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Antibacterial Efficacy of Chitosan Nanoparticles-Incorporated with Different Endodontic Irrigation Solutions: An In-Vitro Study

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Aim: The Purpose of this investigation was to evaluate antimicrobial effectiveness of two concentrations of nanochitosan (NC 0.2%, 0.5%) combined with sodium hypochlorite (NaOCl 5.25%) and chlorhexidine (CHX 2%).
Materials and methods: Seventy two ,without caries, single rooted mandibular first premolars with developed apices extracted for orthodontic purposes used. The roots of all teeth will be sectioned 14mm from the tip of the root. The entire root surface was covered by two thin layers of nail polish, and the apical ends of the roots were sealed with flowable composite. All samples were prepared using the Protaper Ni-Ti rotary system.Ten microliters (10 µl) of Enterococcus faecalis suspension were injected inside root canals and incubated for 48 hours.Roots were divided at random into nine groups (n = 8).Group I: NC 0.2%, Group II: NC 0.5%, Group III: NaOCl 5.25%, Group IV: CHX 2%, Group V: NC 0.2% + NaOCl 5.25%, Group VI: NC 0.5% + NaOCl 5.25%, Group VII: NC 0.2% + CHX 2%, Group VIII: NC 0.5% + CHX 2%, Group IX: Distilled Water(D.W.). After disinfecting the canal, intracanal bacterial samples were collected and counted in order to establish the number of colony-forming units (CFUs).
Result: According to the study, a statistically significant difference was observed in mean of CFUs of the experimental groups and control group. Highest antibacterial activity was when NC was mixed with NaOCl.
Conclusion: This research demonstrates that there is synergistic antimicrobial activity when NC irrigation solution (0.2%, 0.5%) is mixed with NaOCl 5.25% and CHX 2%.

Keywords: Antibacterial Efficacy, Nanochitosan, Enterococcus Faecalis, Chlorhexidine, Endodontic Irrigation.

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Introduction

The main goal of endodontic therapy is to eventually clear the root canal cavity. This mostly depends on successfully eliminating the bacterial biofilm.[1]

A variety of microbial flora, including spirochetes, filaments, cocci, rods, and fungi , are present in infected root canals. *Enterococcus faecalis* is responsible for a considerable percentage of clinical treatment failures and recognized as a primary origin of secondary root infection. It exhibits high resistance to antimicrobial agents used throughout management.[2] A number of laboratory investigations evaluating *E. faecalis's* susceptibility to endodontic therapy revealed the bacteria's strong resistance to antibiotics. Moreover, *E. faecalis* can endure under extremely severe conditions with a limited nutrition supply and a high pH(11.5). The ability of *E. faecalis* to multiply as a mono-infection in treated canals and as biofilm on walls of root canal without the aid of other bacteria is the cause of high resistance to root canal therapy.[3] The major root canal's microbial population is decreased and the bacterial biofilm is effectively disrupted by the instrumentation. Nonetheless, to maximize the cleaning and disinfection of canal system, the aid of irrigation with chemical materials is required.[4] In addition to their antibacterial properties, which promote better adherence and penetration of root canal sealants and obturation materials, irrigations have a significant function in eliminating debris and smear layers from root canal. The most commonly utilized irrigation solutions in root canal treatment include sodium hypochlorite, chlorhexidine, and EDTA. Each has limitations and drawbacks, such as toxicity, allergic response, and dentin erosion, so search for new materials with stronger antibacterial effectiveness and fewer adverse effects is continuing in the field of endodontic.[5] Numerous studies have evaluated new irrigants that are both more effective in their disinfection properties and less irritating to periapical tissues. These studies have explored various natural substances such as herbal solutions, propolis, and chitosan, as well as antibacterial nanoparticles. These alternative substances are believed to possess comparable antibacterial efficacy to NaOCl, lower toxicity, and reduced irritation.[6]

Chitosan is a multifunctional biopolymer , can be produced as powder, capsules, films, or beads. It possesses a potent ability to bind various metal ions in acidic environments; chitosan has a broad range of antimicrobial properties.[7] It was found that nano-chitosan solution have effective antibacterial efficacy against *E. faecalis* and inhibiting the growth of biofilms. Another study, found that while planktonic bacteria were fully removed, biofilm bacteria endure after 72 hours, suggesting that the antibacterial efficacy of the solution may rely on the state of the bacteria.[8]

Thus, the aim of the investigation was to examine the antimicrobial efficiency of various irrigation solutions. The research adopted the null hypothesis which there would be no difference in antibacterial efficacy between irrigation solutions.

Materials and Methods

Sample Size Calculation

The predicted sample size was a total of (72) samples (which mean 8 samples for each group). The statistical calculation of sample size was performed using G*power analysis 3.1.9.4 using an alpha(α) level of 0.05, beta (β) level of 0.95 and an effect size (f) of 0.564.

Sample Preparation

This research will use a total 72 single-rooted teeth that will be extracted for orthodontic purposes. The teeth were taken from the University of Mosul/College of Dentistry. Ethical consent for the use of excised human teeth was obtained according the research guidelines followed at the University of Mosul/College of Dentistry (Code: UoM.Dent.23/27, Date:

28/11/2023). Mandibular premolars with single root canals and mature apices will be selected. The roots of all teeth will be sectioned (14mm from the tip of the root). Nail polish will be applied to the external surface of the roots for preventing bacterial penetration and material diffusion into the dentin. After that, root end will be sealed by flowable composite (COM N FLOW Nanohybrid, Hysbor, India) to avoid bacterial and irrigation leakage. K-File No.10 (Denco Medical Co., Shenzhen, China) will be pushed into the canal until the tip is visible from root apex in order to guarantee patency. The root canal will be mechanically instrumented by a ProTaper (NiTi) rotary file system (Denco Medical Co., Shenzhen, China) up to size F3. Normal saline was used as an alternative irrigant during biomechanical preparation. Teeth were sterilized using an autoclave at a temperature of 121ºC for 20 minutes.

Inoculation of *Enterococcus faecalis*

The microbial suspension was prepared by a standard strain of *E. faecalis* , which was obtained from the microbiology laboratory at the University of Mosul/College of Dentistry, isolated and identified by Vitek 2 system. The bacterium was cultured on M Enterococcus agar for 18 hours, a single colony was picked by loop and cultivated in a brain heart infusion broth (BHIB) (Hi Media, Wagle Industrial Area, India) for 18 hours at 37˚ C, turbidity of the broth were adjusted to be equal to tube 0.5 McFarland (1.5×10^8) cell. About 10µl of bacterial adjusted suspension was used to contaminate the root canals in a laminar flow cabinet by using a micropipette and then incubated at 37ºC for 48 hours.[9]

Preparation of Nano-chitosan irrigation solution (0.2%, 0.5%)

The 0.2% and 0.5% nano-chitosan irrigation was prepared by dissolving 0.2 and 0.5 gm of the chitosan powder, respectively, in 100 ml of distilled water and 1% acetic acid, stirred for two hours using magnetic stirring machine at room temperature until a crystalline homogenous solution (PH =4 , measured by a digital PH meter).[10]

Irrigation of Specimens

*** Specimens were categorized into nine groups(n= 8) which were:**

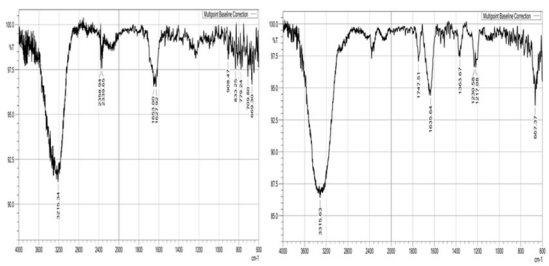
Group I: Nano-chitosan(NC) 0.2%.
Group II: Nano-chitosan(NC) 0.5%.
Group III: NaOCl 5.25%.
Group IV: CHX 2%.
Group V: Nano-chitosan(NC) 0.2%+ NaOCl 5.25%.
Group VI: Nano-chitosan(NC) 0.5%+ NaOCl 5.25%.
Group VII: Nano-chitosan(NC) 0.2%+ CHX 2%.
Group VIII: Nano-chitosan(NC) 0.5%+ CHX 2%.
Group IX: D.W.

***Fourier-transform infrared spectroscopy (FTIR)**

Fourier transform infrared (FTIR) has been established for identifying organic content (such as protein, carbohydrate, and fat) and organic components (such as chemical bonds).[11]

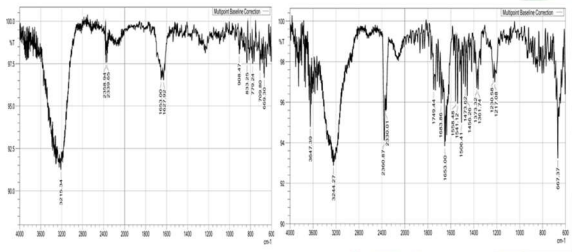
Nanochitosan irrigation solution was mixed in an equal amount (1:1) with NaOCl 5.25% (Aqua Medical, Sultangazi, Istanbul, Turkey) and CHX 2% (Microvem, Akyazi, Turkey) for 10 minutes by using a magnetic stirrer device.

The resulted mixture of nanochitosan with 5.25% NaOCl was characterized using IR spectroscopy. Consequently, it was confirmed that an oxidation of (OH) group to aldehyde group (CHO) was occurred after the mixing. Where, a band at (1747 cm^{-1}) showed a present of a carbonyl group which meant that a reaction between the two solutions was happened as in Figure (1).While there was no reaction occur between nanochitosan and 2% CHX after mixing them, where IR spectrum clearly showed a mixture of the two solutions without any reaction as showed in Figure (2).



A B

Figure 1: A- FTIR result of NC 0.5% , B- FTIR result of NC 0.5% + NaOCl 5.25%



A B

Figure 2: A- FTIR result of NC 0.5%, B- FTIR result of NC 0.5% + CHX 2%

*Irrigation protocol

Three ml of each irrigating solution was placed in root samples by using a disposable irrigating syringe with an irrigation needle(gauge 30) for one minute and allowed to remain in root canal for 3 minutes.

Sampling after irrigation

After single paper point dryness, 3 sterile paper points for each tooth were inserted in root canal for 60 seconds, then they were put in a sterile Eppendorf tubes with BHIB.Vortexing has been done for all samples and 100 microliters of liquid media has been inoculated on M. enterococcus selective media(Hi Media, Wagle Industrial Area, India). The samples then incubated (at 37°C) for 48 hours and (CFUs) has been calculated according to this equation.[12]

Number of colonies (CFUs/ml) = (number of colonies * total dilution factor)/volume of culture plated in ml.

Statistical Analysis

It has been carried out by "SPSS software" (SPSS version 20, IBM, USA). Descriptive statistics of bacterial counts have been represented as mean and standard deviation. '' One-way ANOVA " and " Duncan's multiple range " tests utilized to compare antibacterial efficacy of tested endodontic solutions at significance level ($p \leq 0.05$) .

Results

Mean and standard deviation between different experimental groups were computed, and results of this study are listed in Table (1) and illustrated in Figure (3).

Table 1: Descriptive statistics and Duncan's multiple range test for bacterial count after final irrigation by tested solutions.

Treatment group	N	Mean	SD	Duncan Test	P-value	
NC 0.2%	8	1040.00	270.19	D	0.000*	Sig. difference
NC 0.5%	8	820.00	192.35	C		
NaOCl 5.25%	8	68.50	31.14	E		
CHX 2%	8	2200.00	320.94	B		
NC 0.2%+NaOCl 5.25%	8	62.00	29.87	E		
NC 0.5%+NaOCl 5.25%	8	46.00	27.93	E		
NC 0.2%+CHX 2%	8	158.00	54.50	E		
NC 0.5%+CHX 2%	8	152.00	52.63	E		
DW	8	204000.00	28809.72	A		

Significance level $p \leq 0.05$, * significant, different letter refer to significant difference between groups at ($P \leq 0.05$).

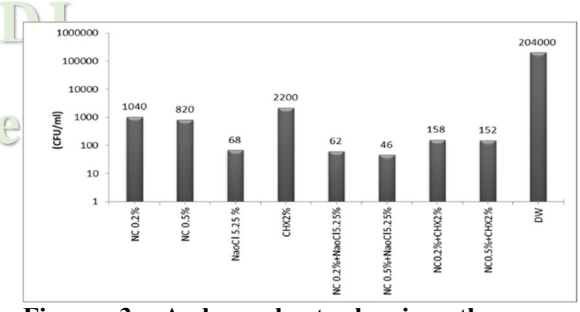


Figure 3: A bar chart showing the mean difference of bacterial count after irrigation by tested solutions.

It showed a statistically significant difference among tested groups at $P \leq 0.05$. The least number of CFUs was found in NC 0.5%+NaOCl 5.25%, followed by NC 0.2%+NaOCl 5.25% and NaOCl 5.25%.

Discussion

The oral cavity contains more than 700 types of bacteria, with each individual containing 100–200 of these species. Historically, the main pathogens in endodontic infections were believed to be the black-pigmenting bacteria of the genera Bacteroides, Prevotella, Enterococcus, and Porphorymonas. Nonetheless, due to its unique combination of virulence characteristics, *E. faecalis* was chosen as the test bacterium since it is the facultative anaerobe most frequently linked to root canal failure cases and persistent apical periodontitis.[13]

This investigation assessed antimicrobial efficacy of several irrigants on *E. faecalis* as well as the effect of mixing of irrigations on the reduction in bacterial count.

The majority of the characteristics of an ideal irrigant are fulfilled by NaOCl, the most widely utilized endodontic irrigant. The processes of saponification , amino acid neutralization and chloramination which take place in existence of microbes and organic tissue provide antimicrobial effect and tissue dissolving process. In the current investigation, NaOCl outperformed CHX in terms of antibacterial activity. The outcomes matched the findings of the Karale *et al*. investigation, which employed 3% NaOCl.[14] In contrast, 5.25% NaOCl was utilized in this investigation since some in vitro research indicated that higher doses of hypochlorite were more effective.[15]

Because of its substantivity as a root canal irrigating solution and broad-spectrum antibacterial activity, 2%CHX was utilized in this study. It also overcomes NaOCl's disadvantages.[16]

Anjali et al.'s study states that the chitosan nanoparticles are made up of clusters of particles that range in size from 10 nm to 80 nm. Since. The size of these nanoparticles is thought to be the defining characteristic; they have a higher charge density and large contact surface area than bulky powder. Because it permits a greater degree of interactions and contact between positive charged nano-particles and negative charged bacterial surfaces, it also contributes to antibacterial activity.[17]

Chitosan was employed as root canal irrigant solution in research conducted by Jaiswal N., and it demonstrated good antimicrobial efficacy against *E. faecalis*. The way in which nanochitosan acts is that it has positive charged NH3+ group of glucosamine interact with negative charged substances of bacterial surface to cause extensive cell surface attraction, intracellular substance leakage, and damage to essential bacterial activities, might be the cause of the antimicrobial action of chitosan.[18]

The current study shows that antibacterial efficacy of nano-chitosan is lower than 5.25% NaOCl ,unlike an investigation done by Khatija *et al*. ,which demonstrates that anti-microbial efficacy of nano-chitosan nearly similar to 5.25% NaOCl.[19] The average CFU value of nano-chitosan irrigation closely resembles that of 2% CHX. The outcomes resemble those of the Suzuki *et al.* study.[20]

Mixing of nano-chitosan with 5.25% NaOCl or CHX 2% enhance its antibacterial efficacy according to the result of this study. The least number of CFUs was seen in groupVI(NC 0.5%+NaOCl 5.25%) .This study's findings indicate that when chitosan is combined with CHX or NaOCl, there is synergistic antibacterial activity; another study found that using CHX in conjunction with chitosan improves the sustained release property. The combination between nano-chitosan and CHX can eradicate *E. faecalis* from root canal by creating of membrane barriers at peri-radicular area .Also, these nanoparticles can increase the antibacterial activity of NaOCl by enhancing its penetration deeper in dentinal tubules.[21]

Additional study plans are being made to examine the in vivo antibacterial effectiveness of nano-chitosan alone and in combination with other endodontic irrigation solutions and on teeth with multiple root canals. A more research on

possible interaction between the mixed irrigants is another study's limitation.

Conclusion

On the evidence of the current re-search, there was synergistic antimicrobial action as nano-chitosan irrigation solution (0.2%–0.5%) was mixed with NaOCl 5.25% and CHX 2%. Nano-chitosan irrigant reduces the side effects of NaOCl irrigation solution and enhances the sustained release property of CHX, so it can serve as a promising endodontic irrigation solution.

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Data availability

Data related to this research will be available upon request to corresponding author.

Ethics approval and consent to participate

Ethical consent for the use of excised human teeth was acquired from the ethics committee of the University of Mosul/College of Dentistry (Code: UoM.Dent.23/27, Date: 28/11/2023).

Conflict of interest

The authors declare that they have no conflicts of interest.

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