EVALUATING ANTIBACTERIAL EFFICACY OF ANTIBIOTIC, ANTI-INFLAMMATORY NON ANTIBIOTICS AND CALCIUM HYDROXIDE AGAINST ENTEROCOCCOUS FAECALIS IN AN ENDODONTIC MODEL: AN IN-VITRO STUDY

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ABSTRACT:

Aims and Objective: Current study aims to evaluate and compare the antibacterial efficacy of Amoxicillin, Ibuprofen, Diclofenac and routinely used intracanal dressing calcium hydroxide against E faecalis with the aid of an in-vitro endodontic model.

Materials and method: Eighty single rooted mandibular premolar teeth were selected and decoronated to standardize the root length to 14 mm. Then all the roots were prepared biomechanically using rotary up to F3 Protaper; followed by 3% NaOCl irrigation. Final irrigation was done with 17% EDTA; followed by a flush of irrigation with 2ml of saline. After irrigating all the root canals were dried and then the apical foramen was sealed. Then all the samples were autoclaved. 5μl of E faecalis bacterial suspension was transferred and inoculated into the prepared and autoclaved sample. Inoculation procedure was done in every 72 hr. for 14 days and the samples were kept in oven at body temperature (37°C). After 24 hrs of incubation period of all samples counting of bacterial colonies on agar plates was done. All the samples were then randomly divided into 4 groups: Group I - Calcium hydroxide + distilled water, Group II - Ibuprofen + distilled water, Group III - Diclofenac + distilled water and Group IV - Amoxicillin + distilled water. After 7 days, in each sample again colony factor count was recorded and compared.

Results: The Amoxicillin group showed the least bacterial colonies value after 7 days, followed by Diclofenac group, then Ibuprofen group and maximum bacterial colonies were in Calcium hydroxide group.

Conclusion: After Amoxicillin, Ibuprofen and Diclofenac have significantly more pronounced antibacterial activity against E faecalis in comparison with Ca(OH)2. Thus, Anti-inflammatory non antibiotics (Ibuprofen and Diclofenac) can be used as an alternative to antibiotics.

Key Words: Antibiotic, Anti-Inflammatory, Calcium Hydroxide, Enterococcus Faecalis, Endodontia

I. INTRODUCTION

During endodontic treatment, it is mandatory that all the dentinal debris, pulpal tissue, and most importantly the persistent bacteria should be removed; as the microorganisms and their by-products play a major role in pulpal
and periapical disease development [1,2]. In root canal treatment, proper irrigation measures and medicament placement are the important steps which on the whole decide the future and success of the treatment; as alone instrumentation of the root canal is not capable to eliminate the bacteria. Without these steps, there will be increased chances of treatment failure due to persistence of microorganism in the canal. E faecalis is a most common opportunistic gram-positive bacteria found in the root canal of the necrotic pulp. It is also associated with persistent or secondary endodontic infection [3]. E faecalis has the ability to penetrate deeply in the dentinal tubules which enables it to escape from the endodontic instruments and irrigants used during chemomechanical preparation, to tolerate high pH and to form biofilms [4]. Various measures are involved to reduce microorganisms from the infected root canal, including use of proper irrigants (eg: chlorhexidine, sodium hypochlorite, saline etc.), rinses and application of inter appointment medicament [2,5]. The most common intracanal inter-appointment dressing is calcium hydroxide due to its harmless action and bactericidal effects caused by its high pH (12.5-12.8) it also denatures and detoxifies bacterial products, such as lipopolysaccharide [2]. Though, considered as the gold standard ; E faecalis is resistant to calcium hydroxide as it resist high pH which is considered to be related to proton pump functioning, which acidify the cytoplasm and drives protons into the cell [6]. Thus, to substitute calcium hydroxide various agents have been proposed [2]. Antibiotics (amoxicillin, gantamycin , triple antibiotic paste etc) have been used variably as systemic or intracanal application. But the irritational and extensive antibiotic use can lead to antibiotic resistance [7]. Recently studies have shown that some medicines such as antipsychotics, antihypertensive, antihistamines, local anaesthetics have antibacterial activity [8,9]. the drugs which exhibit low to high antimicrobial activity was termed as ‘non-antibiotics’ by Kristiansen and Amaral in 1997 [10]. Nonsteroidal anti-inflammatory drugs (NSAIDS) are most commonly used to lower down inflammation and pain management in dentistry. They have potent antibacterial action, as they cause impairment of ,membrane activity and inhibit DNA synthesis [11,12]. Also, has antiplasmid activity and interfere with quorum sensing of bacteria thereby preventing bacterial colonization and formation of biofilm. [13]Commonly used NSAIDS as interappointment dressing include ibuprofen and diclofenac due to their potent antibacterial and anti-inflammatory action [3]. Thus, the aim of this study is to evaluate and compare the antibacterial efficiency of antibiotic (amoxicillin), Anti-inflammatory non antibiotics (ibuprofen and diclofenac) and routinely used intracanal dressing calcium hydroxide against E.faecalis with the aid of in-vitro endodontic model.

II. MATERIAL METHODOLOGY

In this study, efficacy of antibiotic (amoxicillin), Anti-inflammatory non antibiotics (ibuprofen and diclofenac) and routinely used intracanal dressing calcium hydroxide against E.faecalis was determined; by evaluating the total number of bacterial counts. (e. faecalis) Eighty single rooted mandibular premolar teeth were selected with minimum 14 mm of root length and completely formed apex, any tooth defect like caries, resorption, fracture or endodontic treatment were discarded.

Preparation of endodontic model

All the teeth were cleaned, dried and stored in saline. They are then decoronated to standardize the root length to 14 mm. Working length will be taken with 15 K-file (Mani, Japan) with passive instrumentation into the canal upto the apical foramen until it is visible and thus, the working length will be determined by subtracting 1mm from the actual canal length. Then all the roots were prepared (biomechanical preparation done) using rotary upto F3 Protaper (Dentsply,) to standardize the instrumentation; followed by 3% NaOCl (Dentpro india pvt Ltd.) irrigation after every instrumentation. Final irrigation was done with 17% EDTA (Amdent Canallarge EDTA) allowing it to remain it for 2 minutes; followed by a flush of irrigation with 2ml of saline. After irrigating all the root canal were dried with sterile paper point (Dentsply) and then the apical foramen was sealed with bonding agent (Single Bond Universal Adhesive;3M India.) and composite resin which were light cured respectively. Nail varnish application was done on all surfaces except coronal surface to prevent any kind of contamination. All the samples were autoclaved at 121° C with 15 Psi for 20 minutes. The Eppendorf tubes (1.5 ml) were used for specimen contamination as well as medicament placement.

Root canal contamination with E. Faecalis

The E. faecalis (bacterial strain- ATCC 29212) was revived on blood agar medium plates and transferred to sterile brain heart infusion broth and incubated overnight once again. With the micropipette, 5μl of E. Faecalis bacterial suspension were transferred and inoculated into the prepared and autoclaved teeth. The inoculum density was adjusted to 0.5 McFarland standard turbidity (1.5 × 10^8 bacteria/ml). Inoculation procedure
(introduction of bacterial teeth) were done in every 72 hr. for 14 days and the teeth were kept in oven at body temperature (37°C).

Confirmation of tooth Contamination with E. faecalis

After the contamination period of 14 days, irrigation of each tooth was done with 100 μl of sterile saline. Followed by that, sterile absorbent paper point (Dentsply, India) of size 30 was used and placed in the canal for 5 minutes in each tooth. All these paper points were transferred into test tube which contained 1ml saline solution and 4 diluted solutions were made out of that saline solution. The 25 μl aliquots of diluted material were then added to Muller-Hinton agar plate. Finally, counting of bacterial colonies (CFU 1) an agar plates after 24 hr of incubation period was done.

Test intracanal medicament application

- All the teeth were then randomly divided into 4 groups (n=10) and then each group different medicament was placed.

4 groups were:-

- Group I - Calcium hydroxide powder + distilled water
- Group II – Ibuprofen powder + distilled water
- Group III – Diclofenec powder + distilled water
- Group IV – Amoxicillin powder + distilled water

A creamy mix, of intracanal medicament, was prepared on a sterile glass slab by mixing distilled water with test material. Lentulo spirals was used to place the medicament into the canal till working length, plugged with cotton pellet and sealed with temporary restoration (Cavit) and were kept for 7 days in an incubator at 36° temperature.

Microbiological analysis of test medicament after treatment

After 7 days, each tooth was irrigated with 5 ml saline and again colony factor count (CFU 2) was recorded and compared.

III. STATISTICAL ANALYSIS

Kruskal–Wallis ANOVA test was used to compare the four groups. Mann–Whitney U-test was used for pair-wise comparison, and Wilcoxon matched pair test for comparison within the group. (Level of significance at p < 0.05)

IV. RESULTS

Comparison between CFU counts at baseline (CFU-1) and after 7 days (CFU-2) in the four groups and inter-group comparison are given in Table 1 and Table 2, and graphically represented in Figures 1 and 2.

When intergroup comparison was done using Kruskal Wallis at baseline. The results were not found to be significant which showed comparability of findings among 4 groups. However, after 7 day, the results were non-significant. The group IV showed the least CFU 2 value after 7 days, followed by group II, then group III and maximum in group I.

Pairwise comparison was done among four groups and it was found to be significant for group 4 and group 1 only (0.049; p < 0.05)

<table>
<thead>
<tr>
<th>Colony count X 10^5</th>
<th>CFU 1 At baseline</th>
<th>CFU 2 After 7 days</th>
<th>p(^{&lt;}) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Calcium hydroxide</td>
<td></td>
<td></td>
<td>0.01,S 2&lt;1</td>
</tr>
<tr>
<td>Mean</td>
<td>22.63</td>
<td>9.34</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.56</td>
<td>2.34</td>
<td></td>
</tr>
<tr>
<td>Group 2 Ibuprofen</td>
<td></td>
<td></td>
<td>0.04,S 2&lt;1</td>
</tr>
<tr>
<td>Mean</td>
<td>24.26</td>
<td>6.42</td>
<td></td>
</tr>
</tbody>
</table>
### Table 1: Comparison of CFU Counts for Different Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>n</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>p&lt;value</th>
<th>p&lt;value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>-</td>
<td>20</td>
<td>9.34</td>
<td>6.42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.45</td>
<td>20</td>
<td>22.63</td>
<td>6.33</td>
<td>0.001,S</td>
<td>2&lt;1</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.36</td>
<td>20</td>
<td>23.08</td>
<td>3.01</td>
<td>0.001,S</td>
<td>2&lt;1</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.049*</td>
<td>20</td>
<td>23.85</td>
<td>1.14</td>
<td>0.22,NS</td>
<td>0.011,S</td>
</tr>
</tbody>
</table>

#### Mann whitney u test (p<value)
- Group 1 < Group 4

### Figure 1: Intergroup Comparison of CFU Counts

- **Calcium hydroxide**
  - Group 1: 22.63
  - Group 2: 24.26
  - Group 3: 23.08
  - Group 4: 23.85

- **Ibuprofen**
  - Group 1: 9.34
  - Group 2: 6.42
  - Group 3: 6.33
  - Group 4: 2.21

- **Diclofenac**
  - Group 1: 22.63
  - Group 2: 24.26
  - Group 3: 23.08
  - Group 4: 23.85

- **Amoxicillin**
  - Group 1: 22.63
  - Group 2: 24.26
  - Group 3: 23.08
  - Group 4: 23.85

*Kruskal wallis (intergroup comparison), Mann Whitney u test (intergroup comparison-pair wise), Wilcoxon paired Test (intragroup comparison: CFU1 and CFU2), Level of significance at p < 0.05*
In various studies it was analyzed that in failed endodontically treated cases, the predominant specie is E. faecalis and was about 45.8% according to Pinheiro ET et al. in previously treated cases[14]. Similar results were also obtained by Sedgley et al and Siqueira and Roças using the polymerase chain reaction (PCR) and observed E. faecalis prevalence was 79.5% and 77% and respectively [15,16]. Sedgley et al. also determined that in retreatment cases the prevalence of E. faecalis was significantly higher (89.6%) as compared in primary infection (67.5%) [15]. Thus, E. faecalis was used in this study as the test bacterium as it is mainly associated with resistant endodontic infections[2]. Routinely used intracanal dressing calcium hydroxide was used in the present study. The antimicrobial action of calcium hydroxide is mainly due to the release of hydroxyl ion when comes in contact with aqueous fluids. Hydroxyl ions are highly oxidant free radicals that exhibit extreme reactivity with biomolecules, that causes protein denaturation, damage to DNA or protoplasmic membrane damage. However, it was insisted that Ca(OH)\(_2\) is less susceptible against E. faecalis. In this study also calcium hydroxide has exhibited maximum colony forming unit even at 7 day. It was in support with the study conducted by Milani et al (2013) and Peters et al which showed weak antibacterial effect of Ca(OH)\(_2\) against E.faecalis [2,17].

This study has demonstrated profound antibacterial activity of Diclofenac and ibuprofen against E. faecalis after amoxicillin. To the best of our knowledge, the first research against NSAIDS anti-bacterial efficacy was conducted by Domenico et al., against Klebsiella pneumoniae with sodium salicylate [18]. In vivo and in vitro animal studies by Dastidar et al. [19]., Dutta et al. [20,21].nd Annadurai et al. [22], exhibited the antibacterial efficacy of diclofenac against Listeria monocytogens, Salmonella typhimurium and Mycobacterium tuberculosis and . Ibuprofen efficacy against Helicobacter pylori was demonstrated by Shirin et al [23]. The exact mechanism of diclofenac and ibuprofen antibacterial activity remains unclear. Studies have proposed that there is inhibition of bacterial DNA synthesis, impairment of membrane activity, Anti-plasmid activity, Alteration in genes encoding transport/binding proteins, DNA synthesis and cell envelope,Down-regulation of efflux pumps and reduced quorum sensing-controlled motility leading to reduced biofilm [3].

According to some studies, incorporation of anti-inflammatory agents such as diclofenac or corticosteroids in intracanal dressings composition reduces the interappointment pain [24,25]. Salem-Milani et al. was the first to perform an in vitro study to assess anti-bacterial efficiency of Ca(OH)\(_2\),ibuprofen and diclofenac and recommended NSAIDs to be used as intracanal medicaments.\(^2\) Therefore, diclofenac or ibuprofen with both antibacterial and anti-inflammatory activity may theoretically be proposed as the main component of the intracanal medication in substitute for Ca(OH)\(_2\). NSAIDs other than the aforementioned which may serve the role of a nonantibiotic are namely, Ketoprofen [26].and Indomethacin [27]. Nonantibiotic agents (other than NSAIDs) that have shown antibacterial activity against E. faecalis are lignocaine (local anesthetic/anti-arrhythmic),chlorpromazine (anti-psychotic/anti-emetic) and amiloride-HCl (potassium-sparing diuretic). [3]. In regard to effective drug dose, Anti-bacterial activity of Ca(OH)\(_2\), demonstrated by Blanscet et al. was at 400 and 600 μg/ml and Salem-Milani et al. evaluated that at 50 μg/ml and above for diclofenac and ibuprofen against E.
Within the limitation of this in vitro study it was concluded that:

- Ibuprofen and Diclofenac have significantly more pronounced antibacterial activity against E. faecalis in comparison with Ca(OH)₂ but less than antibiotics (amoxicillin).

- Due to higher chance of development of antibacterial resistance, Anti-inflammatory non antibiotic i.e Ibuprofen and Diclofenac can be used as an alternative intracanal medicament against E. faecalis.

**REFERENCES**


