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Impact of Nanotechnology as Irrigation Protocol for Revascularization of Immature Necrotic Teeth on Postoperative Pain

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ABSTRACT
Aim: This study aimed at comparing postoperative pain following photosensitized chitosan nanoparticles versus conventional protocol of disinfection technique in regenerative endodontic treatment (RET) of necrotic immature teeth.
Methods: Double-blind randomized clinical research evaluated the disinfection efficiency of sodium hypochlorite (1.5%) versus photosensitized chitosan nanoparticles (2%) in 22 patients regarding postoperative pain.
Results: The clinical trial demonstrated no statistically significant difference in postoperative pain between both disinfection techniques.
Conclusion: photosensitized chitosan nanoparticles has a positive effect on the postoperative pain in revascularization of necrotic immature teeth.

Keywords: Regenerative endodontics, Nano-chitosan, diode laser, postoperative pain.

1. INTRODUCTION

Regenerative endodontic therapy is biologically based treatments used in management of necrotic immature teeth as it aims to replace damaged tissue structures. microbial elimination mainly dependent on antimicrobial agents for proper canal disinfection, thus determining the prognosis of the regenerative endodontic therapy(Kim et al., 2018).

Studies have shown that Sodium hypochlorite is potent irrigation material in root canal therapy, but there are major drawbacks associated with its use; being irritant to the periapical tissues and toxic to the stem cells present in the region(Kim et al., 2018).

Bashetty& Hegde, 2010 compared the effect of NaOCl and chlorhexidine on the postoperative pain via using the modified visual analogue scale to assess the severity of pain at 6 and 24 hours and seventh day after treatment. Results showed more pain was associated with NaOCl group.

Chitosan shows a remarkable effective broad spectrum antibacterial action attributed to its cationic nature. It interacts with the negatively charged bacterial cell membranes, leading to leakage of the intracellular constituents and ultimately cell death due to increasing the membrane permeability. Furthermore, it possesses biological properties as being biodegradable, biocompatible and has chelating abilities, making it an interesting alternative to modern root canal irrigants(Yang et al., 2009).

The main concept of photodynamic therapy (PDT) is sensitizing a certain photosensitizer to produce toxic reactive oxygen species that possess an effective antibacterial effect.(Abdel Hafiz Abdel Rahim et al., 2020) Based on this and to overcome the limitation of NaOCL, it was suggested that PDT using Diode laser (810 nm) to activate the photo sensitized nanoparticle chitosan can be used as an endodontic irrigant(Hu et al., 2019).

The null hypothesis is that there is no difference in postoperative pain when using sodium hypochlorite or photosensitized chitosan nanoparticles in disinfection protocol in revascularization of necrotic immature teeth.

2. Participants and methods

Trial design

The trial design was set up as a randomized, 1:1, parallel arm, double blinded, controlled trial.

Ethical consideration
This trial was carried out with the approval of the research ethics committee (REC) no. 86, Clause 2, Research No. 568 of 2022 of the Faculty of Dentistry, Minia University. The trial was registered at Clinicaltrial.gov (NCT06631885).

Sample size
A power analysis was designed (G*Power version 3.1.9.7(Faul et al., 2007)) to assess the null hypothesis. The predicted sample size (n) was a total of (20) cases (i.e.10 cases per group).
Participants: Patients were selected from the outpatient clinic according to the following Inclusion criteria; Medically free Patients., Necrotic Immature maxillary anterior teeth with open apex and Patients who agreed to the consent and committed to follow-up period.

Allocation
- Sequence generation was done using computer-generated random numbers. On (https://www.random.org/), all patients were randomly assigned to one of two groups with a 1:1 allocation ratio
- Group I: Multiple visits standard revascularization technique using sodium hypochlorite (1.5%) – (Clorex, Egypt LTD, Egypt) - for canal disinfection.
- In Group II: Photo-sensitized chitosan nanoparticle (2 % CNPs) - (Nanogate, Egypt) - in the disinfection protocol.

Blinding
The patients were told about the treatment steps (as stated on the consent form) but not which of the treatment protocols would be used.

Preparation and Characterization of chitosan nano particles
Preparation
Chitosan nano particles (CNPs) were prepared according to the ionotropic gelation process (Hasanin et al. 2018). CNPs solution was stored in the refrigerator at 8°C

Characterization
Transmission electron microscope (TEM) was used to check the size and shape of the produced nanoparticles using a JEOL JEM-2100 high resolution TEM at a 200 kV accelerating voltage.

Interventions
Intraoperative procedures: (For all groups)
Patients were anaesthetized using 1.8 ml Mepivacaine HCl 3% - Adrenaline 1:100,0000 – (Alexandria Company for Pharmaceuticals, Egypt). Access cavity was done under rubber dam. Working length was determined using 2D digital intraoral radiography (New IDA intraoral radiographic sensor, Atlanta, Brazil).

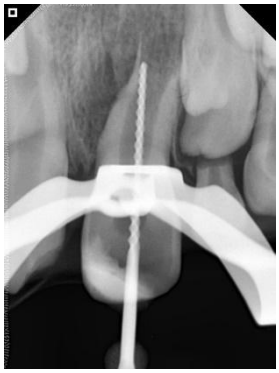


Figure 1: verification of working length using digital intraoral radiographic sensor.

Multiple visits revascularization technique with an interappointment dressing according to the A.A.E Guidelines (2021)(American association of endodontics Clinical Considerations for regenerative endodontics, 2021) was conducted as follows:

First visit
- In Group I: copious, gentle irrigation with (20mL/canal, 5 min) 1.5% NaOCl.

- In Group II: copious gentle irrigation with four cycles of 5 mL each (total of 20 mL/canal, 5 min) of chitosan nanoparticles (2% CNPs) each cycle was followed by disinfection was performed with (810 nm) diode laser - Elexion AG/GmbH, Germany - with power 1 w, 20 ms pulse length, and 20 ms interval duration. for 15 seconds.
- Dryness of the canals was obtained with absorbent paper points (GAPADENT CO., LTD., TianJin City, P.R.China).
- Metapaste Calcium hydroxide (MetabiomedCo.LTD, Korea) was injected into coronal half of the canal(Báez et al., 2022; Bose et al., 2009).
- Access cavity was sealed via resin modifies glass ionomer as a temporary filling till the next visit in two weeks.

Second visit

- The treated tooth was isolated using rubber dam, the temporary restoration was removed, and the canal was reopened and irrigated with saline solution to washout intracanal medication.
- The final irrigation was performed with 20 mL 17% EDTA - (Inodon, Porto Alegre, RS, Brazil) - followed by 10 mL saline solution. Then, a manual K-file 25# - (K-files, Mani, Japan) - was inserted into the root canal and placed 1–2 mm beyond the root apex to induce bleeding for blood clot formation that was stabilized 3-4 mm below the level of the cementoenamel junction where the Gelatemp cellulose matrix (COLTENE/WHALEDENT GmbH + Co. KG) was directly put against which 3-4 mm of the well root putty bioceramic root repair material was placed.
- The access cavity was sealed immediately using Equifil resin modified glass ionomer restoration capsule.
- After the completion of both regenerative endodontic procedures, standardized periapical radiographs were obtained by using the paralleling technique.
- All patients were given postoperative instructions to call the operator if they experienced moderate or severe pain,

Figure 2: Showing immediate postoperative Bioceramic coronal plug.

Follow up: Postoperative pain

All patients were asked to fill out a pain diary accurately and honestly at 24 hours, and 1 week after regenerative endodontic procedures, then get it back to the operator on time. The pain levels were recorded using NRS that was translated into Arabic. The NRS is an 11-point scale with two extremities: "no pain" and "pain as awful as it gets." None (0), Mild (1-3), Moderate (4-6), and Severe (7-10) were used to categorize the pain levels. Participants were asked to choose the score that best reflected their pain level. (McCaffery et al. 1989).

Statistical methods

Statistical analysis was performed using a commercially available software program (SPSS Chicago, IL, USA). Categorical data will be described as frequencies. Data was compared using chi square test. The level of significance will be set at $P < 0.05$.

RESULTS

A total of 24 male patients were assessed for eligibility, 4 of them declined to participate. Twenty teeth in twenty patients were randomly allocated into two equal groups: control and test groups (n=10 each).

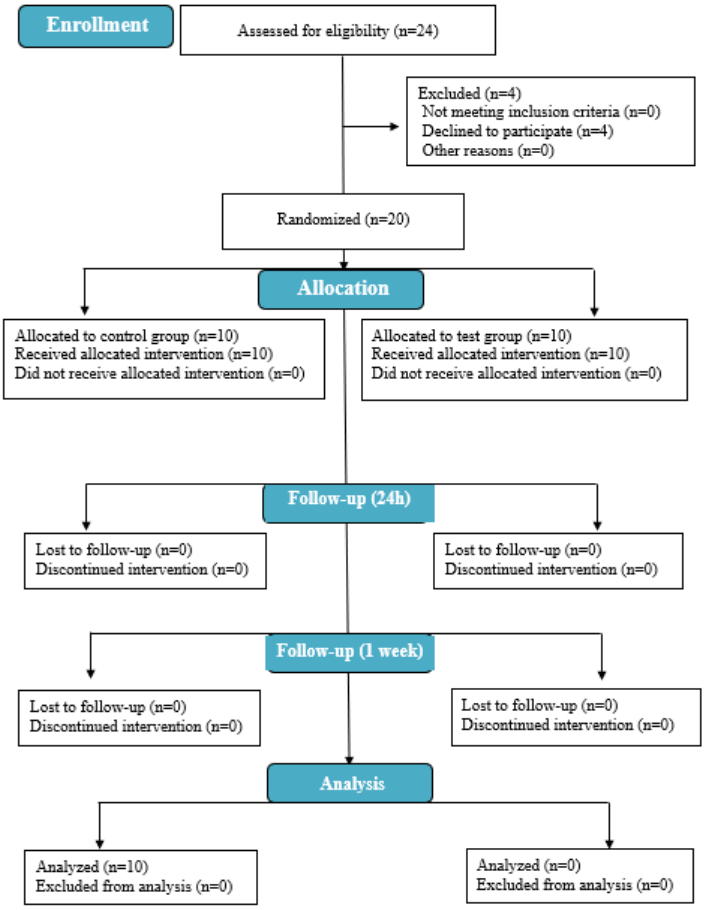
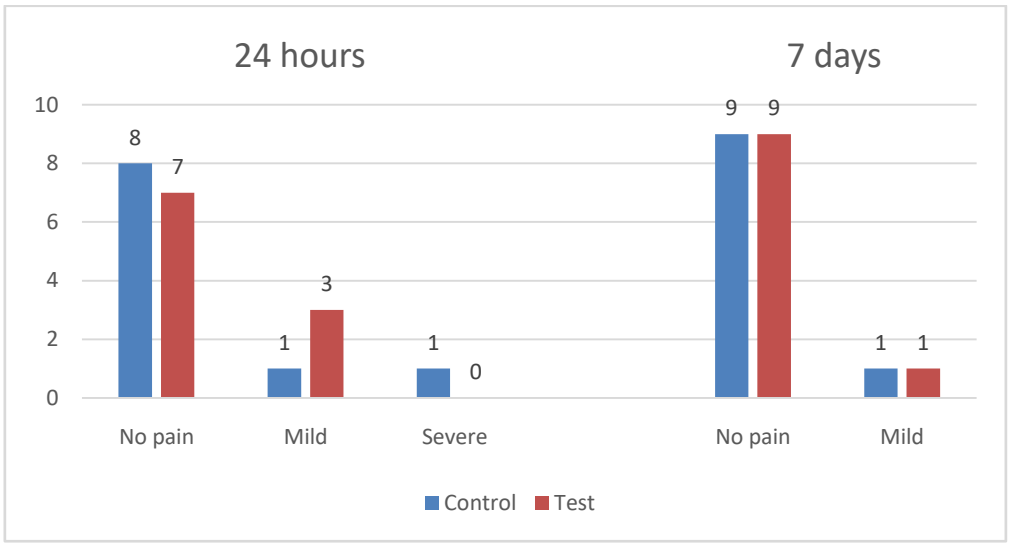


Figure 4: Consort flow chart of the study

Pain incidence categories at 24 hours, 7 days postoperatively:
no statistically significant difference in incidence of pain categories one week postoperatively between the two groups. (P > 0.05). (Table 2.) (Figure 5).
Regarding the change in pain intensity, among the control group; the pain was reduced in one patient who had a mild pain in the 24 hours follow up to no pain in the one week follow up, and another patient who had severe pain in the 24 hours, the pain intensity changed to mild pain in the one week follow up. while among the test group the pain intensity changed in two patients from mild pain in the 24 hours follow up to no pain in the one week follow up.

Table 2: Frequencies, percentages and the results of Chi square test for comparison of incidence of pain categories at 24 hours, 7 days, postoperatively between the two groups:

		Control		Test		P-value
		Frequency	%	Frequency	%	
24 H	No pain	8	80%	7	70%	0.356 ns
	Mild	1	10%	3	30%	
	Severe	1	10%	0	0%	
7 days	No pain	9	90%	9	90%	0.763 ns
	Mild	1	10%	1	10%	



Figurer 5: Pain incidence categories at 24hours, 7 days postoperative

DISCUSSION

Regenerative endodontic procedures are defined as a biologically based procedure to replace damaged tissues in the dentin-pulp complex with viable tissues. Its primary goal is the elimination of clinical signs and symptoms and the resolution of apical periodontitis (Jung et al., 2019).

In REP, stem cell preservation is crucial. However, regeneration and repair will not take place if infection is not controlled. By harming both tissue-forming cells and stem cells in the periapical tissues, a previous infection may have a detrimental effect on the regeneration of pulp tissue(Kim et al., 2018).

Therefore, disinfection of the root canal system seeks to eliminate bacteria, remove irritants, and produce an environment that is conducive to tissue regeneration. In addition to being bactericidal, irrigation solutions need to encourage the survival and development of stem cells. (Elgendy & Fayyad, 2017).

Sodium hypochlorite is the main endodontic disinfecting irrigant owing to its high antibacterial effect and tissue dissolving effect, however it possesses a major drawback due it's detrimental effect on stem cell survival and differentiation when used in high concentrations. This justifies the continuous search for new biological irrigation solutions and techniques.

Positively charged nanoparticles like chitosan can break down negatively charged bacterial cell walls through electrostatic contact(Yang et al., 2009). Chitosan nanoparticles were able to penetrate the biofilm structure (seven-day-old E. faecalis biofilm) and considerably decrease the number of bacteria present, according to a discovery made using confocal scanning microscopy(Shrestha et al., 2010).

Owing to chitosan's unique qualities and potential use in dentistry, particularly in endodontic treatment, deacetylated polysaccharide chitosan has been the focus of extensive research. Regarding to the effect of chitosan on the dental stem cell viability, it was proved by (Belkadi et al., 2024) that chitosan nanoparticles irrigation solution (0.2%) showed the least cytotoxic to Dental stem cells compared to 2.5% sodium hypochlorite which showed the most cytotoxic followed by 17% EDTA. Additionally, (Mostafa et al., 2022) reported that chitosan nanoparticles (2%) were able to encourage periodontal ligament stem cell viability.

Regarding the postoperative pain, both groups showed no statistically significant difference in incidence of pain categories at both 24 hours, and one week postoperatively. This result came in accordance with the previously evidence based published clinical trials in REP (Alfahadi et al., 2022; Youssef et al., 2020).

The significant decrease in postoperative pain may reflect the antibacterial effectiveness of the used disinfection protocol which has a great role in enhancing the host immunomodulation, decreasing the tissue damage and reduction of the release of proinflammatory cytokines and pain mediators, and enhancing the periapical wound healing mechanism(Hussein & Kishen, 2022).

CONCLUSION

Photosensitized chitosan nanoparticles can be used as a biocompatible alternative in disinfection of root canal in revascularization of immature necrotic teeth.

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