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Research Article

COMPARATIVE EVALUATION OF THE ANTIFUNGAL EFFICACY OF SILVER NANOPARTICLES AND 3% SODIUM HYPOCHLORITE AGAINST CANDIDA ALBICANS WHEN USED AS ROOT CANAL IRRIGANTS –AN INVITRO STUDY

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A B S T R A C T

Introduction: Residual microorganisms in the root canal system after endodontic therapy such as Candida albicans are a major cause of endodontic failure. In this study new nanobased irrigant silver nanoparticles is used as root canal irrigant and its efficacy is compared with sodium hypochlorite which is considered as a gold standard root canal irrigant against candida albicans.

Materials and Methodology: Thirty extracted human single rooted teeth were collected and decoronated. Biomechanical preparation was done and root surfaces were coated with nail varnish. Teeth were sterilized in autoclave. Specimens were inoculated with candida albicans strain and incubated for 3 days and were randomly divided into 3 groups. The specimens were irrigated as follows GROUP A- 0.9 % saline, GROUP B- 3% sodium hypochlorite (NaOCl), and GROUP C-0.005% silver nanoparticles (AgNP). Aliquots from experimental teeth were transferred onto sabourauds dextrose agar using H files and colony forming units were counted.

Results: The data was statistically analyzed with Willcoxon signed rank test and Mann Whitney test. There was a statistical significant reduction of candida albicans when NaOCl and AgNP were used as irrigant. But when intergroup NaOCl and AgNP were compared, there was no statistical significant difference.

Conclusion: The results obtained concluded that 0.005% AgNP is equally efficient as 3% NaOCl.

INTRODUCTION

Microorganisms are the main causes of pulp and periapical diseases. Hence, root canal treatment mainly focuses on thorough elimination of these microorganisms from the complex three dimensional root canal system. Yeasts can be detected in 7-17% of infected root canals. Among yeasts candida albicans is the most commonly isolated organism from the root canals.[1] A variety of virulence factors enable C. albicans to adhere to and penetrate into dentine. C. albicans has been associated with root canal infections resistant to non-surgical therapy and is considered as a potent pathogen to infect periapical lesions. They are commonly associated with primary and persistent cases of apical periodontitis. They are capable of forming biofilms and are resistant to commonly used root canal irrigants.[2,3] Many irrigating solutions have been used for root canal treatment along with mechanical instrumentation to achieve better debridement.

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However, because these solutions act through direct contact with the targets and have a limited penetration depth into irregularities of root canal walls, they are unable to eliminate microorganisms from deeper layers of the dentin. Sodium hypochlorite is probably the most widely used irrigant in endodontics. It is a potent disinfectant, killing microorganisms in concentrations between 0.5% and 5%, in clinically relevant periods of time. It has a wide antimicrobial spectrum, and is active against C. albicans.[4] But negative findings regarding toxicity prompted recommendations to dilute 5.25% NaOCl to lower concentrations. Diluting 5.25% NaOCl reduces antimicrobial properties.[5] Instrumentation and irrigation with sodium hypochlorite could eliminate bacteria in 50 to 75% of infected root canals at the end of the first treatment session.[6] Moreover, Nair *et al*. (2005) concluded that even after instrumentation and irrigation with NaOCl in 88% of cases, infection in the root canals could be detected after obturation in one-visit treatment.[7]Nanotechnology is an important field of modern research dealing with the particles size ranging from approximately 1-100 nm.[8] Among the most promising nanomaterials with antibacterial properties are metallic nanoparticles, which exhibit increased chemical

activity due to their large surface to volume ratios of which silver nanoparticles has the highest antimicrobial activity.[9] Silver nanoparticles have been demonstrated to have broad spectrum bactericidal, fungicidal and virucidal activity. Recent studies show that silver nanoparticles are effective against candida albicans.[10] There were only few studies comparing the antifungal efficacy of silver nanoparticles and sodium hypochlorite and hence aim of the present study is to compare the antifungal efficacy of silver nanoparticles and 3% sodium hypochlorite when used as root canal irrigant.

METHODOLOGY

Thirty extracted human single rooted teeth were selected and stored in saline until use. Teeth were decoronated with diamond disc under copious water irrigation to obtain a standardized root length of 15 mm from apex. Working length was determined 0.5 mm short of apex. Biomechanical preparation was done by step back technique upto size 50 k file. During instrumentation 17% EDTA and 3% sodium hypochlorite was used to remove smear layer. All the teeth were finally irrigated with 5 ml of 0.9% Normal Saline to wash out the residual irrigants from the root canal. Then all the specimens were sterilized in an autoclave at 121^0c for 15 min. Root apices were sealed with sticky wax and were coated with nail varnish with specific color coding for group identification. All microbial procedures were performed under aseptic conditions, in laminar flow chamber. Candida albicans was previously cultured in sabouraud's dextrose agar medium. Microbial suspension was prepared to match turbidity of $1.5x10^8$ cfu /ml (equivalent to 0.5 Mc. Farland standard). 10µl of microbial suspension was inoculated into each root canal with automated micropipette (Fig 1) and cervical openings were sealed with modelling wax. All the teeth were suspended in eppendorf tubes and incubated at 37^0 c for 72 hrs (Fig 2). All the teeth were replenished with fresh candida albicans for every 24 hrs. After this period of incubation confirmation of contamination of Candida was done as follows 1) direct visualization which appeared as creamy/white colored, smooth pasty colonies. 2) CFU/ml.

Fig 1 Inoculation of microorganisms using automated micropipette

Fig 2 Incubator

Grouping was done and Samples were divided into 3 groups. Samples were irrigated with respective irrigants using 5 ml syringe. GROUP A: Irrigated with 5 ml of 0.9% saline for 3 min (Fig 3). GROUP B: Irrigated with 5 ml of 3% sodium hypochlorite for 3 min (Fig 4). GROUP C: Irrigated with 5 ml of 0.005% silver nanoparticles for 3 min (Fig 5).

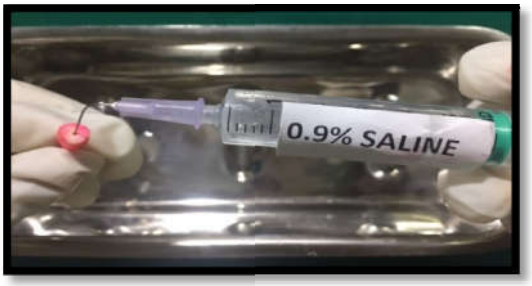

Fig 3 Group 1 – Saline irrigation

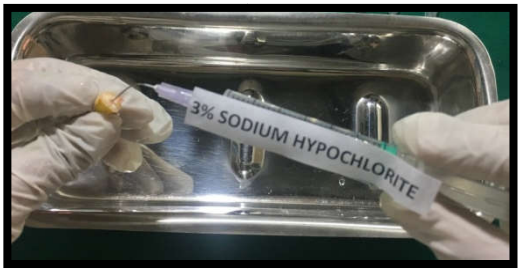

Fig 4 Group 2- Sodium hypochlorite irrigation

Fig 5 Group 3- Silver nanoparticles

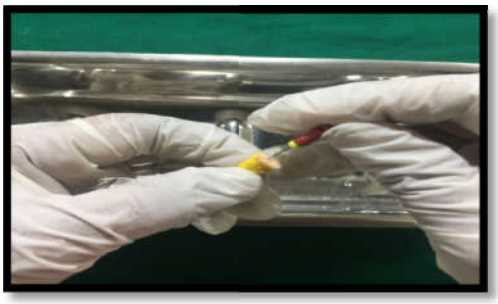

Fig 6 Dentinal shavings collected with H files

Fig 7 Cyclomixer

All the samples were finally irrigated with 5ml of 0.9% saline to remove the residual irrigants and were dried with paper points for 1 min. Sampling is done with 35# H file (Fig 6). Dentinal scrapings were collected from all root canals using H-files and transferred into eppendorf tubes containing 1 ml of peptone water which is the nutrient medium for growth of microbes. Then the tubes were subjected to agitation for 1 min in cyclomixer (Fig 7). Aliquots of 0.1 ml were seeded into petridishes containing sabouraud's dextrose agar medium and streaking was done with microloops and incubated at 37ºC for 24 hrs. After this period microbial growth was measured by CFU/ml.

RESULTS

The data was statistically analyzed with Willcoxon signed rank test and Mann Whitney test. Mann Whitney test for intergroup comparison and Willcoxon signed rank test for intragroup comparison. A 'p' value of 0.05 or less was considered as statistically significant. The initial sample revealed similar CFU/ml for all the groups. The p value in Group B (NaOCl) and Group C (AgNP) was 0.028 indicating a statistical significant reduction of cfu (Table 1). The p value when Group B and Group C compared with control group (Group A) was 0.004 which indicates statistical significant difference. p value when Group B and Group C are compared with each other after irrigation was 0.17 indicating no statistical significant difference (Table 2) which indicates both are equally effective.

DISCUSSION

One of the goals of root canal treatment is to completely disinfect the root canal system three dimensionally. Use of mechanical instrumentation alone cannot sufficiently debride and clean this complex tubular network. Hence use of topical antimicrobials like irrigants and medications are suggested for effective elimination of bacteria within the root canals. The current level of evidence showed that despite the advancements in treatment strategies, the success rates have not increased for the past four to five decades. This could be mainly attributed to the limitations of current technologies to deal with the challenges of the disease process. Even today in the ongoing war against antibiotics, the bacteria seem to be winning, and the drug pipeline is verging on empty. It is important for treatment options to possess a 'nip-in-the-bud' approach where key pathogens are specifically suppressed. Hence in this study nanotechnology has been tried where it has the ability to interfere with biofilm formation and also has less bacterial resistance.

Table 1 Willcoxon signed rank test

	Group A	Group B	Group C
Pretest	155.0±7.74	154.16±6.64	153.33±7.53
Post test	150.83±7.36	9.833±3.25	11.33±3.20
Z value	1.89	2.201	2.201
P value	0.059	0.028	0.028

Table 2 Mann whitney test

	P values	P values
	Pre test	Post test
group A vs group B	0.799	0.004
group A vs group C	0.737	0.004
group B vs group C	0.865	0.17

In this study antifungal efficacy of nanosilver is compared with sodium hypochlorite against candida albicans. Candida albicans is a pleomorphic and versatile organism. A variety of virulence factors favor adherence and tissue penetration of candida albicans into dentinal tubules. The various virulence factors include property of thigmotropism, production of proteolytic enzymes and biofilm formation. Candida albicans has the ability to tolerate harsh environmental conditions like extremes of pH and nutrition depreivation.[10,11]

For comparing the antimicrobial efficacy of the new nano based irrigant, Sodium hypochlorite (NaOCl) was selected as it is considered as the gold standard and is still the most widely used root canal irrigant. It is employed in different concentrations and higher concentrations are more potent in eliminating microorganisms, but increased concentration can also increase toxicity. Sodium hypochlorite acts by saponification reaction with fatty acids, amino acid neutralization and chloramination reaction with amino acids. It has a high ph (>11) which interferes with the cytoplasmic membrane integrity.[4,12]

In this study silver nanoparticles were used as various studies show that silver nanoparticles show broad spectrum antimicrobial activity. Silver nanoparticles of <10 nm was used as smaller silver nano-particles show stronger and better bactericidal effect than larger particles as they have a larger surface area for interaction.[13,22] Lara *et al* showed that silver nanoparticles were potent candida biofilm inhibitors. Silver nanoparticles interact with protein thiol groups and membrane bound enzymes. They interact with phosphorous and sulfur containing compounds such as DNA and cell membrane and disrupts the cell membrane causing pits and pores and causing ion leakage .They interfere with respiratory chains.[14] In a study loefti *et al* compared the antibacterial efficacy of silver nanoparticles with NaOCl and CHX at different concentrations against E faecalis. Their findings found that decrease in Nanosilver concentration led to decrease in antibacterial properties but at different rates when compared with NaOCl. They found that 0.005% nanosilver has antibacterial effect similar to 5.25% NaoCl.[15] Hence in this study 0.005% silver nanoparticles were used. There were only few studies comparing the antifungal efficacy of Nanosilver and NaoCl against candida albicans. In a study by Mohammad *et al* showed that calcium hydroxide and CHX has superior antifungal activity when compared to silver nanoparticles when used as intracanal medicament.[16] In this study 0.005% silver nanoparticles showed equal efficacy as 3% sodium hypochlorite.

There were various studies showing that silver nanoparticles are effective against Enterococcus faecalis. Abbaszadegan *et al* has conducted a study to test positively charged imidazolium based ionic silver nanoparticles against E faecalis. This study concluded that silver nanoparticles have promising antibacterial activity against E faecalis.[17] A study by Samiei *et al* has showed that silver cross linked hydrogel nanocomposite has sustained antibacterial property and low toxicity when compared to chlorhexidine and sodium hypochlorite solutions.[18] Joao *et al* has conducted a study where he has synthesized and evaluated the antimicrobial properties of silver nanoparticles to be used in root canal formulations. This study has showed that silver nanoparticles are potential antiseptic agents to be used in root canals.[19] Gonzalez-Luna *et al* has determined the bactericidal effect of silver nanoparticles, sodium hypochlorite, silver nanoparticles + EDTA and sodium hypochlorite + EDTA. They concluded that silver nanoparticles and sodium hypochlorite has antibacterial

efficacy with no significant difference between them. They also tested the smear layer removal ability of the irrigants and concluded that silver nanoparticles have smear layer removal capacity comparable to EDTA.[20] This is an additional advantage of silver nanoparticles.

In a study Kim *et al* has tested the oral toxicity of silver nanoparticles over a period of 13 weeks (90 days) in rats. He found a NOAEL (no observable adverse effect level) of 30 mg/kg and LOAEL (lowest observable adverse effect level) of 125 mg/kg.[21] Gomes *et al* has conducted a study comparing the biocompatibility of silver nanoparticles with 2.5% NaOCl to the rat tissues when used as root canal irrigant and found that after 30 days the reaction of silver nanoparticles was mild similar to NaOCl indicating both are equally biocompatible.[22]
By this study it can be concluded that 0.005% silver nanoparticles possess equal antifungal efficacy as 3% sodium hypochlorite and can be used as an alternative root canal irrigant. Moreover silver nanoparticles are biocompatible in lower concentration, possess less bacterial resistance and also have ability to remove smear layer. But various other aspects like cost effectivesness, not discoloring the tooth, tissue dissolving ability also should be compared and weighed against sodium hypochlorite which requires further research.

References

1. Priyanka Ghogre. Endodontic Mycology. A new perspective of root canal infection. *J Dent Res.* Jan Mar 2014:2(1).
2. T. Waltimo, Markus haapasalo, Matthias zehnder, Ju rg meyer. Clinical aspects related to endodontic yeast infections. *Endod Topics*. 2004:9;66–78.
3. Saketh Rama rao, yoshaskam agnihotri, pavithra bai, prabeesh padmanabhan, sambrata das. Isolation of fungal hyphae in periapical infections. *Journal of Indian academy of oral medicine and radiology*. jul-sep. 2013:25(3); 221-224.
4. Zahed mohammadi and Sousan shalavi. Antimicrobial activity of sodium hypochlorite in endodontics. *J Mass Dent Soc.* 2013.
5. Mohammadi Z. Sodium hypochlorite in endodontics. An update review. *Int Dent J.* 2008: 58;329-41.
6. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* . Feb 2005;99(2):231-52.
7. A. Byström and G. Sunvqvist. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J.* Jan 1985:18(1);35-40.
8. Hassan Korbekandi and Siavash Iravani. *Silver Nanoparticles-Delivery of nanoparticles*.
9. Jolanta Pulit, Marcin Banach, Renata Szczygłowska and Mirosław Bryk. Nanosilver against fungi. Silver nanoparticles as an effective biocidal factor. 2013:60(4);795-798.
10. Jose. F. Siqueira, Isabella N, Helio P. Lopes, Carlos N. Elias, and Milton de Uzeda. Fungal infection of the radicular dentin. Nov 2002:28(11).
11. Waltimo, Markus Haapasalo, Matthias Zehnder & Ju Rg Meyer. Clinical aspects related to endodontic yeast infections. *Endod Topics*. 2004:9;66-78.
12. Carlos Estrela, Cyntia. Estrela, Eduardo Barbin, Julio Cesar, Melissa A. Marchesan, Jesus D. Pecora. Mechanism of action of sodium hypochlorite. *Braz Dent J.* 2002:13(2);113-117.
13. Rene Garcıa, Liliana Argueta, Cynthia Mejıa, Rocio Jimenez-Martinez, Sahamanta Cuevas, Paola Ariselda *et al*. Perspectives for the use of silver nanoparticles in dental practice. *Int Dent J.* 2011:61;297-301.
14. Humberto H. Lara, Dulce G. Romero, Christopher Pierce, Jose L. Lopez, M. Josefina, Miguel Jose. Effect of silver nanoparticles on Candida albicans biofilms: an ultrastructural study. *J Nanobiotechnol*. 2015:13:91.
15. Mehrdad Lotfi, Sepideh Vosoughhosseini, Bahram Ranjkesh, Sajjad Khani, Mohammadali Saghiri, Vahid Zand. Antimicrobial efficacy of nanosilver, sodium hypochlorite and chlorhexidine gluconate against Enterococcus faecalis. *Afr. J. Biotechnol*. Jul 2011:10(35);6799-6803.
16. Mohammad Ali Mozayeni, Ali Hadian, Pedram Bakhshaei, Omid Dianat**.** Comparison of antifungal Activity of 2% Chlorhexidine, Calcium Hydroxide, and Nanosilver gels against Candida Albicans**.** *J Dent.* February 2015:12(2).
17. A. Abbaszadegan, M.Nabavizadeh, A Gholami, Z.S. Aleyasin, S. Dorostkar *et al*. Positively charged imidazolium – based ionic liquid protected silver nanoparticles : a promising disinfectant in root canal treatment. *Int Endod J.*2014.
18. Samiei, Davaran S, Valipour F , Davari A , Ghiasian T *et al* . Antibacterial efficacy of silver -crosslinked hydrogel nanocomposite versus sodium hypochlorite and chlorhexidine on Enterococcus faecalis for use in root canal infection. *Int J Biol Pharm Allied Sci.* Nov 2014: 3 (11); 2316-2332.
19. Joao Felipe, Yara Teresinha, Guimaraes Lara, Andre Pitondo, Andrea Marcia, Carlos Eduardo. Development of intracanal formulation containing silver nanoparticles. *Braz Dent J* 2014:25(4);302-306.
20. Gonzalez-Luna, Gabriel-Alejandro, Martinez-Castanon, Norma-Veronica, Zavala-Alonso, Nuria Patino *et al*. Bactericidal Effect of silver nanoparticles as a final irrigation agent in endodontics on Enterococcus faecalis: An Ex Vivo Study. *J Nanomat*. 2016.
21. Yong Soon Kim, Moon Yong, Jung Duck, Kyung Seuk, Hyeon Ryol, Yong Hyun. Sub chronic oral toxicity of silver nanoparticles. *Part Fibre Toxicol*. 2010:7(20).
22. Joao Eduardo, Fernando Oliveira, Simone Watanabe, Karina Vanessa, Luana Godoy, Sara Vieira. Evaluation of silver nanoparticles as irrigating solution. *Dental Press Endod.* May-Aug. 2013:3(2);16-23.