Molecular Method of Detection of Primary Endodontic Infection- An Ex Vivo Study

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ABSTRACT

BACKGROUND
The removal of microorganism and debris from the root canal system is the prerequisite for success of treatment. This can be achieved by thorough cleaning, shaping and disinfection of the root canal system. The aim of the present study is to investigate the presence of microorganism in primary endodontic infection in South Canara population using PCR technique.

METHODS
Fifty patients with primary endodontic infection were selected for the study. Access cavity preparation was done followed by working length determination and first sample was collected by placing the paper point near the root apex for 1 min and immediately the samples were placed in Tris-EDTA buffer solution, stored at -20°C, followed by PCR analysis of the sample using specific primers for detection of microorganisms.

RESULTS
A total of 50 cases with primary endodontic infection were analysed for the presence of microorganism within the root canal system. Percentage analysis was done, and the positive results were obtained only for Porphyromonas endodontalis in 50 % of cases.

CONCLUSIONS
Porphyromonas endodontalis was the prevalent organism seen in primary endodontic infection in this particular geographic distribution.

KEY WORDS
Endodontic Infection, Polymerase Chain Reaction, Enterococcus faecalis, Fusobacterium nucleatum, Porphyromonas endodontalis

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The success and failure of root canal treatment is mainly dependent on the presence of microorganisms, their virulence factor, and their pathogenicity. The role of these bacteria in induction of periodontal disease have been well recorded in both animal and human studies. Bacteria play an essential role in the initiation and perpetuation of pulpal and periodontal diseases, the elimination of the source of infection is considered paramount in root canal therapy. The oral microbiota has >400 bacterial species. However only few species have the ability to colonize and induce periodontal disease. The species most commonly isolated from root canal infection is the *Fusobacterium, Prevotella, Porphyromonas, Eubacterium, Peptostreptococcus, Propionibacterium, Actinomyces* and *Streptococcus.*

Despite of the bacterial diversity seen in the necrotic root canals the detection and identification of these microorganisms have been very difficult. The majority of the bacterial species of endodontic infection are anaerobic in nature, fastidious in their growth hence become uncultivable and this organism becomes difficult to be identified and can contribute to disease progressive.

The PCR process was introduced by Kary Mullis in 1983 and ever since has revolutionized the field of molecular biology by being able to amplify as few as one copy of a gene into millions to billions of copies of that gene. The impact of PCR on biological and medical research has been remarkable, dramatically speeding the rate of progress of the study of genes and genomes. Molecular method of detection of endodontic microorganism using the 16SrDNA gene in the newer tool for identification of bacterial phylogeny. PCR is a highly sensitive method which can even amplify even a low quantity of DNA into a billion fold a specific known sequence of microbial DNA and allows its detection through electrophoresis. Hence the focus of current research is to detect the bacterial composition with the root canal system among the South Canara population using PCR technique.

**Study Design**

A randomized, double-blind trial was performed involving a sample of 50 patients (sample size was estimated basing on the results obtained by Vianna et al.). Central ethics committee clearance was obtained from NITTE (deemed to be university) NU/CEC/2019/0253. Informed consent was taken from each patient and then the sample collection was done from fifty adult patients who visited the Department for routine endodontic therapy.

**Inclusion Criteria**

a. Spontaneous pain.
b. Pain on percussion.
c. Swelling.
d. The teeth included in the study were incisors, canines, premolars, first and second molars.

**Sample Collection**

The tooth was isolated using rubber dam, the rubber dam clamp was cleansed with 3% hydrogen peroxide and decontaminated with 2.5% sodium hydroxide. Access cavity preparation was done using Endo-Access bur size followed by working length determination using 15 size K file. The first bacterial sample was collected by placing 25 size paper point near the root apex for 1 min. In case of multirooted tooth the sample was collected from the tooth with larger periapical lesion. A minimum of 5 paper point was used, and the collected samples were immediately placed in Tris-EDTA buffer solution and stored at -20°C.

**DNA Extraction**

The extracted DNA samples were quantified by using a Nano Drop Spectrophotometer (Eppendorf). Each sample (2 μL) was placed sequentially in the spectrophotometer. The DNA concentration was calculated from the 260 nm absorbance value for each replicate using the DNA-50 settings. The software automatically calculated the DNA concentration in ng/μL.

**Microbial Selection**

In the present study the main three microorganism were selected based on the criteria

1. The microorganism frequently isolated from the necrotic pulp - *Fusobacterium nucleatum.*
2. Microorganism with symptomatic endodontic infection - *Porphyromonas endodontalis.*
3. The microorganisms from failed root canal treatment- *Enterococcus faecalis.*

**Microbial Detection (Polymerase Chain Reaction- PCR 16S rDNA)**

Reference bacteria strains which were used in the present study are *Enterococcus faecalis* (ATCC 4034), *Fusobacterium nucleatum* (ATCC 25586), *Porphyromonas endodontalis* (ATCC 35406).

**PCR Amplification**

PCR was performed in a thermocycler containing 5 microliter of aliquots, 1 microliter of primer, 5 microliter of primer, 5 microliter of PCR buffer, 1.25 units of taq polymerase and 0.2 mm of DNTP. The thermocycling conditions of each organisms have been mentioned above in table.
**Gel Electrophoresis**

The amplified product was analysed in 2% agarose gel electrophoresis in TAE buffer followed by staining of the gel with 0.5 g of ethidium bromide for 30 min and flushed with water for 20 min. The anode and cathode electrode were connected at the two ends and electrophoresis were performed at 4 v/cm. A 100 b p DNA ladder served as a molecular weight marker. The documentation and analysis were done using a gel doc.10

**RESULTS**

A total of 50 cases with primary endodontic infection were analysed using the PCR technique with 16S r DNA specific primer the percentage analysis was done, positive results were obtained with only one organism i.e. Porphyromonas endodontalis.

| Target Bacteria          | Amplicon Size | Primers                                                                 | Cycles                                                                 |
|--------------------------|---------------|-------------------------------------------------------------------------|----------------------------------------------------------------------|
| Enterococcus faecalis    | 138 bp        | Forward: CGC AGT GCT TGC ACT CAA TTG G/ Reverse: CTC TTA TGC CAT GGC GCA TAA AC | Initial denaturation at 95°C for 2 min and 36 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 1 min, and a final step 72°C for 7 min |
| Fusobacterium nucleatum  | 1000 bp       | Forward: AGT AGC ACA AGG GAG ATG TAT G/ Reverse: CAA GAA CTA CAA TAG AAC CTG A | Initial denaturation at 95°C for 5 min and 30 cycles of 94°C for 30 s, 40°C for 1 min, 72°C for 2 min, and a final step 72°C for 10 min |
| Porphyromonas endodontalis | 672 bp     | Forward: GCT GCA CCT CAA CTG TAG TC/ Reverse: CGG CTT CAT GTC ACC ATG TC | Initial denaturation at 95°C for 2 min and 36 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 2 min, and a final step 72°C for 10 min |

**Table 1. The Primers and Thermocycling Parameters Used for Identification of Bacterial Species**

| Target Bacteria          | Total No. of Sample | No. of Samples with Positive Results | %     |
|--------------------------|---------------------|--------------------------------------|-------|
| Fusobacterium nucleatum  | 50                  | 0                                    | 0     |
| Porphyromonas endodontalis | 50               | 25                                   | 50%   |
| Enterococcus faecalis    | 50                  | 0                                    | 0     |

**Table 2. Bacterial Percentage for 50 Target Specific Primers in Samples from Primary Endodontic Infection**

**DISCUSSION**

Black pigmented bacteria are gram negative rods which are most commonly present in the oral cavity, respiratory and intestinal tract (Bogen and Slots).11 These organisms serve a challenge for traditional culture technique because they are obligate anaerobes which require a strict anaerobic culturing method. Moreover, these pathogens produce black and identical colonies in culture plate which becomes very difficult to identify these organisms. Therefore, PCR have been a useful tool to directly survey the clinical samples without isolating these organisms from pure culture.12

**Major Factors Influencing the Composition of Bacterial Flora**

1. The origin of infection.
2. The ecological conditions in the infected root canal.
3. The host defense mechanisms.

**CONCLUSIONS**

Within the limitation of this study we conclude that Porphyromonas endodontalis is the prevalent organism seen in root canal infection in the particular geographic area.

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