents. Canal was rinsed with sterile saline to remove the remnants of filling material and debris, and to moisten the canal before sampling. Working length of the canal was determined radiographically using a 20 K-file 0.5 mm short of the radiographic working length. A small amount of sterile saline solution was placed into the root canal, and a sterile endodontic file was introduced to a level approximately 1 mm short of the tooth apex and a gentle filing motion was applied. Sampling was performed by placing a sterile paper point in the canal to its full length for 60 s and was immediately placed in a transport medium containing tris EDTA (TE) buffer. The samples were sent for conventional PCR analysis to Maratha Mandal's NGH institute of Dental Sciences and research centre.

Microbial identification

The DNA was extracted following which the samples were kept in the thermal cycler for amplification of *E. faecalis.* Amplified products were subjected to electrophoresis. The amplified product of 138 base pair for *E. faecalis was* identified and quantified with the help of DNA ladder which were run simultaneously with the samples in each run. Agarose gel image was captured by Gel documentation system and exported to total lab software (UK) for quantification of DNA bands based on the intensity. Intensity of bands of DNA ladder served as reference sample for quantification.

STATISTICAL ANALYSIS

The data collected for each case (clinical features) were typed onto a spreadsheet and statistically analysed. Chi square test compared the association of *E. faecalis* to tenderness to percussion.

RESULTS

Table 1: Association of tenderness to percussion with *E. faecalis*

		E. faecalis		Chi square	p value
		Present	Absent		
TOP	Yes	75	14	12.005	.001
	No	23	18		
Total		98	32		

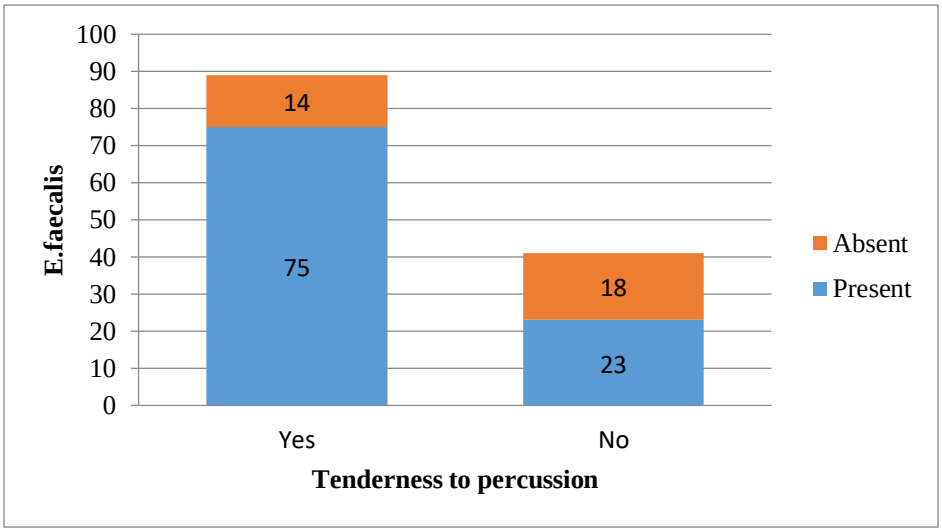

Fig: 1 Presence and absence of *Enterococcus faecalis* and association with tenderness to percussion

The present study included a total of 130 subjects of which 67 were male subjects and 53 were female subjects. *Enterococcus faecalis* was isolated from a total of 98 teeth of the 130 endodontic retreatment cases examined. Chi square test showed a significant association of *E. faecalis* with tenderness to percussion (p=0.001). Tenderness to percussion was associated with 89 root filled teeth with apical periodontitis of which *E. faecalis* was isolated from 75 teeth (57.7%). Fourteen teeth which were associated with tenderness to percussion did not yield *E. faecalis*.

DISCUSSION

Enterococcus faecalis has been found in high prevalence in root-filled teeth with periradicular disease, hence it has been suggested that this species is involved in the pathogenesis of persistent periradicular disease.[4,7,13,14] Various authors have reported the detection of *E. faecalis* by polymerase chain reaction to be consistently high (67% to 77%) compared to culture method (24% to 70%) proving that PCR is a more reliable method in the detection of microorganisms.[4] In addition to being more sensitive than culture, molecular biology technology has emerged as a more effective, accurate and reliable means for the identification of bacteria that are difficult to identify by conventional techniques, usually because of an uncommon phenotypic behaviour.[16,17] Hence conventional PCR was used in this study for the identification of *E. faecalis.*

The pathogenicity of *E. faecalis* has been attributed to its virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid. It possesses the ability to survive in the root canal system since it can endure prolonged periods of starvation. Unlike most putative endodontic pathogens that are frequently found in primary infections, *E. faecalis* may colonize root canals in single infections.[4,18] *Enterococcus faecalis* has an efficient proton pump which maintains pH homeostasis and makes it resistant to intracanal medicaments. When *E. faecalis* is established in the root canal, its eradication by conventional means is extremely difficult.[19,20]

Table 2: Comparison of findings of studies on the association of *E. faecalis* with signs and symptoms in root-filled teeth with apical periodontitis

Authors	Year	Method of identification of *E. faecalis*	Number of cases evaluated	Result
Gomes et al[13]	1996	Culture	60	*E. faecalis* was associated with asymptomatic cases
Rocas et al [4]	2004	PCR	80	11.5% of the asymptomatic teeth and in 3.7% of symptomatic cases.
Fouad et al[21]	2005	PCR	40	*E. faecalis* was associated with nonhealing endodontic retreatment cases (22%).
Sánchez-Sanhueza et al[22]	2015	Culture	20	*E. faecalis* was associated with asymptomatic cases
Vineet et al[23]	2016	Culture	60	*E. faecalis* had a strong correlation with tenderness on percussion in retreatment cases
Present study	2019	PCR	130	*E. faecalis* was isolated from 57.7% of teeth with apical periodontitis.

A significant association of *E. faecalis* with tenderness to percussion was observed in this study. In the present study *E. faecalis* was isolated from 57.7% of symptomatic cases and from 17.7% from

asymptomatic cases. Similar to this study, Vineet et al have found a significant association of *E. faecalis* with the clinical sign of tenderness to percussion.[23] Gomes et al have reported both Gram positive and Gram negative bacteria to be involved in the pathogenesis of tenderness to percussion.[13,14] In contrast to this study, Rocas et al. detected *E. faecalis* in 11.5% of the asymptomatic teeth and in 3.7% of symptomatic cases.[4] The lipopolysaccharide content of cell wall of gram negative bacteria and lipoteichoic acid (LTA) content of gram positive bacteria in higher concentration are related to periradicular inflammation resulting in tenderness to percussion.[24] Lipoteichoic acid is composed of echoic acid and lipid. It is released as a result of cell lysis and binds to target cells, which interacts with circulating antibodies and activates complement cascade and cause damage.[25,26] The factors predisposing to pain in teeth with persistent apical periodontitis include virulence of the endodontic microflora, presence of complex root canal anatomy, genetic factors such as single nucleotide polymorphisms (SNPs), epigenetic factors and systemic diseases that modulate the immune system.[27]

Kaufman et al have concluded from their study that *E. faecalis* was not associated with root-filled teeth with periradicular disease.[28] The host resistance can differ from subject to subject and may spark different patterns of response to microbial infection.

CONCLUSION

The present findings indicate that tenderness to percussion of a tooth is an indicator for the presence of *E. faecalis* in root filled teeth with apical periodontitis. However, some aspects, including the limitations of this and previous studies must be considered before a pathogenic role for *E. faecalis* in endodontic failures is attributed. Further research must be directed towards evaluation of this clinical sign of apical periodontitis in root filled teeth in subjects with systemic disease.

REFERENCES

1. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31: 1-7. 5.
2. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
3. Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North Am population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:579 – 86.

4. Rôças IN, Siqueira JF, Santos KRN. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004;30:315–20.
5. Peciuliene V, Balciuniene I, Eriksen H, Haapasalo M. Isolation of Enterococcus faecalis in previously root-filled canals in a Lithuanian population. J Endod 2000;26:593–5.
6. Pinheiro ET, Gomes BPFA, Ferraz CCR, Sousa ELR, Teixeira FB, Souza Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J 2003;36:1–11.
7. Siqueira JF, Rôças I. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:85–94.
8. Kakehashi S, Stanley HR, Fitzgerald RJ, The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surgery, Oral Medicine and Oral Pathology, 1965; 20: 340–9.
9. Sundqvist G, Bacteriological studies of necrotic dental pulps. Dr. Odont. Thesis, University of Umea˚, Umea˚, Sweden. 1976.
10. Abbott PV. Classification, diagnosis and clinical manifestations of apical periodontitis. Endodontic topics. 2004 Jul;8(1):36-54.
11. Kirkevang LL, Ørstavik D, Hörsted-Bindslev P, Wenzel A. Periapical status and quality of root fillings and coronal restorations in a Danish population. International endodontic journal. 2000 Nov;33(6):509-15.
12. Huumonen S, Suominen AL, Vehkalahti MM. Prevalence of apical periodontitis in root filled teeth: findings from a nationwide survey in Finland. International endodontic journal. 2017 Mar;50(3):229-36.
13. Gomes BP, Lilley JD, Drucker DB. Clinical significance of dental root canal microflora. Journal of dentistry. 1996 Jan 1;24(1-2):47-55.
14. Gomes BP, Drucker DB, Lilley JD. Association of specific bacteria with some endodontic signs and symptoms. International endodontic journal. 1994 Nov;27(6):291-8.
15. Sanghavi TH, Shah N, Shah RR, Sanghavi A. Investigate the correlation between clinical sign and symptoms and the presence of *P. gingivalis*, *T. denticola*, and *T. forsythia* individually or as a "Red complex" by a multiplex PCR method. J Conserv Dent 2014;17:555-60.
16. Preethee T, Kandaswamy D, Arathi G, Hannah R. Bactericidal effect of the 908nm diode laser on Enterococcus faecalis in infected root canals. Journal of conservative dentistry: JCD. 2012 Jan;15(1):46.

17. Lau L, Sanz M, Herrera D, Morillo JM, Martín C, Silva A. Quantitative real-time polymerase chain reaction versus culture: A comparison between two methods for the detection and quantification of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythensis in subgingival plaque samples. Journal of clinical periodontology. 2004 Dec;31(12):1061-9
18. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics. 2006 Feb 1;32(2):93-8.
19. Sedgley CM, Nagel AC, Shelburne CE, Clewell DB, Appelbe O, Molander A. Quantitative real-time PCR detection of oral Enterococcus faecalis in humans. Archives of oral biology. 2005 Jun 1;50(6):575-83.
20. Figdor D, Davies JK, Sundqvist G. Starvation survival, growth and recovery of Enterococcus faecalis in human serum. Oral microbiology and immunology. 2003 Aug;18(4):234-9.
21. Fouad AF, Zerella J, Barry J, Spångberg LS. Molecular detection of Enterococcus species in root canals of therapy-resistant endodontic infections. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2005 Jan 1;99(1):112-8.
22. Sánchez-Sanhueza G, González-Rocha G, Dominguez M, Bello-Toledo H. Enterococcus spp. isolated from root canals with persistent chronic apical periodontitis in a Chilean population. Brazilian Journal of Oral Sciences. 2015 Sep;14(3):240-5.
23. Vineet RV, Nayak M, Kotigadde S. Association of endodontic signs and symptoms with root canal pathogens: A clinical comparative study. Saudi Endodontic Journal. 2016 May 1;6(2):82.
24. Moreillon P, Majcherczyk PA. Proinflammatory activity of cell-wall constituents from gram-positive bacteria. Scandinavian journal of infectious diseases. 2003 Jan 1;35(9):632-41.
25. Hogg SD, Whiley RA, De Soet JJ. Occurrence of Lipoteichoic acid in oral streptococci. Int J Syst Bacteriol 1997;47:62-6.
26. Cohen J. Mechanisms of tissue injury in sepsis: Contrasts between gram positive and gram negative infection. J Chemother 2001;13:153-8.
27. Diogenes A, Hargreaves KM. Endodontic Infections and Pain. Endodontic Microbiology. 2017 Apr; 5:251-67.
28. Kaufman B, Spångberg L, Barry J, Fouad AF. Enterococcus spp. in endodontically treated teeth with and without periradicular lesions. Journal of endodontics. 2005 Dec 1;31(12):851-6.