EFFECT OF USING NANOCHITOSAN VERSUS CALCIUM HYDROXIDE AS INTRACANAL MEDICATION ON THE POSTOPERATIVE PAIN (RANDOMISED CLINICAL TRIAL)

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Abstract

Aim: to evaluate the effect of 0.2% nanochitosan gel versus the gold standard Calcium hydroxide paste as an intracanal medicament on post-operative pain in single rooted previously endodontically treated teeth with post-treatment diseases; namely symptomatic apical periodontitis with evidence of apical radiolucency.

Methods: Forty-two participants were selected and randomly divided into two groups (each n=21) according to the intracanal medication; chitosan nanoparticles group (CNP) using 0.2% nanochitosan gel and Calcium hydroxide group Ca $(OH)_2$. On the first visit, gutta percha was removed using gates glidden burs and H files followed by chemo-mechanical preparation using Protaper Universal files. Intracanal medication application was applied. The post-operative pain levels were recorded at 6, 12, 24, 48, 72 hours, and 7 days post-operatively, using a numerical rating scale (NRS). The analgesic tablet consumption was evaluated for both groups. Obturation was carried out after 7 days using modified single cone technique. Statistical analysis was done, with *P value* for significant set at $P \leq 0.05$.

Results: Comparing median pain scores at each time interval of both groups, there was no statistically significant difference. Regarding pain scores by time within each group, there was a statistically significant decrease. There were no statistically significant differences between the two groups regarding analgesic intake.

Conclusion: The use of 0.2% nanochitosan gel as an intracanal medicament was almost similar to the gold standard Calcium hydroxide paste, in terms of post-operative pain in case of single rooted previously endodontically treated teeth with symptomatic post-treatment diseases and evidence of apical radiolucency.

Key words: calcium hydroxide, chitosan nanoparticles, pain

INTRODUCTION

Generally, the cause of post-operative pain is multi-factorial such as mechanical, chemical, microbial, and psychological. However, in post-treatment diseases the cause is mainly persisting intraradicular bacterial infection resulting from improper primary treatment or re-introduced infection to the root canal system due to inadequate apical or coronal seal ***Sjogren et al., 1997.*** The high possibility of post-operative pain and flare-up are of major concern to patient and dentist ***Torabinejad et al, 1988***. It is revealed that up to 80% of the population report varying degrees of pain.

In retreatment cases, suppression of bacteria is rather harder than primary treatment. The presence of fragments of old gutta percha further complicates treatment. The use of intracanal medication between visits is reported not only to control microbial infection but

also to decrease the post-operative pain. For many years, Calcium hydroxide has been used. It is characterized by high pH and its initial bactericidal effect against common endodontic microbes with less efficiency against some bacteria like *Enterococcus Faecalis* and *Candida Albicans.* It also controls inflammatory process and induces repair.

The use of green dentistry introduces chitosan, a natural polysaccharide from chitin. It has excellent antibacterial, antifungal, and antiviral properties. It has been used as intracanal medication in a vivo study ***Savitha et al., 2019*** and showed efficient bacterial reduction against *Enterococcus Faecalis* in unsuccessful endodontic cases. It can also be used as intracanal medication with high bacterial reduction; with chitosan coating ciprofloxacin that is encapsulated in PLGA (Poly - lactic co - glycolic acid) nanoparticles ***Arafa et al., 2023.*** Furthermore, an invitro study ***Silva et al., 2013*** expressed that 3 min application of 0.2% chitosan had less erosive effect on root dentine with similar smear layer removal as EDTA (15%) and citric acid (10%).

Nanotechnology has many biomedical applications; for example, antimicrobial application, drug delivery, tissue regeneration, and gene transfection. Development of nanomaterials, in the field of endodontics, targets mainly overcoming the microbial challenge via being used in irrigation, intracanal medication and incorporated in sealer. It also progressed to improve the previously diseased dentin matrix mechanical integrity, and to be used in tissue regeneration.

Chitosan nanoparticles CNP could be considered as a new irrigant whose main benefit over chitosan, NaOCl, and 17%EDTA was higher dentinal penetration and better smear layer removal. Chitosan nanoparticles have an advantage over chitosan as they can have access to infected root canal complexities and dentinal tubules thereby improving disinfection.

Up to date, searching in multiple databases including PubMed and Google scholar did not reveal any randomized clinical trial on nanochitosan as an intra-canal medication in endodontics and its effect in post-operative pain. Thus, the present study compared two intracanal medicaments, namely, 0.2% CNP gel versus the gold standard Ca $(OH)_2$ paste on post-operative pain in single rooted previously endodontically treated teeth with post-treatment diseases of symptomatic apical periodontitis accompanied by apical radiolucency.

Trial design:

Registration of protocol was done (Clinicaltrials.gov under ID: ahmed abdelmonam). The trial design of this study is a parallel, randomized, clinical design, with an allocation ratio 1:1. Randomized clinical trial (RCT) is a study in which participants are allocated at random to receive one of several clinical interventions.

Ethical and administrative considerations:

The protocol of the trial, the sample size calculation approval and the approved informed consent from the Research Ethics committee were written according to the Faculty of Dentistry guidelines. The purpose of the study, treatment procedures, possible side effects and treatment alternatives were thoroughly clarified to the participants. Participants were asked to follow the general instructions and to sign a printed informed consent that explains the purpose of the study and obligates the patient to record a pain diary accurately and honestly and to come at follow up intervals.

Participants:

Sample size:

Depending on the results of ***Hepsenoglu et al., 2018***, the estimated percentage differences were 48%. Using alpha (α) level of (5%) and Beta (β) level of (20%) i.e., power = 80%; the minimum estimated sample size was 18 subjects per group for a total of 36 subjects. The sample size will be increased to a total of 42 subjects (21 subjects per group) to compensate for a dropout rate of 15%. Sample size calculation was performed using PS program. Sample size calculations were approved by the Medical Biostatistics Unit (MBU), Faculty of Dentistry, Cairo University.

Eligibility measures:

Inclusion criteria: All Patients reported good general health, had age range from 20-50 years, with no sex predilection and accepted to participate in the study. They had single rooted canals which were previously endodontically treated diagnosed to have post-treatment diseases, namely:–symptomatic apical periodontitis with evidence of apical radiolucency. Lesion size was minimum 2 mm X 2 mm as measured on periapical radiograph by linear measurements tool on the software of digital periapical radiograph.

Exclusion criteria: included medically compromised patients and pregnant female patients, teeth which revealed intracanal separated file or canal transportation or ledge or perforation. Patients who had taken any antibiotic or analgesics during the past 24

hours, or who had periapical swelling or generalized periodontitis (calculus, deep pockets), were also excluded.

Randomization and allocation concealment

A random sequence was generated by computer software (http://www.random.org/). where the intervention and control were denoted I & II and randomly distributed. The random sequence table was kept and only accessed by the main supervisor and concealed from the operator. The intervention and control names were written in 42 sequentially numbered-arranged, opaque, sealed envelopes for allocation concealment.

Blinding:

The patient and assessor did not know which intracanal medication had been used for each group. The operator knew the medication applied for each patient only after completing the instrumentation and just before placement of the medication

Subjects and methods:

The diagnosis of Single rooted previously endodontically treated canal with post-treatment diseases having symptomatic apical periodontitis with evidence of apical radiolucency (minimum # 2mm X 2mm) was based on history taking (personal, medical, dental) and both clinical and radiographic examinations. Digital periapical film using the bisecting angle technique was performed to detect the quality of obturation and any other mishaps. Apical radiolucency was approximately measured to be within 2 mm X 2 mm using the digital linear measurement tool on IDA sensor software.

Participants were asked to record pre-operative pain intensity using numerical rating scale (NRS). Access cavity was reopened. Then the tooth was properly isolated using rubber dam. On the coronal two-thirds of the root canal, previous obturating material was removed using size 1, 2, and 3 Gates Glidden burs while a #15 K[1] file was used to achieve patency to the apical third of the root canal then using H files to withdraw the remaining gutta percha in apical part. Copious irrigation using 2.6% NaOCl solution was employed during gutta percha removal without using of any chemical solvents. Canals were negotiated with stainless steel K-files (size 10 or 15)

MANI-MANI, INC. Industrial Park, Utsunomiya, Tochigi, Japan.

with the help of 17% EDTA[2] root canal conditioner as a lubricant. Working length was determined using an electronic apex locator[3] and confirmed radiographically to be 0.5-1mm short of the radiographic apex.

Reshaping the canals was performed in a crown-down technique using ProTaper Universal rotary instruments[4]. Irrigation during the shaping was performed using 3 ml of 2.6% NaOCl [5] in a 3ml disposable plastic syringe[6] with a 27-gauge. For removal of the smear layer, canals were finally flushed with 5ml 17% EDTA solution for 1min followed by saline then 3ml of 2.6% NaOCl, then distilled water. The canal was dried with paper points corresponding to the master cone tip size, then divided according to the random sequence generation to apply either intracanal medication: Group I (Intervention group): chitosan nanoparticles (0.2% CNPs gel) or Group II (Control group): Calcium hydroxide paste[7]. $(Ca(OH)_2$.

Intracanal medications were injected into the canals through the syringe and a 29-gauge Navitip [8] with a stopper, inserted 2mm short of the working length. Then access cavities were sealed with a dry sterile cotton pellet and a temporary filling material[9]. Finally, periapical radiograph was taken after intracanal medication placement to confirm that the medication reaches to full working length.

Post-operative pain level was recorded after completion of the initial visit at 6 intervals (6, 12, 24, 48, 72 hours and 7 days) accurately and honestly before obturation. A prescription of analgesic (Brufen 200mg) was given to be taken in case of intolerable pain after the 1st visit. In case of flare up, an emergency visit was scheduled, and systemic antibiotic was prescribed.

After 7 days, intracanal medication was removed using a hand K-file corresponding to the size of master apical file and 2.6% NaOCl irrigation. The final flush of the canals was performed as discussed in the first visit Canals were dried with

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Glyde FILE PREP Root Canal Conditioner, DENTSPLY, Tulsa Dental, DENTSPLY Maillefer, TN, USA.

Root ZX, J. Morita USA, Irvine, USA

DENTSPLY, Tulsa Dental, DENTSPLY Maillefer, TN, USA

Clorox®, Household Cleaning Products Of Egypt, 10th Of Ramadn, Egypt

S-S disposable syringe, SUNG SHIM medical Co, Korea.

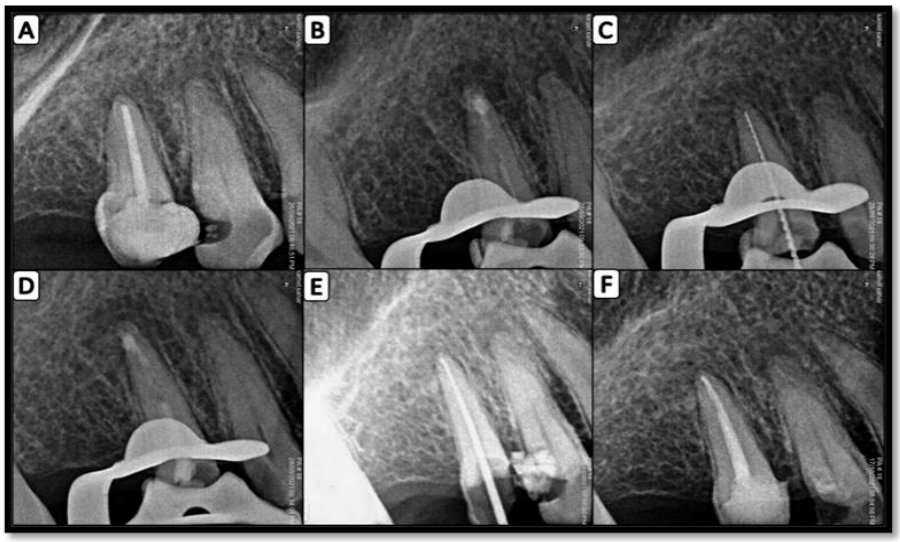
Ultracal XS™, Ultradent Products, Inc, South Jordan, Utah, USA.

Navitip 21mm/29 gauge, Ultradent Products, Inc, South Jordan, Utah, USA

MD-TEMP, META BIOMED CO., LTD, Chungbuuk, Korea

Protaper paper points[10] corresponding to the master cone size and obturated using modified single cone technique with a resin sealer[11] using standard auxiliary gutta percha cones. The access cavity was filled using a composite resin restoration. Post-operative radiographs were taken to confirm the density and length of the obturation.

Representative periapical radiographs showing steps of retreatment of CNPs group, Figure (1) and Ca $(OH)_2$ group, Figure (2).



Protaper® Universal paper Points DENTSPLY, Tulsa Dental, DENTSPLY Maillefer, TN, USA.

ADSEAL, META BIOMED CO., LTD, Chungbuuk, Korea

Fig. (1): Representative periapical radiographs showing steps of endodontic retreatment of maxillary right 2nd premolar from chitosan nanoparticles group (A): Periapical radiolucency of unsatisfactory filling root canal. (B): Confirmation of removal of old gutta percha. (C): Working length determination. (D): **CNPs** intracanal medication. (E): Master cone verification. (F): Final obturation radiograph

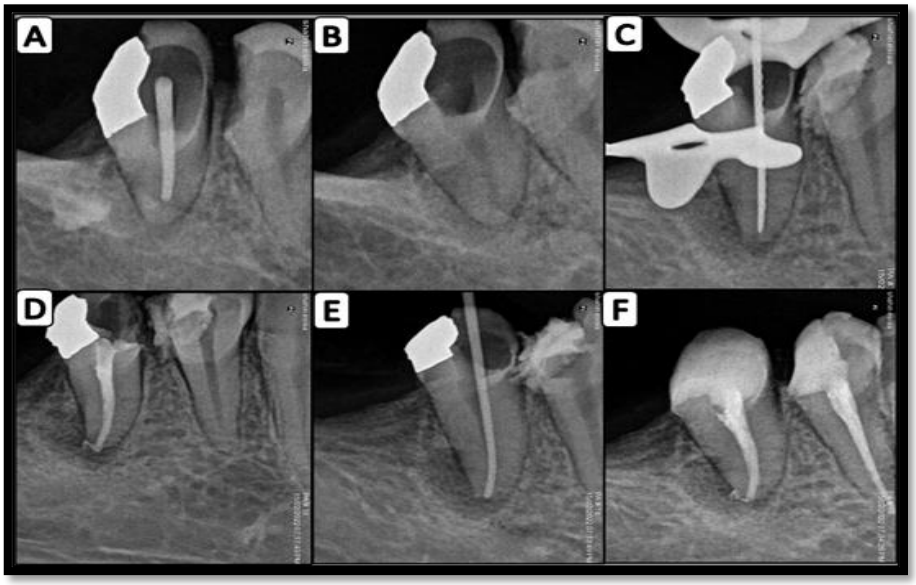


Fig. (2): Representative periapical radiographs showing steps of endodontic retreatment of mandibular right 2nd premolar from calcium hydroxide group (A): Periapical radiolucency of unsatisfactory filling root canal. (B): Confirmation of removal of old gutta percha. (C): Working length determination. (D): **$Ca(OH)_2$** intracanal medication. (E): Master cone verification. (F): Final obturation radiograph

Statistical analysis:

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Age data showed normal (parametric) distribution while pain (NRS) scores and number of consumed analgesic tablets data showed non-normal (non-parametric) distribution. Data were presented as median, range, mean and standard deviation (SD) values. For parametric data, Student's t-test was used to compare between mean age values in the two groups. For non-parametric data, Mann-Whitney U test was used to compare between the two groups. Kruskal-Wallis test was used to compare between more than

two groups. Friedman's test was used to study the changes within each group. Dunn's test was used for pair-wise comparisons when Friedman's test or Kruskal-Wallis test is significant. Spearman's correlation coefficient was used to determine the correlation between age and post-operative pain scores. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. Figure (3) represented flow diagram of the study.

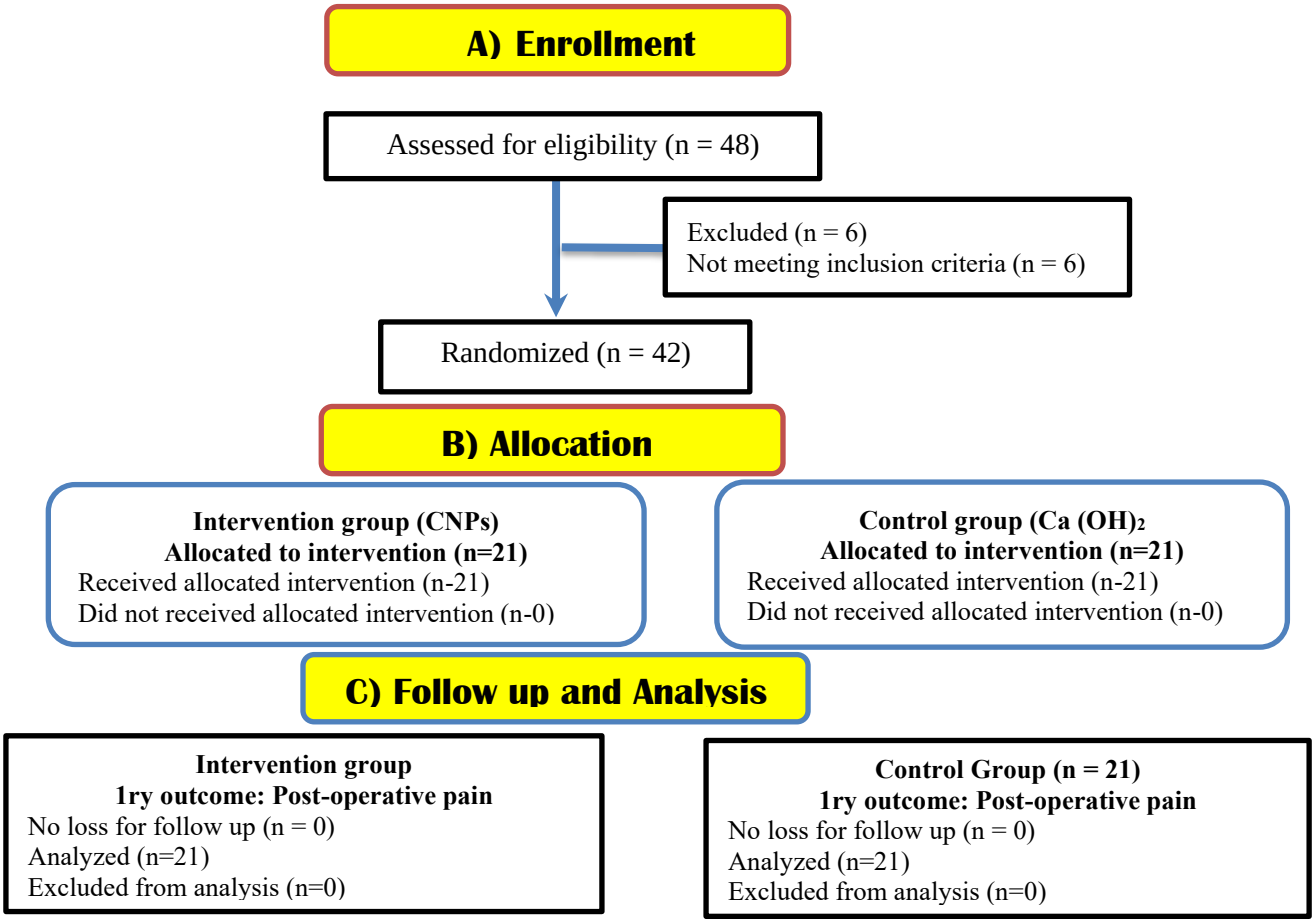


Fig. (3): CONSORT flow diagram of the present study design.

RESULTS

1. Base line characteristics

Statistical analysis of baseline data; age, sex, tooth type, number of canals and pre-operative pain intensity for the three groups are presented in Table (1).

Table (1): Mean, standard deviation (SD), frequencies (n), percentages and results of Student's t-test, Chi-square and Fisher's Exact tests for comparison between base line

characteristics in the two groups

	Chitosan NP (n = 21)	Calcium Hydroxide (n = 21)	*P*-value
Age (Years) Mean (SD)	32.4 (7.52)	30.5 (6.9)	0.395
Gender [n (%)]			
Male	9 (42.9)	4 (19)	0.095
Female	12 (57.1)	17 (81)	
Tooth [n (%)]			
Maxillary central	7 (33.3)	9 (42.9)	
Maxillary lateral	5 (23.8)	5 (23.8)	
Maxillary canine	4 (19)	3 (14.3)	0.962
Maxillary 2nd premolar	1 (4.8)	2 (9.5)	
Mandibular canine	1 (4.8)	0 (0)	
Mandibular 2nd premolar	3 (14.3)	2 (9.5)	
Etiology [n (%)]			
Inadequate filling	17 (81)	18 (85.7)	1
Over-extended filling	1 (4.8)	0 (0)	
Short root canal filling	3 (14.3)	3 (14.3)	
Lesion size Mean (SD)			
mm X mm	2.6 (0.9) X 2.4 (0.8)	2.9 (1.2) X 2.7 (0.9)	0.183
mm^2	6.8 (6)	8.8 (7.4)	
Lesion extension [n (%)]			
Non-perforated	14 (66.7)	15 (71.4)	0.739
Perforated	7 (33.3)	6 (28.6)	

**: Significant at P ≤ 0.05*

2. Outcome data

2.1 Assessment of Pain (NRS) scores:

2.1.1 Comparison between groups at each time point, Table (2).

Pre-operatively, the mean score of *Chitosan NP* (6.29) was *higher* than *Ca (OH)₂* (5.57). At six, 12 hours mean score of Chitosan NP (2.29) was *higher* than $CaOH_2$ (1.86). However, in one, two days the mean score of Chitosan NP (1.76, 1.19; respectively) was *lower* than Ca $(OH)_2$ (2.38, 2.19; respectively). At 3 days, mean score of Chitosan NP (1.33) was again slightly higher than $CaOH_2$ (1.05). At Seven days, both were almost similar (0.71. 0.76)

Statistically, there was no significant difference between pain scores in the two

groups: pre-operatively, after 6, 12 hours, one day, two, three as well as seven days.

2.1.2 Changes along the 6 time points within each group, Table (2), Figure (4, 5)

Regarding Chitosan NP, mean pain scores showed a generally decreasing trend from pre-operative to 6 hours, stability at 6 and 12 hours, decrease along one, two-day time points, however to day 3, there was slight increase, followed by resuming decrease to the 7^{th} day.

There was a statistically significant change in pain scores by time (P-value <0.001, Effect size = 0.519). Pair-wise comparisons between time points revealed a statistically significant decrease after six hours followed by a non-statistically significant change from six to 12 hours. From 12 hours to one day, there was further statistically significant decrease. From one to two, two to three as well as three to seven days, there was no statistically significant change in pain scores.

Concerning Ca $(OH)_2$ group, mean pain scores showed a fluctuating pattern; decreased from pre-operative to 6 hours, stability at 6 and 12 hours, increase along one, two-day time points, followed by resuming the decrease at 3, 7 days.

There was a statistically significant change in pain scores by time (P-value <0.001, Effect size = 0.489). Pair-wise comparisons between time periods revealed a statistically significant decrease in pain scores after six hours followed by non-statistically significant change from six to 12 hours, 12 hours to one day, one to two as well as two to three days. From three to seven days, there was a statistically significant decrease in pain scores.

Table (2): Descriptive statistics and results of Mann-Whitney U test for comparison between pain (NRS) scores in the two groups and Friedman's test for the changes within each group

Time	Chitosan NP (n = 21)	Calcium Hydroxide (n = 21)	*P*-value	*Effect size (d)*

	Median (Range)	Mean (SD)	Median (Range)	Mean (SD)		
Pre-operative	6 (3-10) ^{A}	6.29 (2.17)	6 (3-10) ^{A}	5.57 (1.8)	0.435	0.238
6 hours	2 (0-10) ^{B}	2.29 (2.65)	0 (0-10) ^{B}	1.86 (3.35)	0.169	0.4
12 hours	2 (0-10) ^{B}	2.29 (2.65)	0 (0-10) ^{B}	1.86 (3.35)	0.169	0.4
1 day	0 (0-10) ^{C}	1.76 (3.08)	0 (0-10) ^{B}	2.38 (3.64)	0.944	0.019
2 days	0 (0-10) ^{C}	1.19 (3.01)	0 (0-10) ^{B}	2.19 (3.66)	0.430	0.195
3 days	0 (0-10) ^{C}	1.33 (3.07)	0 (0-9) ^{B}	1.05 (2.44)	0.713	0.082
7 days	0 (0-10) ^{C}	0.71 (2.31)	0 (0-6) ^{C}	0.76 (1.76)	0.684	0.082
P-value	<0.001*		<0.001*			
Effect size(*w*)	0.519		0.489			

**: Significant at P ≤ 0.05, Different superscripts in same column indicate statistically significant change within group*

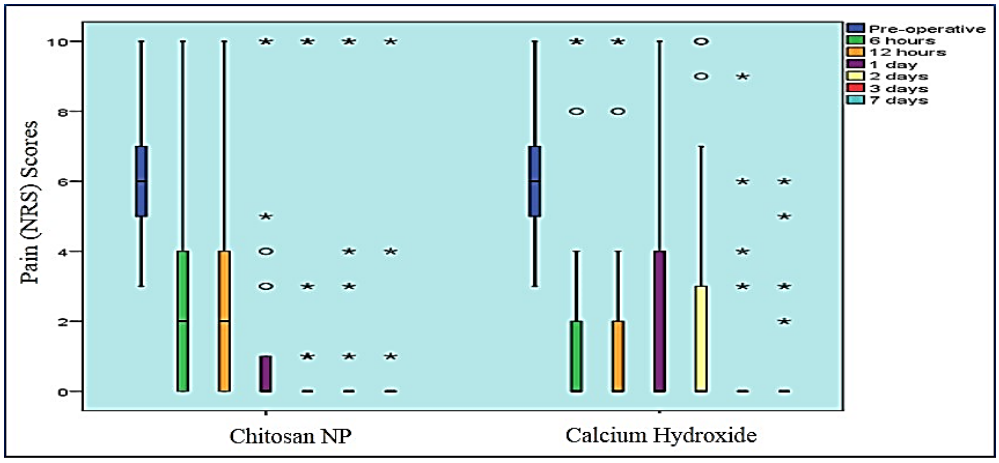


Fig. (4): Box plot representing median and range values for pain (NRS) scores in the two groups (Circles and stars represent outliers)

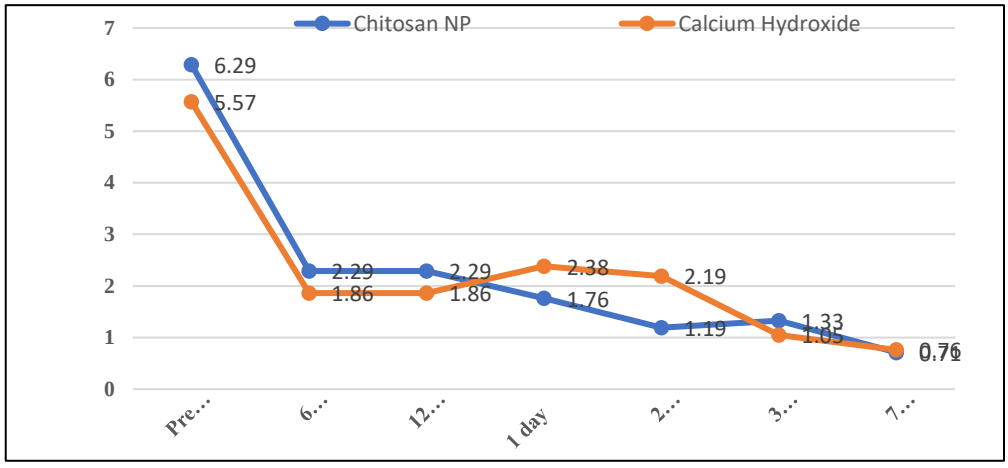


Fig. (5): Line chart representing mean pain (NRS) scores in along the time points in the two group

2.2 Ass **ke**

Regarding analgesic incidence: There was no statistically significant difference between patients who took analgesics in the two groups (*P*-value = 0.726, Effect size = 1.135), represented by six patients of 21 (**28.6%)** in Chitosan NP (two of them had flare up) and five patients of 21 (**23.8%)** in Ca $(OH)_2$ (two of them had flare up).

Regarding Number of analgesic tablets: There was no statistically significant difference between the number of consumed analgesic tablets in the two groups after 6, 12 hours, one, two, three as well as seven days.

2.3 Post-operative complications

Regarding Flare up: no statistically significant difference existed between prevalence of flare up in the two groups (*P*-value = 1, Effect size = 1), represented by two patients (9.5%) per group.

DISCUSSION

Post-endodontic pain following endodontic treatment is mostly attributed to microbial elements. In retreatment cases, suppression of bacteria is rather harder than primary treatment. The presence of fragments of old gutta percha further complicates treatment. Using intracanal medicaments hopefully improves intracanal disinfection in-between-visits and helps to reduce post-operative pain. In fact, the use of intracanal medication ICM is recommended to eliminate any residual bacteria; that could have survived after chemo-mechanical preparation, prevent bacterial regrowth ***Sjogren et al., 1991***, and prevent contamination between appointments. Decreasing the level of pro-inflammatory cytokines and Matrix Metaloprotenase were reported in the presence of intracanal medication. Thereby possesses pain-preventive properties derived from its anti-inflammatory action. Furthermore, intracanal medication can favor healing of the periapical tissues and might accelerate the repair process of bone tissue ***Özdemir et al., 2019*** as revealed by post-operative increased levels of vasoactive intestinal peptide.

Even through Calcium hydroxide paste Ca $(OH)_2$ is the most commonly used medicament in endodontics ***Mohammadi et al., 2012*** , its antibacterial effectiveness has been questioned by ***Sathorn et al., 2007***. Ca $(OH)_2$ showed limited antifungal properties against *Candida Albicans*, and its effectiveness against *Enterococcus Faecalis* biofilm is

controversial ***Mohammadi and Dummer, 2011.*** Furthermore, it might decrease fracture resistance of root dentine over time

Up to date, there is an ongoing search for versatile and optimal intracanal medication. Endodontics has made use of the era of green dentistry by using Chitosan as intracanal medication. Chitosan is a natural polysaccharide known to be a favorable pharmaceutical material because of its biocompatibility and biodegradability, and it forms an ideal hydrophilic carrier system ***Kreuter 1991.*** Chitosan showed, in an invitro model, better efficacy in reducing resistant bacteria, that might be present in retreatment cases, such as *Enterococcus Faecalis biofilm* and *Candida Albicans*. Furthermore, ***Silva et al., 2013*** showed that 3-min application of 0.2% chitosan on root dentin had less erosive with similar cleaning ability as 15% EDTA and 10% citric acid. In a randomized clinical trial, Chitosan was used as drug-delivery intracanal medication by coating Ciprofloxacin hydrochloride encapsulated in PLGA (Poly lactic co-glycolic acid) nanoparticles ***Arafa et al., 2023.*** Unfortunately, Chitosan has limited tubular depth of penetration which might hinder the antimicrobial effect in complex canal areas ***Sireesha et al., 2017***.

The era of nanotechnology produced nanoparticles with a wide variety of polymers and nanotechnology. Chitosan nanoparticles gel CNPs was produced with its possible promising application as intracanal medication. Chitosan nanoparticles (CNPs) have excellent antibacterial, antiviral, antifungal, biodegradable and non-toxic properties with potential of drug delivery vehicles. Chitosan nanoparticles can be transported within the anatomic complexities and dentinal tubules of an infected root canal to improve root canal decontamination ***Shrestha et al., 2009.*** In an invitro study; chitosan nanoparticles as intracanal medication showed less reduction in fracture resistance of root dentine at 1-month interval compared to its micron sized counterpart ***Sireesha et al., 2017***.

Up to date, no randomized clinical studies are available in the literatures that evaluated chitosan nanoparticles intra-canal medication regarding effectiveness on reducing post-operative pain. Therefore, the purpose of the current study was to assess post-operative pain following intra-canal medication with 0.2% chitosan nanoparticles gel compared to calcium hydroxide paste in selected patients of single rooted previously endodontically

treated teeth with post-treatment diseases.

The selected cases for the current study present three clinical challenges in endodontic practice namely: First; endodontic retreatment cases, second; post-treatment diseases having symptomatic apical periodontitis, and third: evidence of apical radiolucency. Retreatment cases are challenging because of the difficulty in gutta percha removal and eliminating bacterial biofilm. Cases of retreatment are harbored by many resistant strains like *Enterococcus Faecalis* and *Candida Albicans*. Symptomatic apical periodontitis condition is liable for more risk of acute post-operative pain and flare ups. ***Mattscheck et al., 2001*** had demonstrated that the cases with periapical lesions showed more post-operative pain, compared to the cases without periapical lesions.

The study was limited to single rooted with single canal cases to enable standardized conditions when comparing the examined intracanal medication materials. Similar selection was reported previously ***Angın et al., 2024***, ***Ahmed et al., 2018)***. The use of multirooted teeth was avoided because of their complex anatomy such as extra roots, extra canals, root curvature and apical ramifications representing confounding factors.

In the present work, initial selection of single rooted endodontically treated cases, was based on the patient's history reporting previous endodontic treatment and on digital periapical radiographic evaluation. On the pre-operative radiograph, the presence and size of the periapical lesion were only screened for inclusion of lesion size of not less than 2mm x 2mm. Linear measurement was done by the tools available on its software. Similarly, ***(Karaoğlan et al 2022a)*** used periapical radiograph in the initial diagnosis of apical lesion.

In the present study, removal of gutta percha during retreatment was performed by Gates Glidden burs and Hedstrom hand files. It is noted that hand instrumentation was associated with less iatrogenic errors like perforation or transportation. Similarly, ***Karaoğlan, et al., 2022*** used Gates Glidden burs and Hedstrom files in retreatment cases.

In the current study, gutta percha solvent was not used, although the use of hand and rotary instruments can be aided by heat, solvents, and ultrasonic instruments for better removal of gutta percha during retreatment ***Zhang et al., 2021.*** Gutta percha solvents are

considered irritating to periapical tissues; this might act as confounding factor and affect results of post-operative pain. Similarly, ***Angın et al., 2024*** avoided the use gutta percha solvent in retreatment to avoid unfavorable results on post-operative pain.

In the current study, working length was determined by an electronic apex locator and confirmed radiographically; as the use of electronic apex locator reduced over instrumentation; causing post-operative pain ***Arafa et al., 2023***. Radiographic confirmation was important to ensure total Gutta perch removal because the accuracy of electronic apex locator can be negatively affected by residual gutta percha in retreatment ***Rivera and Seraji, 1994.***

In the present study, ProTaper Universal system was used for re-instrumentation of root canal, according to manufacturing instructions, in a crown down technique. ***Motlani et al,.2009*** reported less post-operative pain incidence for rotary instrumentation than manual instrumentation. Furthermore, Rotary instrumentation reduced root canal instrumentation time and enhanced obturation and filling quality as compared to manual instrumentation techniques ***Rajkhan et al., 2021***. Similarly, **Angın et al., 2024** used ProTaper Universal rotary files for root canal instrumentation in retreatment cases.

Smear layer removal was essential to promote diffusion of intra-canal medication through the dentinal tubules **Violich and Chandler, 2010** and to eliminate bacteria residing inside dentinal tubules. In the present study, Smear layer removal included removing the inorganic part was done via a final flush of 5 ml of EDTA (17%) for one minute.

For removal of organic part of smear layer; final 3 ml 2.6% NaOCl was used comparable to other studies ***Karaoğlan, et al., 2022, Hepsenoglu et al., 2018, Ahmed et al., 2018)***. However, ***Angın et al., 2024*** recommended using higher concentration of NaOCl (5.25%) because it had higher tissue dissolving potential, bactericidal effect, lower surface tension and subsequently better disinfection. Unfortunately, it showed higher toxicity and tissue irritation ***Marion et al., 2012.*** Thus, in the current research a minimal concentration of 2.6% was preferred to be used.

In the present study, Ca $(OH)_2$ intra-canal medication was applied in a paste form. Similarly, ***Fahim et al., 2022*** used Ca $(OH)_2$ in a paste form as intracanal medication in

retreatment cases. Ultracal syringe delivery system with ready-made paste of suitable viscosity was used. The viscosity of the paste of calcium hydroxide can influence antimicrobial activity.

To ensure complete filling of Ca $(OH)_2$ of the root canal without apical extrusion; application was slowly, and continuously in an apical-coronal direction using Ultracal syringe delivery system. Other methods of calcium hydroxide application are reported such as lentulo spiral***. Siqueira et al., 2002***, reported that there was no statistically significant difference between injection systems, file rotated counterclockwise, a Lentulo spiral or a rotary NiTi file rotated in reverse, regarding the radiographic appearance.

CNPs 0.02% intracanal medication was delivered into the prepared root canal in a gel form which can be easily applied and removed. A little benefit of ionotropic gelation includes: the processing conditions are mild, low toxicity. Poor stability in acidic conditions and difficulty in entrapping high molecular weight drugs are the chief limitations of this method ***Mohammed et al., 2017.***

In the current study, two -visit endodontic retreatment was preferred to ensure post debridement and symptom-free period before obturation ***Yoldas et al., 2004)***. Moreover, patients on a single visit retreatment were more likely to take pain killers and more liable to flare up. Similarly, ***Hepsenoglu et al., 2018*** performed retreatment in two-visit and revealed that post-operative pain of Ca $(OH)_2$ intracanal medication in two-visit technique retreatments was less than CHX intracanal medication.

In the current work, numerical rating scale (NRS) was used to record post-operative pain. It was chosen due to its better compliance, responsiveness, and ease of use ***Fahim et al., 2022***.. NRS was explained to every participant and training was performed before delivering the pain diary for practice to reduce the errors in recording the pain scores during the follow up periods.

Pain was recorded pre-operatively and post-operatively; at six different time points 6, 12, 24, 48 hours, 3, and 7 days ***(Ahmed et al., 2018, Angın et al., 2024).*** Experiencing discomfort after a root canal is usual and recording pain at the first hours (6, 12 hours) is

essential because the highest post-operative pain intensity was observed at this interval ***Pak and White, 2011***. Inflammation is often the offender behind post-procedure tooth pain. This inflammation may stem from the procedure's manipulation of the tooth such as anesthetic injection, preparation of apical area, rubber dam clamp pressure, and staying with an open mouth for a long time ***Shokraneh et al., 2017.*** Inflammatory activity may also originate from infection irritating the periapical area and progressing to inflammation and swelling. Fortunately, as the cause (infection) is eliminated and as time passes, this inflammation tends to diminish, leading to a natural decrease of pain in the days and weeks following root canal treatment.

In the present study, Obturation was performed using modified single cone technique with ProTaper gutta-percha and resin root canal sealer, similar to ***Angın et al., 2024***. Resin sealer is characterized by its high physical properties, proper sealing ability, insolubility in tissue fluids and good radiopacity. Modified single cone technique is simple and does not require specific and expensive instruments ***Peters et al., 2010***.

In the current research, an emergency visit was scheduled in case of flare up and swelling. Intracanal drainage and irrigation were executed to decrease microbial load and intracanal exudate. Intracanal medication was placed again for another week. Augmentin 1gm antibiotic was prescribed twice daily for 7 days to combat the spreading infection and promote resolution.

Analgesics were only prescribed in case of severe pain and not considered a regular prescription of medication. Among the nonsteroidal anti-inflammatory drugs, Ibuprofen was selected due to its effectiveness for treating acute pain and inflammation after the root canal treatment and it is rapidly absorbed and metabolized by the liver**.** Similarly, ***Angın et al., 2024*** prescribed ibuprofen in case of severe pain.

In the present study, comparing the median of post-operative pain between both groups at each time interval stated no statistically significant differences. Emphasizing that the manipulation of 0.2% CNP as an intracanal medicament was almost like the gold standard Ca $(OH)_2$ paste, in terms of post-operative pain.

For both groups, changes from pre-operative (CNP: median: 6, mean: 6.29), (Ca $(OH)_2$

median: 6, mean: 5.57), to 6- and 12-hours post-operative revealed initial effective therapeutic impact and low post-operative pain. At the challenging time points of 6 and 12 hours, though non statistically significant, Ca $(OH)_2$ group revealed lower post-operative pain (median: 0, mean:1.86), than chitosan NP group (median: 2, mean: 2.29). Interestingly, for both groups these values ranged from 0-2 which can be translated categorically to "mild pain". Present study results were similar to ***Ahmed et al., 2018*** who revealed no pain at all time interval 6, 12 hours, 1. 2. 3. and 7 days when using Ca $(OH)_2$ as ICM in retreatment cases. On the other hand, ***Fahim et al., 2022*** revealed that higher median pain scores at 6, 12 hours were 4 and 4, respectively, when using Ca $(OH)_2$ as ICM in retreatment cases.

In flare up cases (n=2/group) pain relieve necessitated unscheduled emergency visit, while in the other cases with moderate or severe pain, the use of analgesic succeeded in controlling this post-operative pain phase similar to previous study ***Holstein et al., 2002***. Thus, it may be recommended to prescribe post-operative analgesic as a preventive measure, while reassuring the patient that pain is going to decrease by time.

Flare up is defined as the occurrence of severe pain and swelling following an endodontic treatment appointment, compelling an unscheduled visit and active treatment. There was no statistically significant difference between the prevalence of flare up in the two groups, represented by two patients (9.5%) in each group. The low incidence of flare up with intracanal medication comes in agreement with ***Hussein et al., 2019*** that showed no significant difference in prevalence of flare up with Ca $(OH)_2$ intra-canal medication compared to Ca $(OH)_2$ /CHX combination. On the other hand, ***Fahim et al., 2022*** showed the highest incidence of flare up after application of Ca $(OH)_2$ in 3 cases (13%).

Effectiveness of Ca $(OH)_2$ can be attributed to its antibacterial property, anti-inflammatory property and pain preventing property that would eliminate bacteria that could have survived after chemo-mechanical preparation**.**

The literature reports the effectiveness of Chitosan can be attributed to unique properties such as its biocompatibility, degradability, nontoxicity, bacteriostasis, anti-inflammatory, hemostasis and helps in wound healing. CNP has excellent antibacterial,

antiviral, antifungal, biodegradable and non-toxic properties**.** Chitosan nanoparticles can be transported within the anatomic complexities of an infected root canal to improve root canal decontamination ***Shrestha et al. 2009.***

Although the advantages of CNP over Ca $(OH)_2$, the results of the present study were not promising regarding post-operative pain as the two groups showed almost the same results. Nevertheless, this limitation of the present study will be prevailed by further randomized clinical trials with varying properties of CNPs as different concentration and combining with other intracanal medication to gain their synergistic antimicrobial effect and to enhance its effectiveness in reducing post-operative pain. Or using CNPs as a carrier for other ICM to gain its benefits as increased dentinal tubular depth of penetration.

CONCLUSIONS

Within the restrictions of this research, the following could be established:

1. The usage of 0.2% nanochitosan as an intracanal medicament was almost similar to the gold standard Calcium hydroxide paste, in terms of post-operative pain and apical bone healing, in case of single rooted endodontically treated cases with symptomatic post-treatment diseases and evidence of apical radiolucency.
2. Both groups are similar regarding analgesic intake and flare up.

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COMPETING INTERESTS

The authors declare no conflict of interest. The Research Ethics Committee, Faculty of Dentistry, Cairo University approved the protocol of this study. Detailed procedures, benefits, and expected harms were discussed with the patient, then informed consent was obtained.

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