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ANTIBACTERIAL EFFICACY OF CALCIUM HYDROXIDE AND CHLORHEXIDINE AGAINST ENTEROCOCCUS FAECALIS–A SYSTEMATIC REVIEW

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ABSTRACT

Aim: To compare the antimicrobial efficacy of calcium hydroxide and chlorhexidine against *E.faecalis*.

Material and Method: MEDLINE, the Cochrane library, Google Scholar and major journals were searched for studies from 1st Jan 2006 to 31st July 2017 to identify appropriate articles. A comprehensive search was designed, and the articles were independently screened for eligibility by two reviewers. In vitro studies comparing antimicrobial efficacy of calcium hydroxide and chlorhexidine against *E.faecalis* were considered for review.

Results: Total of 905 articles were obtained using search strategies out of which 144 articles were selected after reading title and abstract. As a second step, full text publication were obtained. Studies in which calcium hydroxide and chlorhexidine were compared as an intracanal medicament against *E.faecalis* were selected for this review. Finally a total of 31 articles were included out of which 10 articles were excluded on basis of inclusion and exclusion criteria.

Conclusion

1. CHX showed a better antimicrobial efficacy than calcium hydroxide against *E.faecalis*. Though 2% chlorhexidine has been found to be more effective against *E.faecalis* more sophisticated methods such as confocal laser microscopy have shown that bacteria may still be viable but in non-cultivable state so the results are not reliable.
2. The vehicle used for calcium hydroxide would alter its antimicrobial efficacy and substantivity.
3. 2% Chlorhexidine gel has increased bactericidal action when compared to lower concentrations of chlorhexidine gel.
4. Antimicrobial efficacy of calcium hydroxide and chlorhexidine decreases with the time.

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INTRODUCTION

Complete debridement and effective disinfection of the root canal space is an essential step for achieving long term success of nonsurgical endodontics. Elimination of microorganisms is important for management of pulpal disease (Bhardwaj, 2012).

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While treating teeth with a vital pulp, there is no need for intracanal medication as such. However, all root canal treatments cannot be completed in single visit so it is recommended to fill the root canal with an antimicrobial dressing between appoinments, to ensure sterility in the canal space until completion of treatment. If there is persistent pain and exudation, placing an intracanal medicament becomes a necessity (Ingle, 2008). The excellent biologic and antimicrobial properties of calcium hydroxide have made this

medication the choice for the intracanal dressing of infected root canals. It is bactericidal and neutralizes the remaining tissue debris in the root canal system (Bhandari, 2014). The use of calcium hydroxide as an intracanal medicament is very well documented. However its efficacy in clinical practice is controversial (Hargreaves, 2014). Bystrom et al showed that 34 out of 35 canals were bacteria free after 4 weeks of medication period with calcium hydroxide. The study conducted by Sjogren *et al.* (1991), also demonstrated that a 7 day dressing with Calcium hydroxide reduced all bacteria in root canal. However Kvist *el al*. (2004), reported that residual bacteria were detected in 29% of one visit teeth and 36% of two visit teeth treated with calcium hydroxide, with no statistically significant difference in both the groups. Nowdays interest has been developed in the effectiveness of CHX gel alone or its mixture with calcium hydroxide. Chlorhexidine (CHX) is a synthetic cationic bisguanide used in endodontics as an irrigant and intracanal dressing. It has an immediate antimicrobial effect, a wide spectrum of action, substantivity, and relatively less toxicity, but it cannot dissolve organic debris which might interfere with the sealing of the root filling (Carbajal Mejía, 2014). Ercan et al reported that 2% CHX gel was significantly more effective when compared with mixture of CHX gel and calcium hydroxide or calcium hydroxide alone against *E.faecalis*. Kandaswamy Eswar evaluated the antimicrobial efficacy using real-time polymerase chain reaction. This study also showed that chlorhexidine is more effective against *E.faecalis* compared to calcium hydroxide. However some studies states that altering the vehicle increases the antimicrobial effect of calcium hydroxide against *E.faecalis* when compared with chlorhexidine (Vaghela, 2011). The quest for better intra canal antimicrobial protocols must be continued to ensure complete eradication of bacteria from canal prior to obturation. Results of the different studies are inconsistent, because various techniques have been used to asses antimicrobial efficacy such as zone of inhibition, colony forming units, optical densities and polymerase chain reaction technique. This systematic review can clarify conflicting research data and current state of information regarding specific issues about intracanal medicament. So here in this systematic review we are comparing antibacterial efficacy of calcium hydroxide and chlorhexidine against *E.faecalis* when used as an intracanal medicament.

MATERIAL AND METHODS

The methodology used in this systematic review includes

- a literature search strategy,
- selection criteria,
- screening and data extraction.

The PRISMA 2009 flow chart was followed in this systematic review.

PICO

P: Human extracted teeth
I: Chlorhexidine
C: Calcium hydroxide
O: Antimicrobial activity against e.feacalis

Literature Search Strategy

The search strategy covered electronic databases and the reference lists of such articles identified and published from 1st Jan 2006 to 31st July 2017. The electronic databases searched were the following: PubMed (MEDLINE), the Cochrane library and Google Scholar, Google, Clinical trials registry and manual search using DPU college library resources. All cross reference lists of the selected studies were screened for additional papers that could meet the eligibility criteria of the study. The databases were searched up to and including July 2017 using the search strategy. The following combination of key words were used: Calcium Hydroxide AND Chlorhexidine AND Intracanal Medicament, Intracanal Medicament AND Enterococcus Faecalis, Calcium Hydroxide AND Chlorhexidine, Calcium Hydroxide AND Chlorhexidine AND Antimicrobial Efficacy, Calcium Hydroxide AND Chlorhexidine AND Intracanal Medicament OR Intracanal Dressing, Intracanal Medicament OR Root Canal Dressing AND Enterococcus Faecalis, Calcium hydroxide AND chlorhexidine against enterococcus faecalis, Calcium hydroxide AND Chlorhexidine AND Enterococcus faecalis AND Antimicrobial efficacy, Calcium hydroxide AND Chlorhexidine AND Root canal dressing against Enterococcus faecalis.

Inclusion Criteria

- Articles in English language or those having summary in English.
- Studies published in 1st Jan 2006 to 31st July 2017.
- In vitro studies done on human extracted teeth.
- Studies comparing antimicrobial efficacy of calcium hydroxide and chlorhexidine against *E.faecalis*

Exclusion Criteria

- Review, Abstract, Letter to editorials studies are excluded
- An in vivo studies are excluded.
- Any studies done before 1st Jan 2006

Screening and Data Extraction

Initially, potential relevant publications involving calcium hydroxide and chlorhexidine as an intracanal medicament were retrieved independently by two reviewers. All articles were screened for eligibility criteria. Any disagreements were resolved by consensus after discussion with a third reviewer . The extraction of information from studies were conducted by the same reviewers.

RESULTS

The results of the search strategy are presented in table 1. Figure 2 represent flow chart of systematic review process. Preliminary screening consisted total of 905 articles out of which 144 articles were selected after reading titles and abstracts. The papers were screened independently by two reviewers. As a second step, full text papers were obtained when they fulfilled the criteria of the study aim. . For full-text screening, the following criteria were taken into consideration: In-vitro studies in which antimicrobial efficacy was assessed measuring zone of inhibition, colony forming units or by measuring optical densities and by using polymerase chain reaction technique. Finally a total of 31 articles were included out of which 20 articles was finally synthesized in this systematic review. All included articles were summarized in Figure 1.

Sr. No.	Search strategy	Number of articles	Number of selected articles	After Duplicate Removal
Search Strategy 1	Calcium Hydroxide AND Chlorhexidine AND Intracanal Medicament	52	12	8
Search Strategy 2	Intracanal Medicament AND Enterococcus Faecalis	56	11	10
Search Strategy 3	Calcium Hydroxide AND Chlorhexidine	233	28	18
Search Strategy 4	Calcium Hydroxide AND Chlorhexidine AND Antimicrobial Efficacy	57	15	9
Search Strategy 5	Calcium Hydroxide AND Chlorhexidine AND Intracanal Medicament OR Intracanal Dressing	171	13	10
Search Strategy 6	Intracanal Medicament OR Root Canal Dressing AND Enterococcus Faecalis	78	10	7
Search Strategy 7	Calcium hydroxide AND chlorhexidine against enterococcus faecalis	66	10	7
Search Strategy 8	Calcium hydroxide AND Chlorhexidine AND Enterococcus faecalis AND Antimicrobial efficacy	35	12	8
TABLE 1 Search Strategy 9	Calcium hydroxide AND Chlorhexidine AND Root canal dressing against Enterococcus faecalis	5	1	3
Total		753	112	80

Figure 1 is uploaded as excel sheet

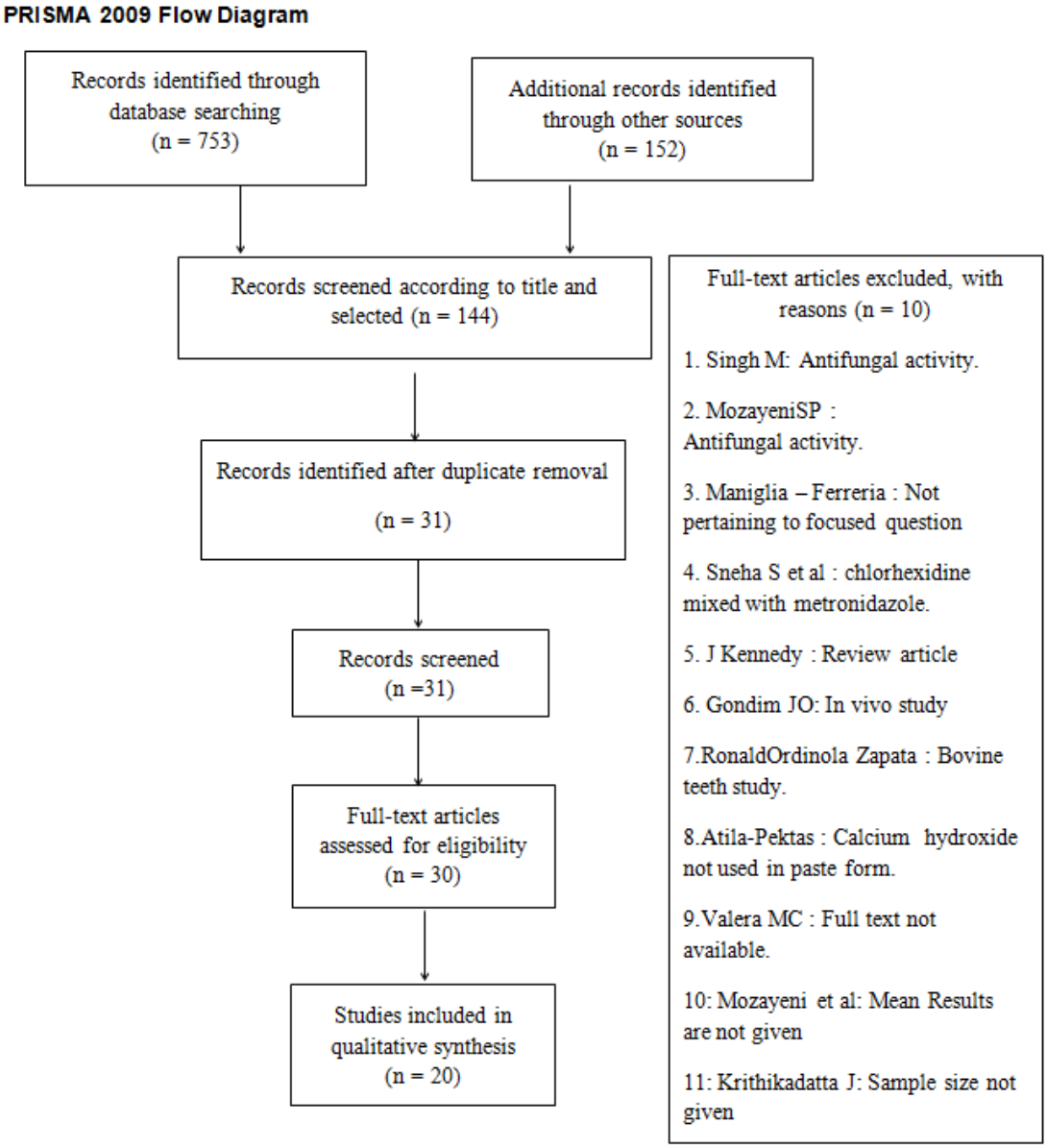

Figure 2.

DISCUSSION

The main objectives of endodontic therapy are to eliminate bacteria from the root canal and to prevent the regrowth of residual micro-organisms. Antimicrobial agents are recommended for intracanal antisepsis, to prevent the growth of microorganisms between appointments (Vaghela, 2011). Despite the antimicrobial properties of the intracanal medicaments and irrigants, elimination of micro-organisms may not be uniform because of the varying vulnerabilities of the involved organisms (Neelakantan, 2007). Studies have shown the poor antimicrobial effect of calcium hydroxide on

E.faecalis (Carbajal Mejía, 2014; Delgado, 2010; Kumar, 2013 and Pavaskar, 2014). However 2% CHX gel demonstrated significant inhibition of *E.faecalis* compared with calcium hydroxide (Bhardwaj, 2012; Bhandari, 2014; Carbajal Mejía, 2014; Neelakantan, 2007; Delgado, 2010; Eswar, 2013 Ballal, 2000; Jhamb, 2010). These differences may have been caused by differences in experimental methodology , concentration of chlorhexidine gel, vehicle used for calcium hydroxide, dressing period of medicament. PrasannaNeelakanthan et al states that the agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medicaments. The advantage of this method is that it allows direct comparison of the materials against the organisms, which indicates that material has the potential to eliminate bacteria in the local micro environment of the root canal system. However, the disadvantage of this method is that the result not only depends on the toxicity of the material for the particular organism, but also is influenced by the ability of the material to diffuse across the medium. Chlorhexidine gel produced the largest inhibitory zones against all the microorganisms whereas Calcium hydroxide was seem to be least effective against *E.faecalis*.

Francisco José de SOUZA-FILHO et al stated that the inhibition zones may be more related to material solubility and diffusibility in agar than to its actual efficacy against the microorganisms. Antimicrobial activity of calcium hydroxide is because of its high pH, which in turn precipitates this medicament on agar, preventing its diffusion. This may be the explanation for poor performance of $Ca(OH)_2$ using the agar diffusion method. This was in accordance with studies conducted on agar plates by measuring zones of inhibition by V Ballal et al, SwathyJhamb et al, RajdeepPavskar et al and Prasanna Neelkanthan *et al.* Chlorhexidine gluconate, a cationic bis-biguanide, is a broad-spectrum antimicrobial agent and is an effective bactericidal and fungicidal agent and its action is related to the binding of the cationic molecule to the negatively charged bacterial cell walls, thereby altering the cell's osmotic equilibrium. A major advantage of chlorhexidine is its substantivity, which allows prolonged residual antimicrobial effect. Another advantage is that it does not produce resistant microorganisms (Neelakantan, 2007). This might be the reason attributed to the largest inhibitory zones against all the microorganisms, even after 72 hours. However Francisco José de SOUZA-FILHO et al stated that it is not an effective intracanal barrier, needing to be changed frequently as it diffuses through the dentinal tubules, leaving an empty canal. Also the study done by SwathyJhamb et al states that Chlorhexidine had a better antibacterial activity in comparison to calcium hydroxide, but loses its property, when used for a long time.

According to study conducted by AR Prabhakar et al in 2013antibacterial activity of $Ca(OH)_2$ can be attributed to direct contact through high pH (12.5-12.8) and its ability to dissociate into hydroxyl ions causing bacterial cell death. $Ca(OH)_2$ showed decreased antibacterial activity over a period of time which may be attributed to several factors. Firstly, the buffering of the alkalinity of $Ca(OH)_2$ by dentin and dentin components.[3,5,10] Secondly, low diffusibility of hydroxyl ions in dentinal tubules.[3,10] Thirdly, E. faecalis colonize within dentinal tubules forming dense biofilms, such that bacteria located within the dentinal tubules can protect those located deeper inside the tubules thus evading the hydroxyl ions (Prabhakar, 2013). The present investigation reviewed the literature for the studies published comparing the efficacy of calcium hydroxide and chlorhexidine against E. faecalis. Most of the studies based on colony forming units showed that both the medicaments have antibacterial efficacy against *E.faecalis* but it was found that calcium hydroxide was less effective than chlorhexidine. Chlorhexidine gel showed maximum antimicrobial activity against *E.faecalis* (Bhardwaj, 2012; Bhandari, 2014; Carbajal Mejía, 2014; Neelakantan, 2007; Kumar, 2013; Prabhakar, 2013; Eswar, 2013; Jhamb, 2010; Ercan, 2006 and Sharifian, 2008). According to results of the study conducted by SwathyJhamb et al in 2009, 2% CHX gel shows higher antimicrobial activity when compared to 0.12 CHX gel. The possible cause could be the high concentration of CHX (2%), lethal bactericidal mode of action and enhanced diffusion into dentinal tubules. Because CHX gel has low contact angle with dentin and thus penetrates the dentinal tubules effectively at faster rate (Bhardwaj, 2012; Prabhakar, 2013; Eswar, 2013). Moreover, SonamBhadari et al stated that the presence of chlorhexidine adds substantivity to the formulation, due to its adsorption capacity and slow liberation of active molecules by dental tissues. Another advantage is that it does not produce resistant micro-organisms. Similar reason was explained by PrasannaNeelkanthanet and Jesion B Carbajal Mejia.

The study conducted by Jesion B Carbajal Mejia states that 2% CHX gel is effective against E.feacalis after 14 days of application. But Ronan J.R. Delgado used confocal laser microscopy to detect the viability of bacteria from which he detected nonculturable E. Faecalis after 14 days of treatment with CHX. However Jesion B Carbajal Mejia's study only checked the colony forming units and did not check the viability of bacteria and hence the results. The antimicrobial action of all the three medicaments increased on the seventh day as compared to that on the second day, for both the organisms tested in the study. This was in accordance with the study conducted by Sonam Bhandari which showed that antimicrobial action of calcium hydroxide increased on third and fifth day. Also in the study conducted by Anuj Bhardwaj et al antimicrobial action of calcium hydroxide increased on 5th day compared to 1st day. But the results of the study by Carbajal Mezia indicated that antimicrobial action of calcium hydroxide decreased on 5th day compared to 1st and 3rd day. Similarly results obtained by AR Prabhakar et al showed that antimicrobial action of calcium hydroxide decreased on 3rd and 5th day compared to 24 hours.

From the study conducted by Dakshita Joy Vaghela et al it was concluded that: The type of vehicle used would alter the antimicrobial property of calcium hydroxide as an intracanal medicament. Addition of propylene glycol to calcium hydroxide as an intracanal medicament showed that calcium hydroxide as an effective antibacterial agent against *E.faecalis* whereas to use aqueous solutions of calcium hydroxide were not as effective against *E.faecalis*. The vehicle may also alter the substantivity of calcium hydroxide. The agar diffusion method detects a zone of inhibition around the wells containing the medicament. It is so far the most commonly used method. Most of the studies done by using agar diffusion method to assess antimicrobial activity against *E.faecalis* have shown that calcium hydroxide is not as effective as chlorhexidine (Neelakantan, 2007; Kumar, 2013; Ballal, 2010; Jhamb, 2010; De Souza-Filho, 2008; Marickar, 2014). However Marickar RF et al stated that the results of agar diffusion tests should be viewed with caution because of the

fact that owing to high pH calcium hydroxide, the medicament may precipitate on the agar and thereby reduce its diffusion and hence the poor performance of calcium hydroxide may be explained. Most of the studies testing the antimicrobial action of calcium hydroxide and chlorhexidine using colony forming units have shown that both the medicaments exerts the antimicrobial activity but chlorhexidine demonstrated significant reduction of colony forming units against *E.faecalis* (Bhardwaj, 2012; Bhandari, 2014; Delgado, 2010; Prabhakar, 2013 and Kandaswamy, 2010). KandaswamyEswar evaluated the antimicrobial efficacy using real-time polymerase chain reactionand they found that chlorhexidine is more effective against *E.faecalis* compared to calcium hydroxide. The viable but non culturable states of E. faecalis under adverse environmental conditions, where the enterococcalcells lose their culturability but are viable which often result in PCR positive/culture negative cases. This was in accordance with the study conducted by Ronan Degaldo who used CLSM to detect viability of bacteria.

Conclusions

- CHX showed a better antimicrobial efficacy than calcium hydroxide against *E.faecalis.*Various methods such as checking zone of inhibition, counting colony forming units or checking optical densities have been used to assess antimicrobial efficacy. Though 2% chlorhexidine found to be more effective against *E.faecalis* more sophisticated methods such as confocal laser microscopy have shown that bacteria may still be viable but in non-cultivable state so the results are not reliable.
- The vehicle used for calcium hydroxide would alter its antimicrobial efficacy and substantivity.
- 2% Chlorhexidine gel has increased bactericidal action when compared to lower concentrations of chlorhexidine gel.
- Antimicrobial efficacy of calcium hydroxide and chlorhexidine decreases with the time.

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