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THE ANTI BACTERIAL EFFECT OF DIFFERENT FORMULATIONS OF CALCIUM HYDROXIDE AGAINST E-FAECALIS

Medhat Taha Elfaramawy* *and* Shady Ali Hussein*

ABSTRACT

Aim of the study: To compare the antibacterial effect of Nano-calcium hydroxide (Nanostreams, Cairo, Egypt) mixed with saline versus conventional calcium hydroxide (GAMA dental lab,Cairo, Egypt) with saline and calcium hydroxide paste with iodoform (Cal plus) (Apexion, Karala, India) against E-faecalis using agar diffusion method. Samples were classified into 3 groups according to the material used. Each group was further divided into 3 subgroups according to the observation time (1day, 3days and 7 days). Each group consisted of 7 agar plates implanted with E-faecalis strain. 3 holes were created in each agar plate, each hole contained one tested material. The results showed that Nano calcium hydroxide had the largest inhibitory zone of E faecalis in agar diffusion test over all observation periods followed by conventional calcium hydroxide. The calcium hydroxide mixed with iodoform had the least average inhibitory zone. Calcium hydroxide Nano particles have superior anti microbial activity against E faecalis compared to other tested materials.

INTRODUCTION

Chemical and biological dynamics of any intra canal medication is an important issue for choosing the desired intra canal medication. Microorganisms infecting the root canal system have a limited virulence when it is acting as an individual species, however it is of high virulence when acting collectively. The success of endodontic treatment is highly dependent on the elimination of the infection from the root canal system. Different intra canal medications has been used for this purpose, however still the calcium hydroxide is considered the most popular and efficient intra canal medication used [1-3]. So many changes were done to increase the antimicrobial action of calcium hydroxide that can speed up or slow down the ionic dissociation. such as the vehicle, addition of other active ingredients such as iodoform or changing of the particle size with use of the Nanotechnology, which allows much more area of exposure to external environment and so much more hydroxyl ions release. The purpose of this study was to verify the influence of changing of the particle size of calcium hydroxide or addition of iodoform on its activity against E-faecalis.

* Lecturer of Endodontics. Faculty of Dentistry. Ain Shams Univ.

MATERIALS AND METHODS

Materials

In this study, the following materials were used:

Bacterial isolates:

A total of (7)*Enterococcusfaecalis* isolates were included in this study.

Substances:

Nano calcium hydroxide.

Conventional Calcium hydroxide.

Calcium hydroxide paste with iodoform.

Media:

The following media were used in this study:

Brain-Heart Infusion Broth:

This medium was used to prepare the suspension of *Enterococcus faecalis*.

Brain-Heart Infusion Agar:

This medium was used to test the effect of the different substances on the growth of *Enterococcus Faecalis* using the diffusion agar method.

Methods:

a) Preparation of Brain-Heart Infusion Broth:

Thirty-seven grams of the medium were suspended in one liter of distilled water. Heating with frequent agitation ensured good mixing and dissolution. The suspension was then boiled for one minute until complete dissolution. It was later dispensed into appropriate containers and sterilized at 121°C for 15 minutes. The prepared medium was stored at 2-8°C. For best results, the medium was used on the same day.

b) Preparation of Brain-Heart Infusion Agar:

Fifteen grams of agar powder were added to 1 liter BHI broth and then heated to dissolve agar before dispensing into appropriate containers. Autoclaving was then performed for 15 min at 121°C to ensure adequate sterilization. The mixture was then poured into Petri dishes, and left to cool and solidify.

c) Preparation of Enterococcus Faecalis Suspension:

A sterile swab was used to transfer bacterial growth from the primary culture into the BHI broth bottle and mixed well to form a homogenous suspension.

d) Classification of samples:

21 samples were classified according to the tested material into 3 groups;

Group 1: Consisted of 7 holes filled with Nanocalcium hydroxide mixed with saline.

Group 2: Consisted of 7 holes filled with conventional calcium hydroxide mixed with saline

Group 3: Consisted of 7 holes filled with calcium hydroxide paste with iodoform

Each group was further divided into 3 subgroups according to the observation period,

Subgroup A: One day.

Subgroup b: Three days.

Subgroup c: Seven days.

e. Implantation of the E-faecalis in the agar plates:

A sterile cotton swab was dipped into the suspension and excess fluid was removed by turning the swab against the inside wall of the tube. The inoculum was evenly spread over the entire surface of dry BHI agar plates by swabbing in three different directions.

f) Mixing and application of the tested substances:

Three holes of 4 mm diameter were made on the agar surface by a metal punch leaving about 10-15

mm away from the edge of the petri dish. These holes were separated from each other by a distance not less than 20 mm to avoid overlapping zones of inhibition.

Each hole contained one of the tested materials and marked as follow, Hole number I for Nanocalcium hydroxide past, hole number II for conventional calcium hydroxide and hole number III for calcium hydroxide paste with iodoform.

All the agar plates were incubated at 37°C in aerobic conditions for the required observation periods.

g) Method of evaluation:

After one day observation the area of microbial growth inhibition (lack of bacterial colonization) around the holes were measured at the largest diameter with a poly gauge millimeter ruler. The agar plates were then re-incubated to take the measurements of the inhibitory zones after 3 days and then after 7 days.

h) Statistical analysis

Data were analyzed by SPSS software (version 16.0, SPSS, Chicago, IL, USA). Data in each group were compared by the ANOVA and Kruskal-Wallis tests. Also the Dunnett's test was performed to compare the results between two groups. The level of significance was set at 0.05.

RESULTS

A-Group one (Nanocalcium hydroxide):

The average of the diameter of the inhibitory zone was 18mm after one day, which was not changed after 3 or 7 days.

B-Group two (Conventional calcium hydroxide):

The average of the diameter of the inhibitory zone was 17mm after one day, which was steady over the whole observation periods.

C-Group three (calcium hydroxide pastes with iodoform):

The average of the diameter of the inhibitory zone was 16mm after one day

Which was not changed after 3 or 7 days.

Table (1) The diameter of the inhibitory zones of tested materials .

Group Subgroup	Nano $Ca(OH)_2$	Conventional $Ca(OH)_2$	$Ca(OH)_2$ paste with iodoform	P value
One day	18 mm	17 mm	16 mm	<0.0001
3 days	18 mm	17 mm	16 mm	<0.0001
7 days	18 mm	17 mm	16 mm	<0.0001

P≤ 0.05 is considered significant.

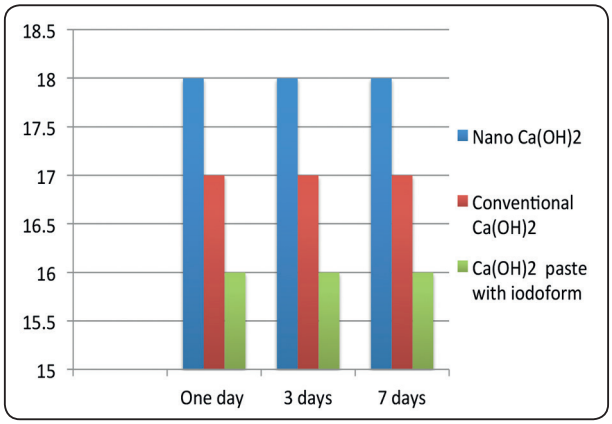

Fig. (1) Histogram showing the diameter of the inhibitory zones of the tested materials.

DISCUSSION

The main purpose of endodontic treatment is the elimination of micobiota from the root canal system, which is the main challenge for all the endodontic practitioners. Sporadic species of bacteria affecting the root canal system have low virulence impact however collectively they have high virulence impact [4,5]. Estrela et al.[1] Suggested the hypothesis of an irreversible inactivation of bacterial enzymes under extreme condition of pH for long period of

time and also a temporary bacterial enzymatic inactivation with the restoration of normal activity when the pH returns to the ideal level. Estrela et al.[3] Suggested the mechanism of action of calcium hydroxide through the release of hydroxyl ions that causing changes in the transport of nutrients and structure of organic component causing bacterial destruction. Lima et al.[6] concluded that all calcium hydroxide medicaments were able to reduce colony forming unit (CFU) values of E faecalis. Chai et al.[7] also concluded that calcium hydroxide is 100% effective in eliminating E faecalis biofilm. Iodoform is composed of some powder with bright hexagonal crystals, it decomposes releasing iodine in nascent state [1]. Compounds containing iodine are employed for infection control in dentistry and high radio-opacity for calcium hydroxide. Nano calcium hydroxide has smaller size and high surface area that enables it to penetrate into the deeper layers of dentinal tubules and being more efficient in elimination of E faecalis[8]. Several studies proved that the bacterial penetration into the depth of 300 to 1500 micrometers in dentinal tubules in these depths the bacteria remain inaccessible for conventional irrigants, medicaments and sealers [9,10]. Nano particles are microscopic particles with dimensions less than 100 nanometers with different properties such as active surface area, chemical and biological reactivities[11]. The higher surface to volume ratio and charge density of these materials result in their greater interaction with the environment and thus cause a higher anti bacterial activity [12-13] . Agar diffusion test is a widely used test with reproducible results for the evaluation of the antimicrobial activity [8]. It is able to demonstrate the activity of freshly mixed intra canal medications. However, it has many limitations as it lacks the ability to test and compare the viability of microorganisms used and the inability to distinguish between the bactericidal and bacteriostatic ability of tested materials [14]. The results of this study showed no privilege of iodoform as an additive to calcium hydroxide on the zone of inhibition of E-faecalis. This finding was in agreement with Filhoetal. [15] . Nanocalcium hydroxide showed superior results through the observation periods for the major diameter of zone of inhibition of E-faecalis growth as compared with conventional calcium hydroxide and calcium hydroxide paste with Iodoform was in agreement withDianatetal. [8]

CONCLUSION

Calcium hydroxide Nano particles have superior anti microbial activity against E-faecalis compared to conventional calcium hydroxide and calcium hydroxide mixed with iodoform in culture media.

REFERENCES

1- Estrela C, Estrela C, Hollanda A, Decurcio A, Pécora J. Influence Of Iodoform On Antimicrobial Potential Of Calcium Hydroxide. J ApplOral Sci. 2006;14(1):33-7

2- Estrela C, MamedeNeto I, Estrela CRA, Pécora JD. Evaluation of density of calcium hydroxide pastes in dog's mandible. BrazEndod J. 1998;3:24-30.

3- Estrela C, Sydney GB, Bammann LL, Felippe O Jr. Mechanism of the action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J. 1995;6:85-90.

4- Estrela C, Bammann LL, Pimenta FC, Pécora JD. Control of microorganism in vitro by calcium hydroxide pastes. IntEndod J. 2001;34:341-5

5- Estrela C, Holland R. Calcium Hydroxide: study based on scientific evidences. J Appl Oral Sci. 2003;11:269-82.

6- Lima R, Tanomaru J, nior N. and M. Filho T. Effectiveness of calcium hydroxide-based intracanal medicaments against Enterococcus faecalis. Int. Endod. J, 2012; 45:311–6.

7- Chai W.L., Hamimah H., Cheng S. C., Sallam A.A. Abdullah M. Susceptibility Of Enterococcus Faecalis Biofilm To Antibiotics And Calcium Hydroxide. J. Oral Sci. 2007;49:161-6.

8- Dianat O, Saedi S , Kazem M , Alam M. Antimicrobial Activity of Nanoparticle Calcium Hydroxide against Enterococcus Faecalis: An In Vitro Study. I.E.J. 2015; 10:39-43

9- Calt S, Serper A. Dentinal tubule penetration of root canal sealers after root canal dressing with calcium hydroxide. J Endod. 1999;25:431-3.

10- Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res. 1987;66:1375-9.

11- Allaker RP, Memarzadeh K. Nanoparticles and the control of oral infections. Int J Antimicrob Agents. 2014;43:95-104.

12- Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod. 2014;40:285-90.

13- Kishen A, Shi Z, Shrestha A, Neoh KG. An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. J Endod. 2008; 34:1515-20.

14- Mazyony S. Batouty K. Elfaramawy M.T. evaluation of antimicrobial effect of three resin based sealers. MSc. Thesis Endodontic Department Ain Shams Uni. 2016.

15- Filho S, Soares A, Vianna M, Zaia A, Ferraz C Gomes B. Antimicrobial Effect and pH of Chlorhexidine Gel and Calcium Hydroxide Alone and Associated with other Materials. *Braz Dent J 2008 19: 28-33*.