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Evaluation of Postoperative Pain after Using Antimicrobial Silver and Chitosan Nanoparticles: A Randomized Controlled Clinical Trial

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Abstract

The study aims to assess and compare the impact of using silver or chitosan nanoparticles on postoperative pain in treating endodontic patients. Seventy-eight patients eligible for endodontic treatment were recruited and divided into three groups (n=26) according to the type of irrigation material used silver nanoparticles (group A), (group B) chitosan nanoparticle irrigation and 2.6% NaOCl and 17% EDTA sol control group (Group C). Pain scores were recorded at 6, 12, 24, 48, and 72 hours post endodontic treatment. The Wilcoxon test was used to compare two sets of samples that were linked and the Kruskal Wallis test was used to compare more than two groups of unrelated samples. There was no statistically significant difference between all three groups preoperatively and after 72 hours at p=0.301 and p=0.081, respectively. While after 6, 12, 24 and 48 hours there was a statistically significant difference between the control group and each of (Silver NP) and (Chitosan NP) groups at ($p<0.001$). However, there was no statistically significant difference between (Silver NP) and (Chitosan NP) groups at the aforementioned different time intervals. Silver and chitosan nanoparticles have comparable effect on pain level after endodontic treatment.

Keywords: Silver-Nanoparticles, Chitosan-Nanoparticles, Clinical Trial, Postoperative pain.

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1. Introduction

The nature of endodontic infection is widely recognized to be polymicrobial, involving both anaerobic and fungal species [1]. Therefore, our aim was to establish a germ-free root canal system, in order to facilitate optimal healing. While complete eradication of microorganisms is challenging due to the complexities of the root canal system, reducing bacterial load through the use of various irrigating solutions may be beneficial. Additionally, obturation helps to encapsulate any remaining resistant bacteria [2]. The colonization of many kinds of bacteria in biofilm, formation of a smear layer during instrumentation, the intricate topology of the root canal system, and the persistence of microorganisms in dentinal tubules are some of the major reasons for failure and reinfection in endodontic treatment [3]. Postoperative pain is also common in endodontic therapy, with rates ranging from 3% to 58% in single and multiple-visit treatment. Sodium hypochlorite exhibits a broad spectrum of antibacterial activity, making it effective against various microorganisms. However, it also has certain drawbacks, including its toxicity, the potential for tissue damage, an unpleasant taste, limitations in eliminating all germs present in infected canals, and the possibility of altering the structure of the dentinal canal walls [4]. Nanoparticles-based antimicrobial agents represent a significant class of nanomaterials with applications in biological settings. The popularity of nanoparticles as antibacterial agents stems from their broad range of action and biocompatibility. Due to their polycationic/polyanionic composition, nanoparticles possess a larger surface area and charge density, enabling increased interaction with bacterial cells. Silver nanoparticles exhibit distinctive physicochemical and biological characteristics alongside their antibacterial properties [5]. Additionally, chitosan nanoparticles are anticipated to have a more pronounced antibacterial effect compared to regular-sized chitosan due to their ability to penetrate and disrupt microbial cell membranes [6]. Thus, the aim of the current study is to assess and compare the impact of using silver or chitosan nanoparticles on postoperative pain in treating endodontic patients.

2. Materials and methods

2.1. Ethical Considerations

This study protocol was approved by the research ethics committee of the Faculty of Dentistry, Minia University in Egypt and was given an approval code (RHDIRB2017122004) (438) on the 11th of September, 2020. The study protocol was also registered on clinicaltrials.gov under the number NCT06172023. All participants were properly informed about the therapy methodology, study purpose, possible side effects, and treatment alternatives. Participants were asked to follow the general instructions and sign a site-specific printed informed consent.

2.2. Study Design and Grouping

The current study took place between the year 2020 and the year 2022. The participants were recruited from the outpatient clinic of the endodontic department at the Faculty of Dentistry, Minia University. A parallel randomized clinical trial design was adopted in this investigation. The participants (n=78) were divided into 3 groups according to the type of irrigation material used. The first experimental group (group A) (n=26) received irrigation with silver nanoparticles. The second group (group B) (n=26) received experimental chitosan nanoparticle irrigation. Finally, irrigation with 2.6% NaOCl and 17% EDTA sol was administered to a third control group (Group C) (n=26).

2.3. Sample Size Calculation

A total sample size of 60 patients was computed and divided into three groups (20 patients each) using a power of 80% and a significance level of 5% according to the end variable of the study of Gundogar et al., (2021) which was the postoperative pain determined by the visual analogue scale (VAS) [7]. This number was increased to 66 patients (22 in each group) to accommodate for the necessity to use a nonparametric test. To compensate for losses during follow-up, the number of patients were increased to 78 (26 in each group). G* was utilized to calculate the sample size in PS (Power and sample size).

2.4. Eligibility criteria

2.4.1. Inclusion criteria

- Medically stable healthy patients.
- The patients ages range from 20 to 40.
- Necrotic pulp with symptomatic apical periodontitis.
- Enough crown structure to provide adequate isolation.
- Single rooted tooth with a single canal.
- Patients' desire to engage in this research.
- Patients' comprehension of the visual analogue scale (VAS).
- Patients' ability to sign an informed consent.

2.4.2. Exclusion criteria

- Endodontic treatment for the tooth previously.
- Teeth with poor conditions for using rubber dams.
- Vital pulp tissue was observed throughout the treatment.
- Patients who have a debilitating medical condition.
- Teeth with open apices that are immature
- Women who are pregnant or breastfeeding.
- Psychologically disturbed patients.
- Patients having a history of allergy to any of the research drugs were barred from participation.
- A periodontally affected tooth with grade 2 or 3 mobility.

2.5. Randomization, Sequence Generation and Blinding

Randomization took place using the random.org website. The sequence generated was concealed and remained secured on a cloud storage owned by the supervisor of the trial. All study participants were blinded as well as outcome assessors and the data analyst.

2.6. Procedural Steps

Recruited patients with eligible tooth to be treated were clinically and radiographically diagnosed. Teeth were tested for vitality by using thermal and electric pulp testing. In the first visit, teeth were isolated using a Hygenic rubber dam – Coltene, Switzerland and the surfaces were swabbed with sodium hypochlorite. The access cavity was then prepared using a coolant. The working length was determined using J. Morita, Japan electronic apex locator and was confirmed with a parallel radiographic image using Acteon Sopro digital sensor, France. Root canal shaping was done using the crown down method using a Saeshin Cordless Traus Endo Motor, Korea with a Revo S rotary system. France, with a speed range of 250 to 400 rpm and torque of 2.6N/cm. The root canals were irrigated at a depth of 2-3 mm short of the working length using a 30-gauge side perforated needle (NaviTip Sideport 31 G/27 mm) and 6 ml irrigating solution (1 ml irrigating solution in between each file with a flow rate of 1 ml per 10 seconds) according to the irrigating solution allocated to each group. Distilled water was used as a final flush to irrigate all root canals in each group. To close the access cavity, a sterile cotton pellet and a temporary filling was utilized till a second visit scheduled after seven days. At that time obturation was carried out using a Protaper Next standard gutta-percha cone and resin sealer.

2.7. Outcome Measures

2.7.1. Postoperative pain assessment

Each patient was given a pain scale chart (VAS scale) before and at 6, 12, 24, 48, and 72 hours post endodontic treatment to record his or her pain level.

2.8. Statistical analysis

The data was tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests, which indicated a non-parametric distribution. The Wilcoxon test was used to compare two sets of samples that were linked. The Kruskal Wallis test was used to compare more than two groups of unrelated samples. The significance threshold was set at P 0.05. For statistical analysis, IBM® SPSS® Statistics Version 20 for Windows was employed.

3. Results

Table 1 & Figure 1 represent pain level at the different tested time intervals.

Pre-operative pain as well as after 72 hours, the results showed that there was no statistically significant difference between all three groups at p=0.301and p=0.081, respectively. After 6, 12, 24 and 48 hours there was a statistically significant difference between (NaOCl and EDTA) group and each of (Silver NP) and (Chitosan NP) groups at (p<0.001). However, there was no statistically significant difference between (Silver NP) and (Chitosan NP) groups at p=0.444, p=0.307, p=0.387 and p=0.311 at the different time intervals, respectively. For Silver NPs group, the mean pain score decreased after 6 hours to 12 hours, then it decreased again after 24 hours. It continued to decrease till it finally reached (0.35±0.80) after 72 hours. There was a statistically significant decrease in the intensity of pain at different time intervals (p<0.001). Similarly, in Chitosan NPs group, the mean pain score decreased after 6 hours to 12 hours, then it decreased again after 24 hours. It continued to decrease till finally it reached (0.00±0.00) after 72 hours. There was a statistically significant decrease in the intensity of pain at different time intervals (p<0.001). For NaOCl and EDTA group, the mean pain score decreased after 6 hours to 12 hours, then it decreased again after 24 hours. It continued to decrease till it finally reached (0.00±0.00) after 72 hours. There was a statistically significant decrease in the intensity of pain at different time intervals (p<0.001).

4. Discussion

The root canal system is intricate due to the presence of isthmuses, ramifications, fins, apical delta, lateral canals, dentinal tubules, and other features. These environments promote the formation of bacterial biofilms and influence the outcome of endodontic treatment [8]. Bacteria cause the development of all pulp and periapical disorders. If the root canal system is thoroughly debrided and the root canal gap is effectively sealed, endodontic treatment may be successful for a long period [1]. Bacteria in biofilm are very resilient to severe growth and environmental conditions [9-10]. Root canal shaping only removes 50% of germs from the root canals, while antimicrobial treatments such as sodium hypochlorite remove 80% of bacteria. As a consequence, irrigation is necessary during the chemo-mechanical approach for removing bacteria and cleansing the root canal system [11]. The most important role of any root canal irrigant is to help in the eradication of any microbes and biofilm from un-instrumented surfaces, which account for around 30-50% of the root canal walls. The optimum root canal irrigation should be efficient against biofilms, non-toxic, and non-caustic on periodontal tissues [12]. Sodium hypochlorite (NaOCl) is considered the gold standard irrigant because to its high antibacterial activity and tissue disintegrating capabilities; yet, it has certain drawbacks due to its caustic and poisonous effects on critical tissues. It also causes dentin collagen denaturation and disintegration, which reduces the flexural strength and elastic modulus of dentin [13-14]. NaOCl cannot entirely destroy the bacteria in the biofilm. Therefore, chelating agents are utilized with NaOCl to remove the smear layer created during the shaping processes and open the dentinal tubules, enabling the antimicrobial solution to reach any inaccessible places. Although ethylene diamine tetraacetic acid (EDTA) is a commonly used agent, its antibacterial efficiency against biofilms is questionable [15]. Chelating substances have the potential to change the unique structure of dentin since prolonged contact may result in significant loss of both peritubular and intratubular dentin, affecting mechanical integrity and increasing the possibility of bacterial adhesion to collagen. Furthermore, EDTA is classified as a pollutant in the environment [6,14]. Therefore, there is always a persistent need for drugs that have antibacterial activity while causing minimal tissue irritation and having little impact on dentin. The current efforts are focusing on natural materials to decrease toxicity and drawbacks. Chitosan is a non-toxic cationic biopolymer produced from the alkaline de-acetylation of crab exoskeleton chitin [16]. When chitosan is placed to exposed demineralized dentinal collagen, its functional phosphate groups are thought to connect to calcium ions, producing a favorable surface for crystal nucleation. Chitosan may also make dentin less susceptible to collagenase degradation [17-18]. Chitosan nanoparticles chelate against the smear layer, giving them a better alternative to EDTA and its dentin-eroding effects [4]. It also possesses antibacterial activities against a broad range of bacteria and fungi, both gram-positive and gram-negative. Because of its polycationic nature, it has antibacterial capabilities. Glucosamine' positively charged NH^{3+} groups interact with the negative charges on the surface of bacteria, generating cell permeability and intracellular component leakage. It may help prevent microorganisms from adhering to dentin [14,19]. Chitosan nanoparticles may inhibit bacterial enzymatic breakdown, making bacterial penetration less likely. It may also help to enhance the mechanical properties of root dentin [14]. Among the nanoparticles, silver nanoparticles have been shown to exhibit simultaneous antibacterial and smear layer removal efficacy. The method of action of silver nanoparticles is mostly due to the controlled release of silver ions in an aqueous environment. Because of their large surface area and strong reactivity, silver nanoparticles exhibit exceptional physical, chemical, and biological properties when compared to their bulk counterparts [20]. Silver nanoparticles with diameters ranging from 10 to 100 nm have been shown in studies to be bactericidal against both Gram-positive and Gram-negative bacteria [21-22]. The antibacterial action of silver nanoparticles is unclear. Ingestion of free silver ions, followed by inhibition of adenosine triphosphate synthesis and DNA replication, production of reactive oxygen species by silver nanoparticles and silver ions, and direct damage to cell membranes are the three most common ways of antibacterial activity recorded [23-24]. Root canal therapy is associated with more frequent and severe postoperative pain than other dental surgical procedures, thus, postoperative pain avoidance and management is a key component of endodontic treatment. It is a multi-factorial phenomenon with no one singular cause. Mechanical, chemical, host, and microbiological factors have all been identified as important in causing pain after root canal treatment [25]. Additionally, prior pain, demographic factors (such as gender and age), the pre-operative pulp status, the type of tooth, the type of treatment (initial or retreatment), and a history of allergy may all have an impact on postoperative pain. Even with the best equipment, complete root canal debridement is reported to be difficult. The bulk of the main root canal wall remains intact, which may harbor pulpal tissue debris, bacteria, and their byproducts.

So, serve as a cause of reinfection or chronic periradicular inflammation. This emphasizes the need to discover new techniques of cleansing and disinfecting all sections of the root canal [26]. According to the research, age and gender have minimal bearing on pain. As a consequence, the present research comprised people of both genders ranging in age from 20 to 40 years. Watkins et al., (2002) discovered that younger age groups anticipate and experience higher pain [27]. However, as shown by Ali et al., (2012) and Jabeen et al., (2013) postoperative discomfort was more prevalent in the older age group [28-29]. Other research has shown that age has little to do with the incidence of post-obturation pain. Cases with necrotic pulp and symptomatic apical periodontitis were selected as major inclusion criteria. Because microorganisms have been identified as the major cause of flare-ups, necrotic teeth were selected [30]. It has been proven that post-operative pain and flare-ups are more common in patients with pulpal necrosis than in vital pulps [31]. Moreover, to limit the influence of differences and complexity on the efficiency of debridement procedures, teeth with a single canal were used in this investigation. In this research, pain intensity was assessed preoperatively as baseline data and postoperatively at different time intervals. The period of 6 hours was selected since this is when the anaesthetic solution's effects begin to fade [32]. Furthermore, pain was recorded at 12, 24, and 48 hours after chemo-mechanical preparation because it was proven that most postoperative pain occurred between these time intervals [33]. Singh et al., (2020) discovered that some patients may experience pain beyond the 48-hour mark after chemo-mechanical preparation, so pain was also recorded at 72 hours [34]. Various measures and approaches have been used to evaluate pain after endodontic treatment. In the present study, the Visual Analogue Scale (VAS) was used to quantify pain intensity.

This scale is straightforward for the patient to comprehend, and if used appropriately, it may offer a rapid and simple evaluation as a numerical measure of pain change that can assist in objectively evaluating the effectiveness of pain alleviation and the degree of pain that certain treatments or procedures may produce [35]. When compared to the other groups, the (NaOCl and EDTA) group had the lowest incidence of discomfort in the present study. These results are consistent with previous research that has shown that bacteria in the root canal are the primary cause of postoperative pain [36]. At 6 and 12-hour time intervals, the degree of postoperative pain rose in all groups. Then it fell within each category during the next several years. Endodontic treatment may be aggravating or generating an inflammatory response in the periapical tissues. Silver and chitosan nanoscale presence may have escaped periapical, causing pressure or irritation of apical tissues. Polymorphonuclear leukocytes (PMNs) begin to enter the injured area within 6 hours, followed by a rise in the production of inflammatory mediators and neuropeptides. After 48 hours, the proliferative process begins, which is characterized by a decrease in the PMN population and the initiation of macrophages entering the wound site [37]. This is consistent with Singh et al., who stated that the majority of post-operative pain occurred on the first day after chemo mechanical preparation [38]. Furthermore, similar findings were observed in a systematic review conducted by Pak and White in which 40% of pain incidence was in the first 24hrs then sharply decreased over the first couple of days following chemo-mechanical preparation [39]. The duration of action and depth of penetration into dentinal tubules of the irrigants used in this research need additional investigation and comparison. More study into all aspects of Silver NP and Chitosan NP is also required to have a better understanding of their potential functions as intracanal irrigant and medicaments.

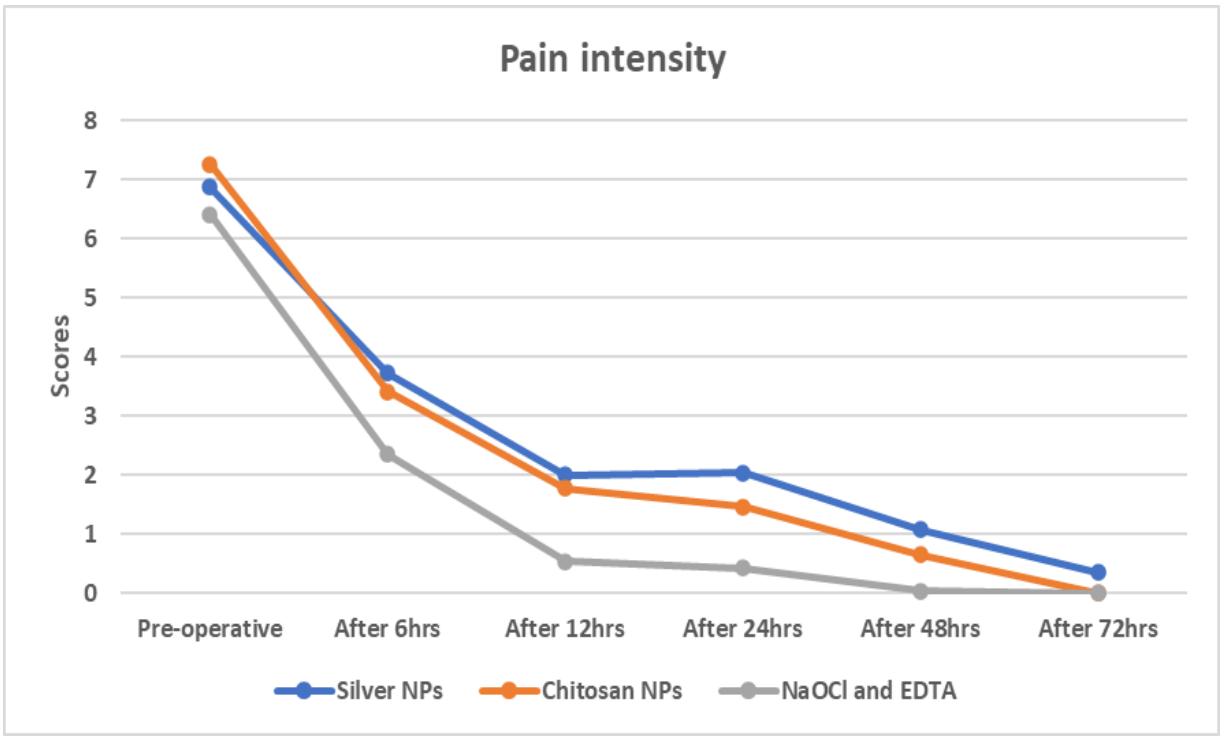

Figure 1: A chart representing the intensity of instrumentation pain at different time intervals for each group.

Table 1: Intensity of pre & post-instrumentation pain of the tested groups after Pre-operative, 6 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hrs.

Period	Pain intensity						
	Silver NPs		Chitosan NPs		NaOCl and EDTA		P-value
	Mean	SD	Mean	SD	Mean	SD	
Pre-operative	6.88	1.45	7.27	0.96	6.42	0.90	**0.301ns**
After 6hrs	3.73	1.51	3.42	1.06	2.35	0.63	**<0.001***
After 12hrs	2.00	1.30	1.77	1.07	0.54	0.58	**<0.001***
After 24hrs	2.04	1.84	1.46	0.71	0.42	0.50	**<0.001***
After 48hrs	1.08	1.16	0.65	0.49	0.04	0.20	**<0.001***
After 72hrs	0.35	0.80	0.00	0.00	0.00	0.00	**0.500ns**
p-value	**<0.001***		**<0.001***		**<0.001***		

*Significant ($p<0.05$), ns: non-significant ($p>0.05$).

5. Conclusions

There is no difference in postoperative pain after using silver nanoparticles and Chitosan nanoparticles as irrigating solutions.

Conflict of Interest

No conflict.

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