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POST-OPERATIVE PAIN AND APICAL BONE HEALING AFTER USING CHITOSAN NANOPARTICLES VERSUS CALCIUM HYDROXIDE AS INTRACANAL MEDICATION (RANDOMIZED CLINICAL TRIAL)

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ABSTRACT

Aim: to evaluate the effect of 0.2% Chitosan nanoparticles NP gel versus the gold standard Calcium hydroxide Ca (OH)2 paste as an intracanal medication on post-operative pain and apical bone healing in single rooted previously endodontically treated teeth with post-treatment disease; namely symptomatic apical periodontitis with apical radiolucency.

Methods: Forty-two participants were selected and randomly divided into two groups (each n=21) according to the intracanal medication; 0.2% chitosan NP gel and Ca (OH)2 paste. On the first visit, gutta percha was removed using Gates Glidden burs and H files followed by chemo-mechanical preparation using ProTaper Universal files and Sodium hypochlorite irrigation. Intracanal medication was applied according to random sequence generation. 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 followed by coronal access sealing. Post-operative apical bone healing was assessed clinically and radiographically. Percent of Lesion-Volume changes were calculated by comparing pre-operative CBCT images with the post-operative ones at 1 and 2-year follow up. Statistical analysis was done, with P value for significance set at $P \leq 0.05$.

Results: Regarding post-operative pain levels at each time point; median pain scores of Chitosan NP ranged from 2 (at 6 & 12 hours) to 0 (at all other time points), while it was 0 at all postoperative time points in Ca (OH)2, with no statistically significant difference $P \geq 0.05$. While for pain scores by time within each group, there was a statistically significant decrease after 6 and 12 hours. Median pain score of chitosan NP dropped from 6 (preoperative) to 2, while pain score of Ca (OH)2 dropped from 6 (preoperative) to 0. Regarding analgesic intake; there was no statistically significant difference between the two groups. Concerning healing assessment, chitosan NP gel, mean percent volume reduction at 2-year follow up was 81.37% versus 99.3% for Ca (OH)2 paste with no statistically significant difference.

Conclusion: Considering the trend to follow green dentistry; 0.2% chitosan NP gel as an intracanal medication can be viable alternative therapy to gold standard Ca (OH)2 paste; for reducing post-operative pain and promoting apical bone healing, in single rooted previously endodontically treated teeth with symptomatic post-treatment disease and apical radiolucency. Advancement in research targeting improvement of chitosan NPs as intracanal medication is encouraged.

Keywords:calcium hydroxide, chitosan nanoparticles, pain, apical healing, periapical periodontits.

INTRODUCTION

Generally, the cause of post-operative pain is multi-factorial such as mechanical, chemical, microbial, and psychologica[l1] . 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. The high possibility of post-operative pain and flare-up are of major concern to patients and dentists. It is revealed that up to 50% of the population report varying degrees of pain [2].

In retreatment cases, suppression of bacteria is rather harder than primary treatment [3]**.** 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 [4]. 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* [5].

The era of green dentistry introduced 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 [6] and showed efficient bacterial reduction against *Enterococcus Faecalis* in unsuccessful endodontic cases. It was used as intracanal medication with high bacterial reduction; with chitosan coating ciprofloxacin encapsulated in PLGA (Poly - lactic co - glycolic acid) nanoparticles [7].

Nanotechnology has many biomedical applications, for example, antimicrobial, drug delivery, tissue regeneration, and gene transfection [8]. Development of nanomaterials, in the field of endodontics, targets overcoming the microbial challenge via being used in irrigation, intracanal medication and incorporated in sealer [9].

Chitosan nanoparticles have an advantage over chitosan as they can have access to infected root canal complexities and dentinal tubules thereby improving disinfection [10]. Chitosan nanoparticles were reported as an efficient new irrigant whose main benefit over chitosan, NaOCl, and 17%EDTA was higher dentinal penetration and better smear layer removal [11].

Up to date, searching in multiple databases including PubMed and Google scholar did not reveal any randomized clinical trial on the effect of chitosan nanoparticles as an intra-canal medication in endodontics on both post-operative pain and long-term healing of periapical lesions in retreatment cases. Thus, the present study compared two intracanal medications, namely, 0.2% Chitosan NP gel versus the gold standard Ca $(OH)_2$ paste on post-operative pain and healing of apical bone in single rooted previously endodontically treated teeth with post-treatment diseases of symptomatic apical periodontitis accompanied by apical radiolucency.

MATERIALS AND METHODS

The trial design of this study is a parallel, randomized, clinical design, with an allocation ratio 1:1. Registration of protocol was done on Clinicaltrials.gov. The protocol of the trial and the informed consent form were approved by the faculty Research Ethics committee. Participants were asked to follow the general instructions and to sign a printed informed consent that explained treatment options, risks, benefits.

Sample size calculation*:* Depending on the results of *Hepsenoglu et al.* [12] 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 was 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.

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 with post-treatment diseases, namely:-symptomatic apical periodontitis with 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.

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. The operator knew the medication to be applied for each patient only after completing the instrumentation and just before placement of the medication. The patient and statistician did not know which intracanal medication that was used for each group.

Diagnosis 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 preoperative quality of obturation and any other mishaps. Apical radiolucency was screened to be within 2 mm X 2 mm using the digital linear measurement tool on IDA sensor software. CBCT was also requested pre-operatively to detect the exact measurement of the apical radiolucency. It was further requested at 1-year and 2-year postoperative follow-up. All images were taken by CBCT machine Promax classic, Planmeca, Filand with 14 mA 90 KVP with the following parameters: Anode voltage: 54-90 KV, 1-14 mA, Focal spot: 0.5 mm, fixed

anode, Image detector: Flat panel, Image acquision: Flat panel, Scan time; 7.5-27 s, Reconstruction time: 2-25 s, Voxel size 75 microns.

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. Copious irrigation using 2.6% NaOCl solution was employed during gutta percha removal without using of any chemical solvents. Canals were negotiated to the apical third with stainless steel K-files size 10 or 15 (MANI-MANI, INC. Industrial Park, Tochigi, Japan) with the help of 17% EDTA (Glyde FILE PREP Root Canal Conditioner, DENTSPLY Maillefer, TN, USA) root canal conditioner as a lubricant. H files were used to retrieve the remaining gutta percha in apical part. Working length was determined using an electronic apex locator (Root ZX, J. Morita, Irvine, USA) 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 (DENTSPLY, Tulsa Dental, DENTSPLY Maillefer, TN, USA). Irrigation during the shaping was performed using 3 ml of 2.6% NaOCl (Clorox®, Household Cleaning Products of Egypt, 10th Of Ramadn, Egypt) in a 3ml disposable plastic syringe (S-S disposable syringe, SUNG SHIM medical Co, Korea) 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.

According to the random sequence generation intracanal medication was applied: Group I (Intervention group): chitosan NP (0.2% gel) (chitosan NP, NANO gate Company, Naser City, Cairo) or Group II (Control group): Calcium hydroxide paste (Ca $(OH)_2$. (Ultracal XS^{TM}, Ultradent Products, South Jordan, USA)

Intracanal medications were injected into the canals through 29-gauge Navitip Ultradent syringe (Navitip 21mm/29 gauge, Ultradent Products, South Jordan, USA) with a stopper, inserted 2mm short of the working length. To ensure complete filling of the root canal without apical extrusion; application was slowly, and continuously in an apical-coronal direction Then access cavities were sealed with a dry sterile cotton pellet and a temporary filling material (MD-TEMP, META BIOMED CO., LTD, Chungbuuk, Korea). An immediate 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 six intervals (6, 12, 24, 48, 72 hours and 7 days); before obturation. A prescription of emergency 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. Intracanal drainage and irrigation were executed to decrease microbial load and intracanal exudate. Same intracanal medication previously applied was placed for another week.

After 7 days, in cases confirmed to be asymptomatic, 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 on the first visit. Canals were dried with ProTaper paper points (ProTaper® Universal paper Points DENTSPLY, TN, USA) corresponding to the master cone size and obturated using modified single cone technique with a resin sealer (ADSEAL, META BIOMED CO., LTD, Chungbuuk, Korea) and 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.

Radiographic assessment for healing: All CBCT images (pre and post) of recalled patients were examined and volumes of periapical radiolucency were measured to calculate the percentage of volumetric reduction to evaluate healing. Measurements of pre- and post-operative lesion volume were done twice by a single observer. During follow up, symptomatic cases with no improvement were scheduled for apical surgery. Radiographic outcome was also presented in 3 categories: completely healed, reduction in size of lesion (incompletely healed) or no improvement according to [13] *Zhang et al., 2021.* Follow up of healing time points were at 1-year +/- 2 months, and 2-year +/- 2 months

Steps for CBCT volumetric measurement were done according to the following three sequences: First: Fusion (superimposition) between pre- and post-operative images, Second: In the fused image, scrolling between pre- and post-operative images to reveal the same level of cut, figure (1), Third: Volumetric measurement of apical radiolucency in pre- and post-operative CBCT images and calculation of percent volume reduction as follow: the lesion-volume was divided into 3 or 4 horizontal cuts in sagittal plane according to size of radiolucency, followed by measuring the volume of each cut separately. The non-uniform area outline was traced manually then the calculated area was multiplied by

the height. For each cut, the procedure was done on pre-operative image and post-operative image to measure pre- and post-operative volumes. Summation of the volumes of pre-operative cuts gave the whole preoperative volume of the radiolucency. While summation of the volumes of post-operative cuts gave the final volume of the whole radiolucency. An example of the detailed steps for one of the cases is shown in Figure (2).

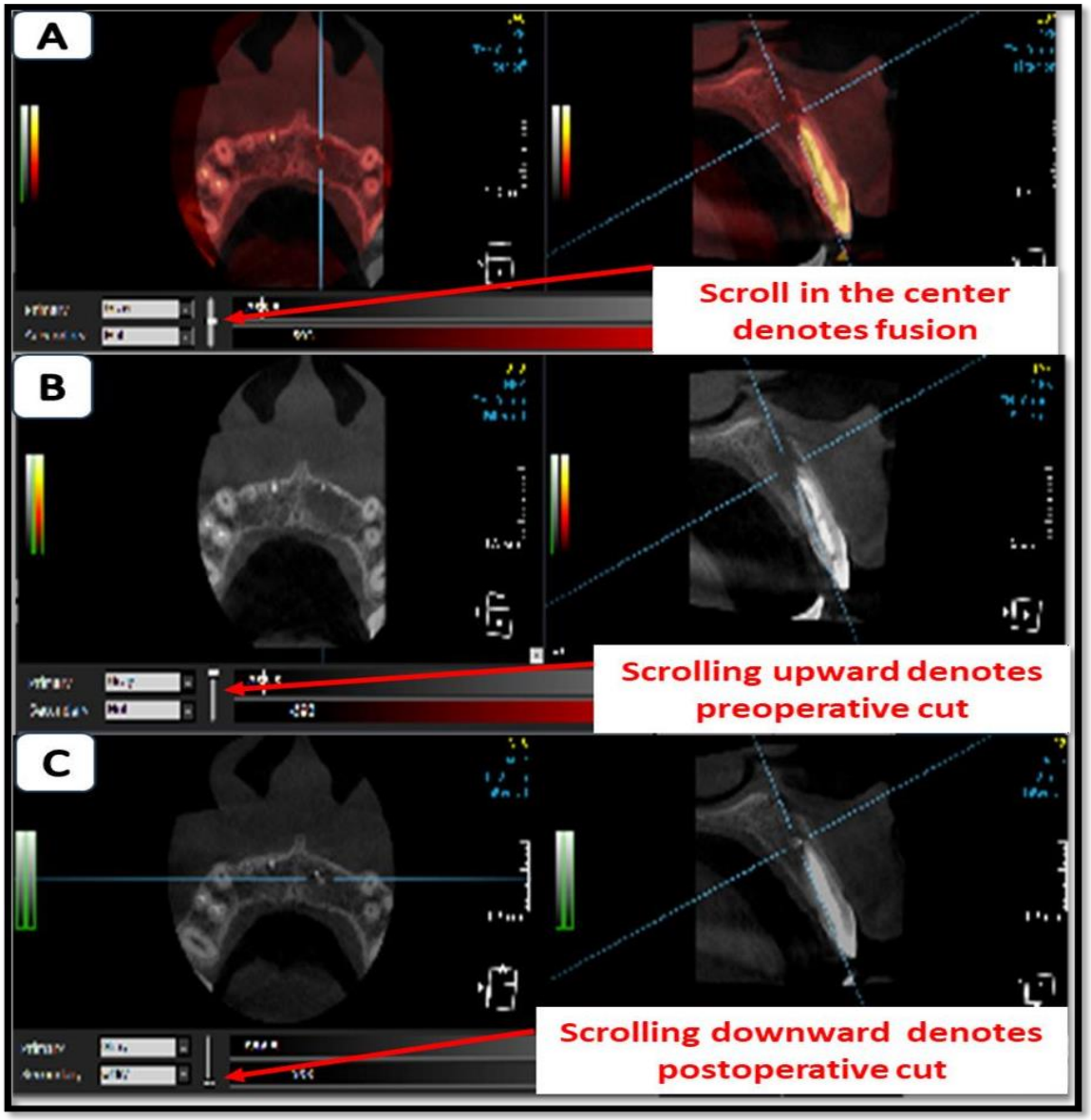

Figure 1. In the fused image; scrolling between pre- and post-operative images to reveal the same level of cut (A) Fused CBCT view. (B) scrolling upward to show Pre-operative CBCT view at certain cut. (C) scrolling downward to show Post-operative CBCT view at the same level of cut of pre-operative one.

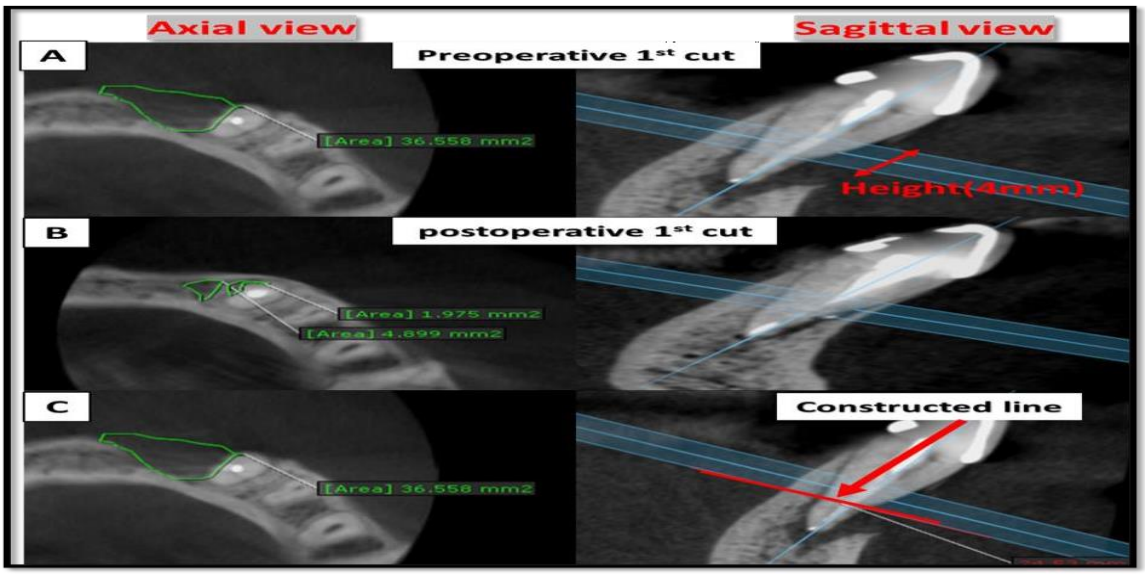

Figure. 2. (continued): (D) Volume of 2nd pre-operative cut (V2) =Height of 2nd pre-operative cut (4mm) from sagittal view multiplied by the traced area from axial view (30.5 mm^2), thus V2= 122 mm^3. (E) Volume of 2nd post-operative cut (P2) = height of 2nd post-operative cut (4mm) from sagittal view multiplied by the traced area from axial view (6.4 mm^2), thus P2 =25.6 mm^3. A Line was constructed to move to 3rd cut. (F) Volume of 3rd pre-operative cut (V3) =Height of 3rd pre-operative cut (4mm) from sagittal view multiplied by the traced area from axial view (14.5 mm^2), thus V3 =58 mm^3. (G) Volume of 3rd post-operative cut (P3) = height of 3rd post-operative cut (4mm) from sagittal view multiplied by the traced area from axial view (5 mm^2), thus P3 =20 mm^3. The following calculations were then done First: total volume of pre-operative radiolucency = sum of volumes of 1st, 2nd and 3rd pre-operative cuts V1+ V2+V3= (146+122+58) = 326 mm^3. Second: total volume of post-operative radiolucency= sum of volumes of 1st, 2nd and 3rd post-operative cuts P1+P2+P3 (26.8+25.6+20) = 72.4 mm^3. Third: % volume in

Statistical analysis: Numerical data were explored for normality using (Kolmogorov-Smirnov and Shapiro-Wilk tests). 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. Chi-square test and Fisher's Exact test were used for categorical data. 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 (4) represented flow diagram of the study.

RESULTS

Regarding postoperative pain assessment, all cases were analyzed with no drop out. Concerning postoperative healing assessment: Initially 42 single-rooted teeth were included in the current study. Figure (3) shows flow charts of recalled cases for healing assessment part. Drop- out involved 10 patients per group at the 2-year time point due to loss of contact and extraction as the process of study was in the period of epidemic COVID-19. Along 1-year and 2-year follow ups a total of five patients reported extraction due to fracture (2 from chitosan NP group and 3 from $Ca(OH)_2$ group.

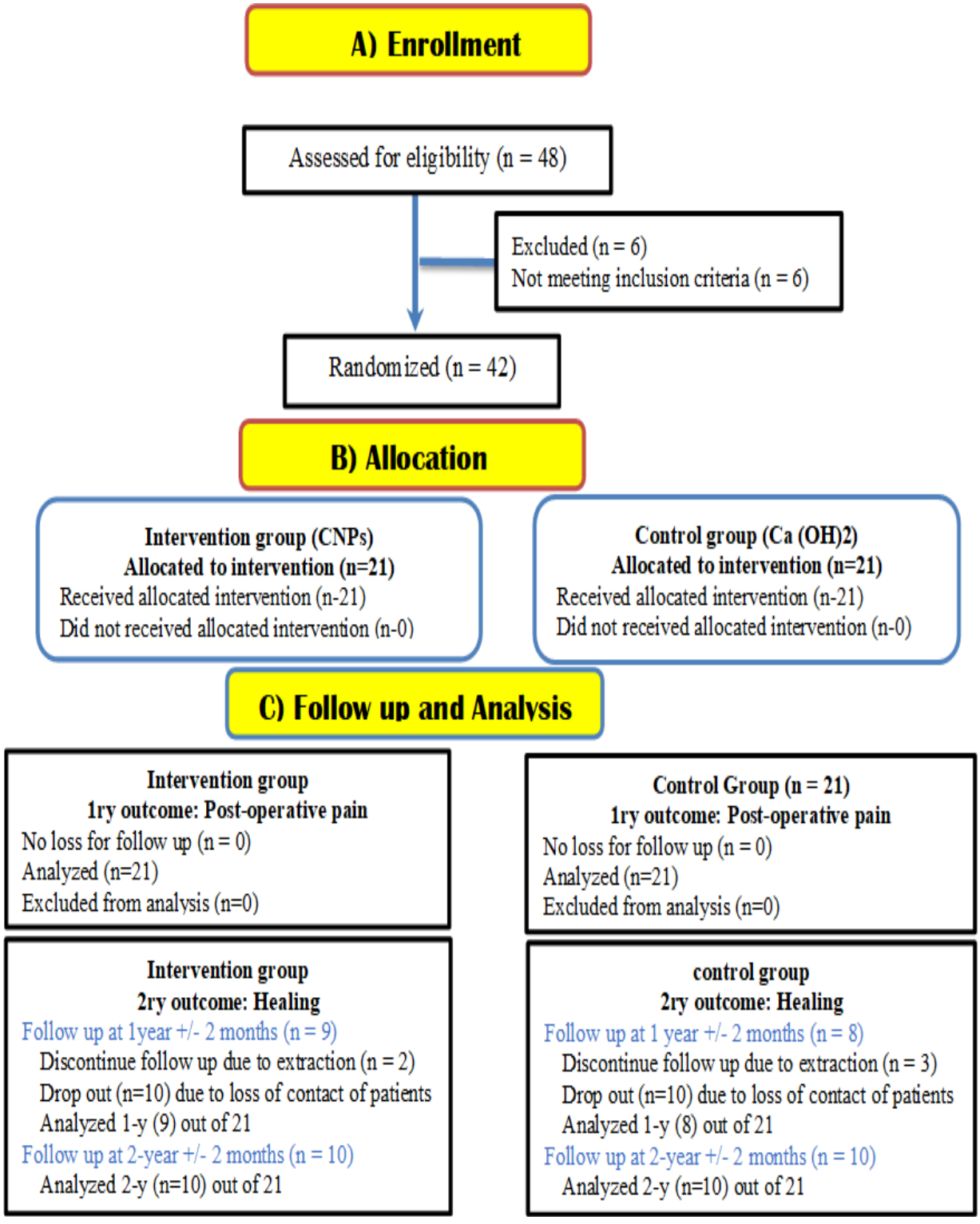

Figure. 3 CONSORT flow diagram of the present study design.

1. **Base line characteristics:** Statistical analysis of baseline data; age, sex, tooth type, number of canals and pre-operative pain intensity for the two 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 and calcium hydroxide $Ca(OH)_2$

	Chitosan NP (n = 21)	$Ca(OH)_2$ (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)	0.962
Maxillary lateral	5 (23.8)	5 (23.8)	
Maxillary canine	4 (19)	3 (14.3)	
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 $P \leq 0.05$*

2. Outcome data

Assessment of Pain (NRS) scores, *Table (2).*

Comparison between groups at each time point, non-significant statistical difference existed between pain scores in the two groups preoperatively (median score 6). At each post-operative time point; median pain scores of Chitosan NP ranged from 2 (at 6 & 12 hours) to 0 (at all other time points), while in $Ca(OH)_2$, it was 0 at all postoperative time points with no statistically significant difference $P \geq 0.05$ between groups at each time point

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

In both groups; there was a statistically significant decrease in median pain scores from preoperative to 6 and 12 hours. Median pain score of chitosan NP dropped from 6 (preoperative) to 2, while median pain score of $Ca(OH)_2$ dropped from 6 (preoperative) to 0. In Chitosan NP; Day-one to day-7, showed non-significant difference; all having median score of 0, range 0-10. On the other hand, in $Ca(OH)_2$, statistically significant decrease existed between day-7 (median 0, range 0-6) and all other postoperative time points; all having median score of 0, range mostly 0-10.

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*

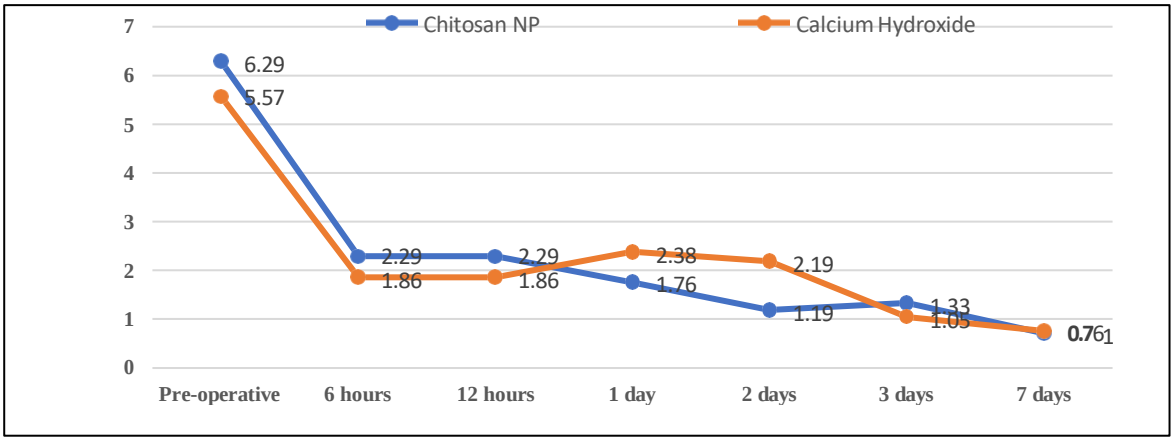

Figure 4. Line chart representing mean pain (NRS) scores in along the time points in the two group. Regarding Chitosan NP, mean pain scores showed a generally decreasing trend starting from pre-operative to 6 hours, stability at 6 and 12 hours, *decrease* along day-one, day-two with sight increase at day 3, followed by resuming decrease to the 7th day.
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 day-one, day-two followed by resuming the decrease at 3, 7 days.

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 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.

1-year and 2-year Healing assessment:

Radiographic CBCT assessment: Assessment of Volumetric changes of periapical lesions of both groups. Cohens Kappa statistical test for intra-observer reliability was calculated to be 0.812

Mean percent volume reduction, table (3): showed non-significant statistical difference between both groups at each time point, (At 1 year: Chitosan NP vs $CaOH_2$ P= 0.591, At 2 year: Chitosan NP vs $CaOH_2$ P= 0.103), as well as within each group at all time points (Chitosan NP 1,2 years P= 0.238, $CaOH_2$ 1,2 years P=0.121).

Number of cases in each category of post-operative healing (completely healed, reduction of lesion size, no improvement) during follow up of recalled cases at 1and 2 years is also shown in, table (3).

Regarding Chitosan NPs group: At 1 year follow up; mean percent volume reduction was 73.38% (total healed 3/9 (33.3%) and incomplete healing 5/9 (55.5%) increased to 81.37% at 2-year follow up (total healed 5/10

(50%) and incomplete healing 4/10 (40%).

Regarding Ca (OH)$_2$ group: At 1 year follow up; mean percent volume reduction, was higher than in Chitosan NP reaching 85% (total healed 4/8 (50%) and incomplete healing 4/8 (50%) increasing to 99.3% at 2-year follow up (total healed 9/10 (90%) and incomplete healing 1/10 (10%).

Table 3. Descriptive statistics and results of Mann-Whitney U test for comparison between percent volume reduction between the two groups/time interval, and for each group at both time intervals, as well as Fisher Exact Test comparing **between incidence of success represented of healed and incompletely healed lesions between the two groups**

Chitosan NP			Calcium Hydroxide			P-value
1-y (n = 9)	% Volume reduction; mean (SD)	73.38% (32.66)	1-y (n = 8)	% Volume reduction; mean (SD)	85% (30.78)	0.591
	Total heal: n(%)	3 (33.3%)		Total heal: n(%)	4 (50%)	
	Incomplete: n(%)	5 (55.5%)		Incomplete: n(%)	4(50%)	1
	No improvement: n(%)	1 (11.1%)		No improvement: n(%)	0	
2-y (n = 10)	% Volume reduction; mean (SD)	81.37% (34.4)	2-y (n = 10)	% Volume reduction; mean (SD)	99.3% (2.21)	0.103
	Total heal: n(%)	5 (50%)		Total heal: n(%)	9 (90%)	
	Incomplete: n(%)	4 (40%)		Incomplete: n(%)	1 (10%)	0.14
	No improvement: n(%)	1 (10%)		No improvement: n(%)	0	

Defining success for cases who attended follow up.

According to *Zhang et al.* [13], Success was defined when the lesion size was completely healed or decreased in size, while the failure was defined when the lesion size showed no improvement or increased in size.

Regarding the Chitosan NPs group, at 1-year follow up, 8 of 9 (88.8%) were considered success, while 1 of 9 (11%) was considered failure. At 2-year follow-up, 9 of 10 (90%) were considered success, while 1 of 10 (10%) was considered failure.

Regarding the Ca (OH)$_2$ group, at 1-year follow up, 8 of 8 (100%) were considered success. At 2-year follow-up, 10 of 10 (100%) were considered successful.

In cases considered to be "success", comparing percent of healed versus incompletely healed at 1- year and 2- year follow up, revealed that in both groups, the percentage of healed cases increased at 2- year follow up relative to 1-year follow up, where **Ca (OH)$_2$ group** was higher (90%) than **Chitosan NPs group** (50%), Table (3)

DISCUSSION

Post-operative 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 medications 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 [14], and prevent contamination between appointments. Decreasing the level of pro-inflammatory cytokines and Matrix Metalloproteinase 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 [15] as revealed by post-operative increased levels of vasoactive intestinal peptide.

Even through Calcium hydroxide paste Ca (OH)$_2$ is the most commonly used medication in endodontics [16], its antibacterial effectiveness has been questioned by Sathorn et al [17]. Ca (OH)$_2$ showed limited antifungal properties against *Candida Albicans*, and its effectiveness against *Enterococcus Faecalis* biofilm is controversial [18]. Furthermore, there is concern that it might decrease fracture resistance of root dentine over time [19].

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 [20]. 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.* [21] 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 [7]. Unfortunately, Chitosan has limited tubular depth of penetration which might hinder the antimicrobial effect in complex canal areas [22].

The era of nanotechnology produced nanoparticles with a wide variety of polymers and nanotechnology. Chitosan NP gel was produced with its possible promising application as intracanal medication. Chitosan NPs 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 [23]**.** 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 [24.]

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 and on long-term healing of periapical lesions in retreatment cases

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; symptomatic apical periodontitis, and third: presence 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 [25] had demonstrated that the cases with periapical lesions showed more post-operative pain, compared to 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 [26,27]. The use of multirooted teeth was avoided because of some confounding factors such as complex anatomy, extra roots, extra canals, root curvature and apical ramifications.

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. Screening of presence the periapical lesion was done on periapical radiograph Similar to *Karaoğlan et al* [28]. Lesion size measurement was selected to be not less than 2mm x 2mm using digital software linear measurement tool.

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.* [28] used Gates Glidden burs and Hedstrom files in retreatment cases.

Gutta percha solvent was not used in the current study, 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.* [26] avoided the use gutta percha solvent in retreatment to avoid unfavorable results on post-operative pain.

In the present 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 [7]. 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 [29].

ProTaper Universal system was used for re-instrumentation of root canal, according to manufacturing instructions, in a crown down technique. Motlani et al [30] 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 [31]. Similarly, Angın et al [26] 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 32 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 [12, 27, 28]. In the current research a minimal concentration of 2.6% was preferred to be used to avoid higher toxicity and tissue irritation reported by Marion et al.[33].

In the current study, two -visit endodontic retreatment was preferred to ensure post debridement and symptom-free period before obturation[34]. Moreover, patients on a single visit retreatment were more likely to take pain killers and more liable to flare up. Similarly, *Hepsenoglu et al.* [12] 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.

Pain was recorded pre-operatively and post-operatively; at six different time points 6, 12, 24, 48 hours, 3, and 7 days [26, 27]. 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 [35]**.** 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 [36]. Inflammatory activity may also originate from infection irritating the periapical area and progressing to inflammation and swelling. As the cause (infection) is eliminated and as time passes, this inflammation tends to diminish, leading to a natural decrease of pain in days and weeks following root canal treatment.

Analgesics were only prescribed in case of severe pain. 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*. [26] prescribed ibuprofen in case of severe pain.

Regarding results of the present study, comparing post-operative pain scores between both groups at each time interval stated no statistically significant differences. Emphasizing that the manipulation of 0.2% chitosan NP as an intracanal medication had almost similar effect as the gold standard Ca $(OH)_2$ paste, in terms of post-operative pain.

For both groups, changes from pre-operative (chitosan NP: median: 6, mean: 6.29), (Ca $(OH)_2$ median: 6, mean: 5.57), to 6- and 12-hour 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). Present study results were similar to *Ahmed et al.* [27] who revealed no pain at all time interval 6, 12 hours, 1. 2. 3. and 7 days when using Ca $(OH)_2$ as intracanal medication in retreatment cases. Interestingly for Chitosan NP, score 2 can be translated to clinically acceptable level of "mild pain" category.

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. Chitosan NP 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 [23].

Both chitosan NP and Ca $(OH)_2$ reduce postoperative pain to patient-tolerable levels, selection of chitosan NP might be the recommended choice because of the trend nowadays to utilize natural products and due to extra versatile properties over Ca $(OH)_2$. Pain free treatment is still a dentist's dream which should be prevailed by further randomized clinical trials with varying properties of Chitosan NP 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 Chitosan NP as a carrier for other ICM to gain its benefits as increased dentinal tubular depth of penetration.

The study revealed low incidence of flare up with both intracanal medications, with no statistically significant difference between the two groups, represented by two patients per group (9.5%). This comes in agreement with *Hussein et al.* [37] that showed no significant difference in prevalence of flare up with Ca $(OH)_2$ intra-canal medication. *Fahim et al* [38] showed the incidence of flare up after application of Ca $(OH)_2$ in 3 cases (13%).

The use of prescribed emergency analgesic succeeded in controlling this post-operative pain phase similar to previous study [39]. 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.

In the present study, the method used for volumetric measurement of apical lesion size, depending on CBCT, was a combined manual and semi-automatic technique starting by fusion of pre-operative and post-operative CBCT images to ensure exact superimposition. Standardization of the level cut of pre- and post-operative images ensured measuring at the exact lesion levels. Volumetric measurement of apical radiolucency in pre- and post-operative CBCT images used a simple mathematical rule: volume = area X height. The lesion area at each level was traced manually and its area calculated then

multiplied by the measured height. This method was easy, reliable, and did not need expensive software. It was even suitable for this study because of the presence of some cases with labial perforated lesions with discontinued outline. In these cases, the outline was emphasized and projected manually before the procedural steps for volume measurement.

The volume was calculated twice by the same operator. Cohen's kappa coefficient test was used to detect intra-observer reliability [40]. Intra-observer reliability was referred when repeated measurements on the same subject by the same examiner were taken. Kappa (κ) is a statistical measure used to quantify the level of agreement. Present study Cohen's Kappa (κ) test gave a positive correlation as the value was 0. 812.Cohen's, which ensured intra-observer reliability.

On the other hand, there are several CBCT software with full automated methods for volumetric measurement of periapical lesion like AMIRA software [13] Or OsiriX Lite software [41]. Automated segmentation software volumetric measurement takes less time to be performed. It presents slightly higher precision. However, they are costly and may still need a degree of manual intervention for non-outlined lesions; as in cases of the present study having perforated lesions involving labial cortical bone.

In the present study, percent volume reduction was calculated, furthermore successful lesion healing was dichotomized into complete healing and incomplete healing. Success was adopted from studies that provided definition of success to be based on both healed (complete regression of periapical lesion) and healing cases (decreasing in periapical lesion size) [13], [28]. Though *Mosquera -Barreiro et al.* [41] considered the treatment to be successful when complete resolution of periapical lesion presented (total lesion healing).

In the present research, nearly 50% were not available for follow-up of healing; and were defined as "dropouts. They could not be contacted or due to extraction as the study was conducted during epidemic Covid 19 condition and afterwards. In fact, there was an initial attempt to set follow up at 6 months and at one year, however, only three patients were able to be recalled at 6 months, while a relatively larger number were recalled at 1 year ± 2 months. Also, *Zhang et al.* [13] reported 35% drop-out when evaluating the healing in retreatment cases for 4 years.

In the present study, both groups presented high mean lesion volume reduction at 1- and 2- year. Furthermore, at 2-year follow up both groups (Ca (OH)2 = 99.3%, CNPs = 81.37%) presented higher values than at 1-year (Ca (OH)2 = 85%, CNPs = 73.38%).

In the current work, at 1-year follow up, in Ca $(OH)_2$ group mean percent Lesion volume reduction was 85%. *Castro Rizzi-Maia et al.* [42] revealed a close value of 79.25% by CBCT after 1-year in 2-visit retreatment cases using Ca $(OH)_2$ as intracanal medication.

While at 2- year follow up, mean percent Lesion volume of Ca $(OH)_2$ increased to 99.3**%.** *Karaoğlan et al.* [28] revealed a slightly lower cumulative success rate was 91.1% for two-visit retreatment at 2 years follow up using calcium hydroxide as intracanal medication. On the other hand, *Zhang et al.* [13] revealed that even after 4 -years follow up the percent lesion volume reduction by CBCT was 84.3%.

In the present study, at 1-year follow up, percentage of completely versus incompletely healed cases of Ca (OH)2 group was 50% versus 50%. However, *Ercan et al.* [43] revealed higher rate of 64.1% of complete healed as verified by periapical radiograph after 1-year in retreatment cases using combined Ca $(OH)_2$ / 1% CHX as intracanal medication. This might be due to synergistic effects of combined Ca $(OH)_2$ / 1% CHX. [41] revealed higher rate of completely healed 76% as verified by CBCT in a mean healing time of 19 months. This might be due to longer follow-up period.

In the current research, 2-year follow up, Ca $(OH)_2$ showed percentage of completely versus incompletely healed cases of Ca (OH)2 group was 90% versus 10%. Similarly, *Karaoğlan et al* [28] showed almost similar healing percent; 86.7% "healed", 4.4% "incomplete healing" at 2 years follow up when assessing two visit root canal treatment with Ca $(OH)_2$ intracanal medication. Moreover, *Zandi et al.* [44] revealed that even after 4 years follow up, percent of healing by digital periapical radiograph in 2-visit retreatment cases using Ca $(OH)_2$ as intracanal medication was 81%.

In the current study, there was one case of no improvement in apical lesion size in Chitosan NPs group at 1 and 2- year follow up. A reason why an apical lesion endures over time despite bacterial eradication, could be related to the different types of lesions [45], or microbial communities in extra-radicular infection [46]**.** Thus, this case of no improvement in apical lesion was scheduled for apical surgery.

According to the present study results: the use of chitosan NP gel is promising. However, still advancement in research targeting improvement of chitosan NPs is encouraged. Further investigations are recommended by combining with other intracanal medication to gain their synergistic antimicrobial effect. It was previously reported Chitosan can be mixed with another intracanal medication to give more antibacterial effect. Merging of Ca $(OH)_2$ and

chitosan NPs as intracanal medication showed highest significant percentages of bacterial death at 14 days compared to Ca $(OH)_2$ alone [47]. Moreover, combination of CHX and chitosan as ICM showed highest bacterial reduction against *Enterococcus Faecalis* after 7 days during endodontic retreatment procedure compared to 2% CHX gel or 2% chitosan when they were used alone [6].

CONCLUSIONS

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

Considering the trend to follow green dentistry; 0.2% chitosan NP gel as an intracanal medication can be promising viable alternative therapy to gold standard Ca $(OH)_2$ paste; for reducing post-operative pain and promoting apical bone healing, in single rooted previously endodontically treated teeth with symptomatic post-treatment disease and apical radiolucency. Advancement in research targeting improvement of chitosan NP as intracanal medication is encouraged.

DECLARATIONS

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ETHICAL APPROVAL

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.

Competing Interests

The authors have no competing interests to declare.

Informed Consent

Not applicable.

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