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Evaluation of Antibacterial Effect of Obturating Material and Different Sealers on the Expression of Enterococcus Faecalis

Dr. Vandana James[1], Dr.Sundaresan Balagopal[2], Dr.C. Charanya[3], Dr. S. T. Gowtham[4], Dr. M. Anisha Sebatni[5]

[1]*MDS, Reader, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai - 127
[2] MDS.,M.Sc., Professor & HOD, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai - 127
[3]M.D.S., Senior lecturer, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai - 127
[4]Post graduate, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai - 127
[5]Reader, Department of Conservative Dentistry and Endodontics, Tagore Dental College and Hospital, Rathinamangalam, Chennai - 127

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Corresponding author: Dr. Vandana James
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Introduction

The most important aspect of a successful endodontic treatment relies on elimination of bacteria from the root canal. This could be achieved through different procedure such as bio mechanical preparation, irrigation, obturation, and post endodontic restoration [1]. Despite an attempt to eliminate the microorganism from the root canal space, many researchers have proven that there are microorganisms present in dentinal tubules and cementum even after the treatment. This could be due to ineffective intracanal irrigation, biomechanical preparation or ineffective hermetic seal due to anatomical limitation. Presence of bacteria after root canal therapy can lead to endodontic failures over the years [2].

Enterococcus faecalis (E.faecalis) , facultative Gram-positive cocci, is present in over one third of the canals of teeth with persisting periapical lesions. The clinical resistance of this microorganism is partly related to some of its virulence factors. The importance of this virulence factors are that they play an important role in adherence of E. faecalis to host tissues, it's invasiveness, and abscess formation as well as enhance biofilm formation. These virulence factors include aggregation substance (AS), serine protease (sprE), enterococcal polysaccharide antigen (epa), gelatinase (gelE), enterococcal surface protein (esp), general stress protein (gls24) and E. faecalis endocarditis antigen (efaA). Among these efaA is considered the most potent virulence factor. E. faecalis when lodged in the dentinal tubules of the canal, is difficult to remove through root canal medicaments alone. Thus a good root canal filling material with adequate antimicrobial efficacy is needed to further reduce the effect of persistent microorganisms [4]. Root canal sealers can be classified according to the chemical composition as zinc oxide-eugenol-based, calcium hydroxide-containing, glass ionomer-based, epoxy resin-based, mineral trioxide aggregate (MTA)-based sealers and bio ceramic sealers. The use of

endodontic sealers with antibacterial abilities may be advantageous especially in clinical situations of persistent or recurrent infection and achieve a successful root canal therapy [1]. In this study Sealapex, AH plus and CeraSeal sealers were used as they have shown better antibacterial efficacy compared to other sealers from previous studies.

Many studies have been done to assess the antimicrobial efficacy of different root canal sealers. Agar diffusion test is the most commonly used method to evaluate the antimicrobial efficacy but it was found to have many drawbacks as it depends on diffusion and physical properties of the materials used. To overcome these difficulties recently introduced molecular diagnostic methods can be used as it is based on investigation of bacterial DNA and RNA. The techniques such as reverse transcriptase polymerase chain reaction have improved the sensitivity for microbial detection compared to culturing and have therefore enabled the identification of enterococci with greater precision [5]. Won Young Lee et al (6) compared the culture-based method with PCR methods for estimating bacterial abundance and stated that PCR estimates of bacterial abundance were 10 times higher than culture-based estimates, and the culture-based technique failed to detect bacteria at lower densities.

Therefore the aim of this study is to compare and evaluate the antibacterial effect of Gutta percha with three different sealers on the expression of Enterococcus *faecalis* virulence factor (efaA)- E. faecalis endocarditis antigen(efaA) using RT PCR.

Materials and Methods:

The study was conducted in Tagore Dental College and Hospital after approval of ethical committee of the institution. Forty non-carious, single rooted, single canalled human mandibular first premolar teeth with relatively straight roots were selected for the study. The anatomy of the roots was confirmed by taking straight and angulated radiographic images, and the teeth with anatomical abnormalities and calcified canals were excluded. Teeth were immersed in 1.3% sodium hypochlorite (NaOCl) (Prime Dental Products Pvt., Ltd., India)for 20 minutes and the root surfaces were debrided using a periodontal curette (GDC fine crafted dental Pvt., Ltd., Hoshiarpur). Access cavities were prepared using Endo Access bur (Dentsply Maillefer, Switzerland) and working length determinations were done using #15 K-file (Dentsply Maillefer, Switzerland). All the root canals wereinstrumented up to apical size of 25 8%by Protaper Gold rotary instruments (Dentsply Maillefer, Switzerland) according to the manufacturer's instruction using endomotor (Dentsply Tulsa dental, USA). 2 mLof saline (Prime Dental Products Pvt., Ltd., India) was used for irrigation between each file. To prevent bacterial leakage, the apical foramina were sealed with sticky wax, and the root surfaces were covered with two layers of nail varnish. The teeth were then sterilized by autoclaving for 15 min at 121°C (15 lbs pressure).

Inoculation of E. Faecalis

A suspension of E.*faecalis* (ATCC 29212) with an optical density (OD) of 0.3-0.4 at 450-nm wavelength was obtained from an overnight culture of E.*faecalis*(106 CFU (colony-forming units)/ml) in brain heart infusion (BHI) broth. The suspension was injected into the root canals and the samples were incubated at 37°C and 100% humidity for 21 days. Before grouping, all the specimens were irrigated with 2mL of sterile saline and the canals were dried with paper points (Dentsply Maillefer, Switzerland)**.** After inoculation with E.faecalis, the samples (N=40) were randomly divided based on the sealer materials into four groups of 10 each. Group A- positive control, Group B- AH plus sealer, Group C – Sealapex and Group D- CeraSeal sealer respectively.

Obturation and Dentin Powder Collection:

The different sealers were mixed according to the manufacturer's instructions, and the sealers were applied to the root canals of the respective groups by using Lentulo spiral (Mani Inc., Japan). Group A was not obturated (positive control). Groups (B, C, D) were obturated using (F2) ProTaper Gutta-percha point (Dentsply Tulsa dental, USA) corresponding to ISO size 25. All the specimens were incubated at 37°C and 100% humidity for one week. After the mentioned time interval, teeth in each group were sectioned parallel to their longitudinal axis using a diamond disc (Kerr dental, USA) and a spatula under aseptic condition was used to gently remove the root filling material. Dentin powder was obtained from the middle thirds of the root canals using a low-speed handpiece and a #5 round bur (*MANI*, INC, Utsunomiya, Tochigi, Japan). The dentin powders were then collected in microcentrifuge tubes.

Dna Isolation and Rt- Pcr

One milliliter of lysozyme stock solution was added to the dentin powder that was collected from all the groups and mixed, and incubated at 37°C for 30 min. This is followed by the addition of an equal volume of phenol-chloroform and mixed by vortexing at 10,000 rpm for 10 min. The final supernatant was collected and dried. Thirty microliters of sterile water were added to dissolve the DNA which was then subjected for the RT-PCR analysis. RT-PCR was performed by monitoring the increase in fluorescence intensity of the SYBR Green dye with a Rotor-Gene 3000 RT-PCR apparatus (Corbett Research) according to the manufacturer's instructions. Triplicate reactions were done on each sample. RT-PCR data were represented as cycle threshold (Ct) values, where Ct was defined as the Ct of PCR when amplified product was first detected. The Ct or threshold value of the target sequence is directly proportional to the absolute concentration when compared with the threshold value for reference gene.

Statistical analysis:

The values were entered into an Excel sheet (Microsoft) for calculation. One way analysis of variance followed by Tukey'spost-hoc tests were used to compare CFU among the groups. The p-value<0.05 was consideredsignificant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results:

PCR determines the results in Ct. Ct are inversely proportional to the amount of target DNA. The different groups of root canal sealers were compared with the values attained in the control group and were found to be statistically significant ($P < 0.005$). Gutta percha with Sealapex showed statistically significant antibacterial efficacy against expression of E. *fecalis* virulence virulence factor (efaA) followed by Gutta percha with AH plus and Gutta percha with CeraSeal. The least antibacterial efficacy was seen in control group. (Graph-1)

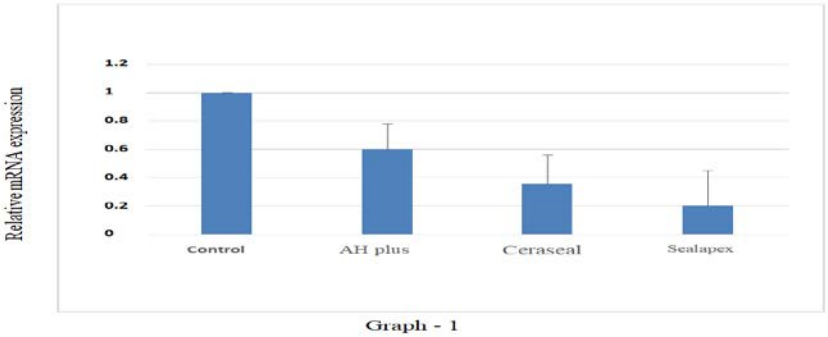

Figure 1: Effect of different sealers on expression of virulence gene in Enterococcus faecalis

Discussion:

Chemomechanical preparation is considered one of the most important steps in successful root canal treatment. It is difficult to completely remove microorganisms from the root canal system, even after debridement, shaping, and irrigation of the root canals with antimicrobial agents. The persistence of bacteria in the root canal system often leads to failure of the root canal treatment. However, this does not rule out the importance of obturation, in which the sealer plays a key role. Root canal sealers aid in minimizing leakage, reducing the possibility of microbial activity by the residual bacteria, and also in some cases resolve periapical lesion. Therefore, the use of root filling materials with antimicrobial activity might help to achieve this goal.[1]

In the present study, *E. Faecalis* was chosen as the test microorganism because it is the most commonly isolated species from oral infections such as infected root canals, periradicular abscesses, and marginal periodontitis. Its prevalence in such infections ranges from 24% to 77% (7).E.faecalis possess several potential virulence factors. It has been understood from the previous studies that these virulence factors could synergically increase bacterial virulence and cause a deeper bacterial invasion as well as greater tissue damage.[7]. In the present study efaA virulence factor expression is calculated because it has been proven as a potent virulence factor which can be found in E. faecalis strains detected in failed root canal of treatment resisted endodontic infections (8)

A variety of laboratory methods can be used to evaluate or screen the *in-vitro* antimicrobial activity of different materials. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. Limitations of these methods include poor growth of bacteria in the media, inability to estimate minimum inhibitory concentration (MIC) and manual errors during the procedures are inevitable. In order to overcome the limitations associated with culture techniques like agar diffusion test and direct contact test, molecular biology methods have been introduced. These methods are based upon the investigation of bacterial DNA and RNA. RT-PCR has the advantage of detecting not only cultivable species, but also of uncultivable microbial species or strains. It possess higher specificity and sensitivity in indentifying microbial strains with different phenotypic behaviour, including divergent or convergent strains. It detects microbial species directly in the clinical samples without the need for cultivation and is less time-consuming. RT-PCR offers rapid diagnosis, which is particularly advantageous in cases of life-threatening diseases or diseases caused due to slow growing microorganisms. [5,1]

A proper obturation with good selection of sealer will help to provide excellent hermetic seal and antimicrobial activity with would prevent reinfection as well as the treatment failure. A variety of endodontic sealers are available in the market which include zinc oxide-eugenol, calcium hydroxide, glass ionomer, silicon, resin, and bioceramic. These sealers have antimicrobial effect depending on their chemical composition. In this study Sealapex, AH plus and Ceraseal sealers are used, Sealapex which calcium hydroxide-based sealer has shown the highest antimicrobial activity which is in accordance with the previous study by swatidalmia et al (1). Calcium hydroxide-containing sealers was introduced to improve the biological properties and also to ensure a good seal of the root canal system. The antimicrobial activity of hydroxide-based sealers may be due to hydroxy ions releasing property as well as its high solubility which helps to create an alkaline environment leaving the environment unfavourable for microbial growth [1]. E. faecalis has shown penetrate to a depth of about 821.91 µm to 1061.79 µm into the dentinal tubules. (11). A study by Ronaldo ordinola-zapata et al have shown that Sealapex demonstrated deeper penetration of about 616.19µm to 650.55 µm into

dentinal tubule compared to GuttaFlow and Sealer 26(12).

AH plus showed the second highest antimicrobial efficacy against E. faecalis in this study. Huang et al compared the antimicrobial activity of GuttaFlow2, AH Plus, ProRoot MTA and RealSeal) against E. faecalis and stated that AH plus showed highest antimicrobial efficacy initially and diminished over the week (13). The antimicrobial activity of AH plus is due to the presence of bisphenol- a-diglycidylether. Extracts of paste a (containing epoxy resin) and paste b (containing amines) are mixed together which exhibits antibacterial ability by reducing the cell viability. Ah plus sealer has a good flow, thereby diffusing into the dentinal tubules and creating microbial inhibition by means of entrapment. [1,9].

Ceraseal which is a calcium silicate-based bioceramic sealer showed the least antimicrobial ability next to control group. It contains calcium phosphate, calcium silicate cement, and calcium oxide. On reacting with dentinal moisture the calcium silicates undergo a hydration reaction which results in the formation of calcium silicate hydrogel and calcium hydroxide. This calcium hydroxide partially reacts with the calcium phosphate to form hydroxyapatite and water. The water formed, in turn, re-starts the cycle to produce more calcium silicate hydrogel and calcium hydroxide, resulting in an increase in ph (> 12.5). By the time the sealer sets, its pH also reduces to about 9.14, consequently reducing its antibacterial efficacy. Salah M Abduljabbar et al (14) compared the antimicrobial activity of Endosequence/BC Sealer, CeraSeal, and BioRoot RCS and stated that CeraSeal showed the least antibacterial efficacy which is accordance with this study. These sealers also exhibit hydrophilic properties, and it also has been stated that their antibacterial properties may be due to a combination of their high pH, active calcium hydroxide diffusion, and hydrophilicity.[10]

The antimicrobial ability of root canal sealer is important as it may help to destroy the remaining microbes that are not removed by cleaning, shaping, and intracanal medicaments. The selection of sealer is very important for the healing of peri radicular tissues as well as for the antimicrobial activity inside the root canal.

Conclusion:

Based on this study it could be concluded that the antibacterial efficacy against the expression of E. fecalis virulence factor (efaA) was highest for the calcium hydroxide based sealer (Sealapex). Sealapex along with gutta percha can be used for obturation in teeth with persistent infections to yield better results.

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