J Oral Res
research in oral sciences

ARTICLE

IN VITRO ASSESSMENT OF INTERACTION BETWEEN LIDOCAINE HYDROCHLORIDE AND IRRIGATING SOLUTIONS ON ROOT CANAL DENTIN: A SCANNING ELECTRON MICROSCOPE STUDY.

Evaluación *in vitro* de la interacción entre el clorhidrato de lidocaína y las soluciones de irrigación en la dentina del conducto radicular: Un estudio con microscopio electrónico de barrido.

Gurmeen Kaur.[1,2]
Chitharanjan Shetty.[1]
Shalin Ann Saji.[1]
Sunheri Bajpe.[1]

AFFILIATIONS:
[1]Department of Conservative Dentistry and Endodontics, AB Shetty Memorial Institute of Dental Sciences, NITTE University, Mangalore, Karnataka, India.
[2]Department of Conservative Dentistry and Endodontics, Dr. D. Y. Patil Dental College & Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, India.

CORRESPONDING AUTHOR:
Gurmeen Kaur. Department of Conservative Dentistry and Endodontics, Dr. D. Y. Patil Dental College & Hospital, Dr. D. Y. Patil Vidyapeeth, Pune, India. Phone: (91) 8605335888. E-mail: gurmeenk36@gmail.com

CITE AS:
Kaur G, Shetty C, Saji SA & Bajpe S. *In Vitro* assessment of interaction between lidocaine hydrochloride and irrigating solutions on root canal dentin: A scanning electron microscope study. J Oral Res.2022;11(4):1-12. doi:10.17126/joralres.2022.043

ABSTRACT:

Aim: To investigate the precipitate formed from the interaction between 2% lidocaine hydrochloride with adrenaline (LA) with 2.5% sodium hypochlorite (NaOCl) and 0.2% chitosan nanoparticles on root canal dentin, using scanning electron microscopy (SEM).

Material and Methods: Sixty mandibular premolars were decoronated, and the root length standardised. The specimens were randomly distributed into the following groups: Group 1 (control): 2% LA mixed with sterile water without root canal instrumentation, Group 2: 2% LA with 2.5% NaOCl in water without root canal instrumentation, and Group 3: 2% LA with 0.2% chitosan nanoparticles in water without root canal instrumentation. Teeth specimens were split and subjected to SEM analysis at cervical, middle, and apical root thirds. On observing precipitate formation in Group 2, 10 premolars were decoronated and treated with 2% LA and 2.5% NaOCl and subjected to root canal instrumentation.

Results: Group 1 and Group 3 showed patent dentinal tubules and no precipitate formation. Group 2 showed precipitate blocking dentinal tubules in all the three sections, and the precipitate could not be removed completely after cleaning and shaping.

Conclusion: NaOCl forms an insoluble precipitate on interaction with local anaesthetic solution that cannot be removed after chemo-mechanical preparation. Chitosan nanoparticles do not form any such precipitate and show patent dentinal tubules. Hence, chitosan can be used as a flushing irrigant.

KEYWORDS:

Chitosan; nanoparticles; lidocaine; root canal irrigants; smear layer; sodium hypochlorite.

Received: November 22, 2021 | **Accepted:** March 16, 2022 | **Published online:** August 30, 2022.

RESUMEN:

Objetivo: Investigar el precipitado formado a partir de la interacción entre el clorhidrato de lidocaína al 2% con adrenalina (LA), el hipoclorito de sodio al 2,5% (NaOCl) y nanopartículas de quitosano al 0,2% en la dentina del conducto radicular, mediante microscopía electrónica de barrido (SEM).

Material y Métodos: Se decoraron 60 premolares mandibulares y se estandarizó la longitud de la raíz. Los especímenes se distribuyeron aleatoriamente en los siguientes grupos: Grupo 1 (control): 2% la que fue mezclado con agua estéril sin instrumentación del conducto radicular, Grupo 2: 2% LA con 2,5% de NaOCl sin instrumentación del conducto radicular y Grupo 3: 2 % LA con 0,2% de nanopartículas de quitosano sin instrumentación del conducto radicular. Las muestras de dientes se dividieron y se sometieron a análisis SEM en los tercios radiculares cervical, medio y apical. Al observar la formación de precipitado en el Grupo 2, 10 premolares fueron decorados y tratados con LA al 2% y NaOCl al 2,5% y sometidos a instrumentación de conductos radiculares.

Resultado: El Grupo 1 y el Grupo 3 mostraron túbulos dentinarios permeables y sin formación de precipitados. El grupo 2 mostró precipitado que bloqueaba los túbulos dentinarios en las tres secciones, y el precipitado no se pudo eliminar por completo después de limpieza y conformación.

Conclusión: el NaOCl forma un precipitado insoluble al interactuar con la solución anestésica local que no se puede eliminar después de la preparación quimiomecánica. Las nanopartículas de quitosano no forman ningún precipitado de este tipo y muestran túbulos dentinarios permeables. Por lo tanto, el quitosano se puede utilizar como irrigante para el lavado.

PALABRAS CLAVE:

Quitosano; nanopartículas; lidocaína; irrigantes del conducto radicular; capa de barro dentinario; hipoclorito de sodio.

INTRODUCTION.

Achieving successful local anaesthesia is important in patient management during endodontic procedures as it alleviates pain and controls associated anxiety in patients. However, achieving a 100% anaesthetic success rate in nerve-block anaesthesia is a challenge.[1] Local anaesthesia (LA) is even less effective in patients with irreversible pulpitis, especially in symptomatic; 2% lidocaine hydrochloride with epinephrine in the ratio of 1:100000 is commonly used as local anaesthetic solution in endodontics.[2] Literature shows that even after using proper technique, there is a low success rate of a single inferior alveolar nerve block with 1.8 ml of LA solution.[3]

Generally, the inferior alveolar nerve block has a failure rate of around 80%.[1,3-7] In inflamed pulp, this can be attributed to inflammatory activation of nociceptors.[1] Painful stimulus and damage to tissue alter and modulate the peripheral and the central pain pathways.[8-10]

Reduction of activation threshold of peripheral nociceptors results in the firing of these neurons even by a minor stimulus.[1] The dorsal horn undergoes neuroplastic changes due to constant firing of these peripheral nociceptors, which leads to increase in the rate of neuron discharge and size of the receptive field of A-delta fibers.[1,8,11]

Inflammatory mediators also activate the capsaicin-sensitive transient receptor potential vanilloid type 1, which causes the release of neuropeptides like calcitonin gene-related peptide (CGRP).[12,13] This results in the central mechanism of hyperalgesia. Inflammatory mediators are bradykinin, prostaglandins, histamine, and serotonin.[14] As suggested by Nakanishi *et al.*,[15] pulpal fibroblasts play a role in

pulpal inflammation by producing prostaglandin via COX-2 expression, which ultimately leads to irreversible pulpitis.

Chang *et al*.,[16] reported that after proinflammatory cytokines (interleukin-1 and tumour necrosis factor- a) challenge, COX-2 mRNA was detectable in cell lysates till 24 hours and that the synthesis of COX-2 may lead to pulpal inflammation.

Reasons for failure of anaesthesia in inflamed teeth may include the individual's response to the drug administered, differences in operator technique, variations in anatomy, decreased pH of the recipient site, alteration in the excitability of membrane of the peripheral nociceptors and increase in tetrodotoxin-resistant sodium channels.[1]

Following failure, adjuvant local anaesthetic techniques, for instance, supplemental injection using 4% articaine, intraligamentary, intraosseous and intrapulpal injections are used.[17] One of the most commonly used technique is intrapulpal injection in case of *'hot tooth'*. After deroofing the pulp, local anaesthetic solution is administered directly into the pulp chamber using pressure for achieving complete pulpal analgesia enabling pulp extirpation and root canal instrumentation.[18]

Following which, sodium hypochlorite (NaOCl) is routinely used in a concentration varying from 0.5 to 5.25% for cleaning and shaping procedures.[19] Vidhya *et al*.,[20] found that the interaction between NaOCl & LA resulted in reaction of the hypochlorous acid with carbon atoms from lidocaine to form 2,6-xylidine on hydrolysis- which is a known carcinogen, (Figure 1).

Chitosan is a bioactive biopolymer, a derivative of the exoskeleton of crustaceans obtained by partial deacetylation of chitin. It consists of copolymers of β-(1,4)-linked glucosamine units (2-amino-2-deoxy-β-D-glucopyranose) and N-acetyl glucosamine units (2-acetamino-2-deoxy-β-D-glucopyranose) with two free hydroxyl groups and a primary amino group for each C6 structure unit.[21]

It has been brought into limelight with its superior antibacterial properties, biocompatibility, cost effectiveness, superb hydrophilicity, degradability, along with other physicochemical and biopharmaceutical properties. Its biomedical applications include its use as drug delivery system, antioxidant, anti-bacterial, antacid, osteogenesis promoter, haemostatic agent, fat absorbent, healing of ulcers and wounds.[22,23]

Chitosan aids removal of inorganic portion of smear layer by chelation for elimination of dentin calcium ions. The bridge model suggests that one metal ion binds with two or more amino groups of chitosan, whereas pendant model states that the metal ion is linked to one amino group like a pendant. 24 Silva *et al*.,[24] reported that 0.2% chitosan shows effective smear layer removal from the middle and apical thirds of the root canal as compared to routine endodontic irrigants. Penetration of chitosan nanoparticles (CNP) is deeper into dentinal tubules as compared to ethylenediaminetetraaceticacid (EDTA) and NaOCl.

So, the aim of the study was to evaluate the formation of resultant precipitate on interaction between 2% lidocaine hydrochloride LA solution and 0.2% chitosan nanoparticles on root canal dentin.

The null hypothesis tested were:

(i) The combination of local anesthetic solution and sodium hypochlorite does not form any precipitate on root canal walls.

(ii) The combination of local anesthetic solution and chitosan nanoparticles does not form any precipitate on root canal walls.

(iii) if any precipitate is formed, conventional chemomechanical instrumentation will completely remove it from the coronal-third, middle-third, and apical-third of the root canal.

MATERIALS AND METHODS.

This *ex vivo* study was carried out in the Department of Conservative Dentistry and Endodontics, A. B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Nitte Deemed to be University, Mangalore, India. The study was approved by the Institutional Ethical Committee (certificate no. ABSM/EC/12/2020). Sixty freshly

ISSN Print 0719-2460 - ISSN Online 0719-2479.

extracted intact, non-carious human mandibular premolars without any anatomical defect were collected from the Department of Oral and Maxillofacial Surgery, A. B. Shetty Memorial Institute of Dental Sciences, Nitte Deemed to be University, Mangalore.

Scanning electron microscopic evaluation was done in the Department of Metallurgical and Materials Engineering, National Institute of Technology Karnataka, Surathkal, India. Sixty freshly extracted intact permanent mandibular premolars were collected and disinfected as per the guidelines and recommendations laid by Occupational Safety and Health Administration (OSHA) 2004.

For initial collection of teeth, 0.5% NaOCl was used and stored in wide mouthed plastic jars. Teeth were always handled wearing gloves, mask and protective eyewear. Teeth were immersed in NaOCl for at least half an hour to allow the organic tissue to dissolve, and then transferred into physiologic saline in a separate storage jar.

The jars, lids and gloves employed for initial collection were discarded into biohazard waste receptacles. Before preparation, the teeth were removed from jars using cotton pliers and rinsed with tap water.

Selection of teeth samples

Inclusion criteria:

- Teeth with completely formed roots.
- Teeth with normal anatomic roots.
- Absence of caries and root canal fillings.
- Single patent canal.

Exclusion criteria:

- Teeth with fractured roots.
- Multi-rooted teeth.
- Teeth with caries or previous restorations.
- Internal or external resorption.
- Cracks on examination.
- Abrasion/attrition.

The presence of a single root canal and mature root apex was confirmed radiographically.

Specimen preparation

The teeth were decoronated near the cementoenamel junction to standardize the root length to 15 mm. Glide path was obtained with #10K and #15K files. The apical foramen was sealed with composite to prevent extrusion of solutions. All the specimens were rinsed with 17% EDTA for 1 min.

For administration of local anesthesia, standard dental local anesthetic cartridges were used with 27 gauge stainless steel beveled needles. The needle was bent gently in the centre for easy placement into the chamber; 0.5 mL of 2% lidocaine LA administered into the pulpal space with pressure. According to the irrigants being used, teeth were randomly divided into three groups.

After LA administration, Group 1 was irrigated with sterile water, Group 2 was irrigated with 5 ml of 2.5% NaOCl and lastly, Group 3 with 5 ml 0.2% chitosan nanoparticles for one minute each. Close ended side vented endodontic irrigation needle of size 30 gauge, length 25 mm was used to irrigate the canals.

The needle was bent at length 1 mm short of the working length and passively placed into the canal. Manual dynamic activation of irrigants was performed by manual up and down movement of the needle inside the canal. The canals were then dried with sterile paper points and were left uninstrumented.

Scanning electron microscopic evaluation

All the specimens were grooved buccolingually along the entire length without perforating the root canal space, and split along the groove using an enamel chisel. Of the split specimen, one half was randomly selected, sputter coated and evaluated by scanning electron microscope (JSM-6380 LA, JEOL, USA) and observed under x2000 and x5000 at cervical, middle and apical root thirds.

On observing precipitate formation in Group 2, ten additional specimens were treated similar to that of Group 2 and additionally root canal instrumentation was done. After irrigating with NaOCl, size 10 K files were inserted into each canal till the apical foramen, and 1 mm was reduced from the recorded length.

The canals were prepared in a sequential manner

from Sx to F2 using the ProTaper Gold rotary file system using 2 ml of 2.5% NaOCl for one minute between change of each instrument.

The canals were then irrigated with 5 ml of 17% EDTA for one minute. Final irrigation was done using 5 ml distilled water, following which, sterile absorbent paper points were used to dry the canals.

Statistical Analysis

Chi- square test and Fischer's exact test were used for comparison of precipitate formation in uninstrumented groups. McNemar test was used for comparison of outcome (removal of precipitate) in the instrumented teeth that were irrigated with NaOCl. All analyses were conducted at a significance level of $p<0.05$.

Figure 1. Interaction between NaOCl and lidocaine. (Modified from reference 20).

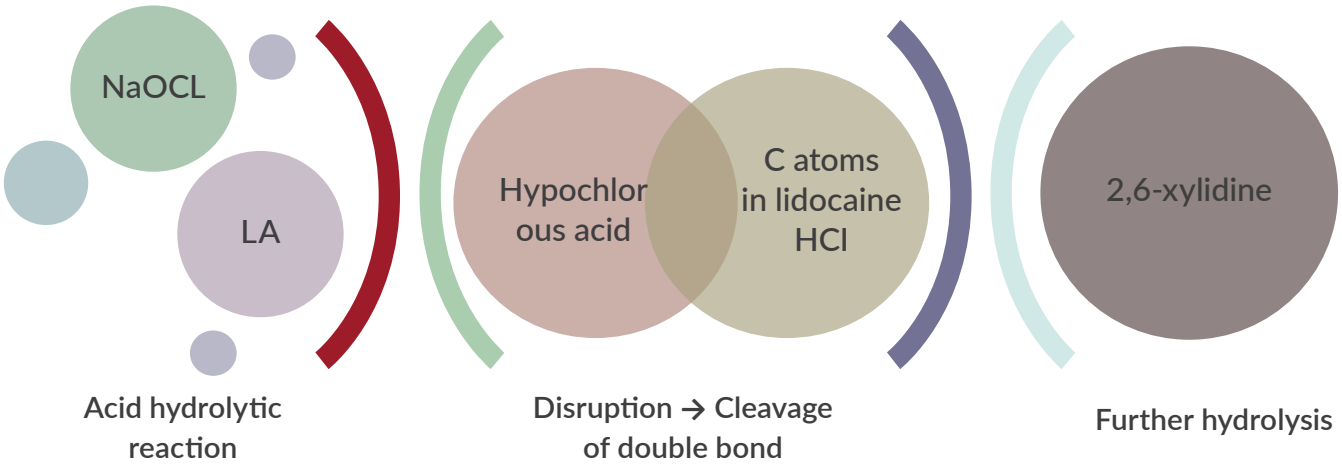
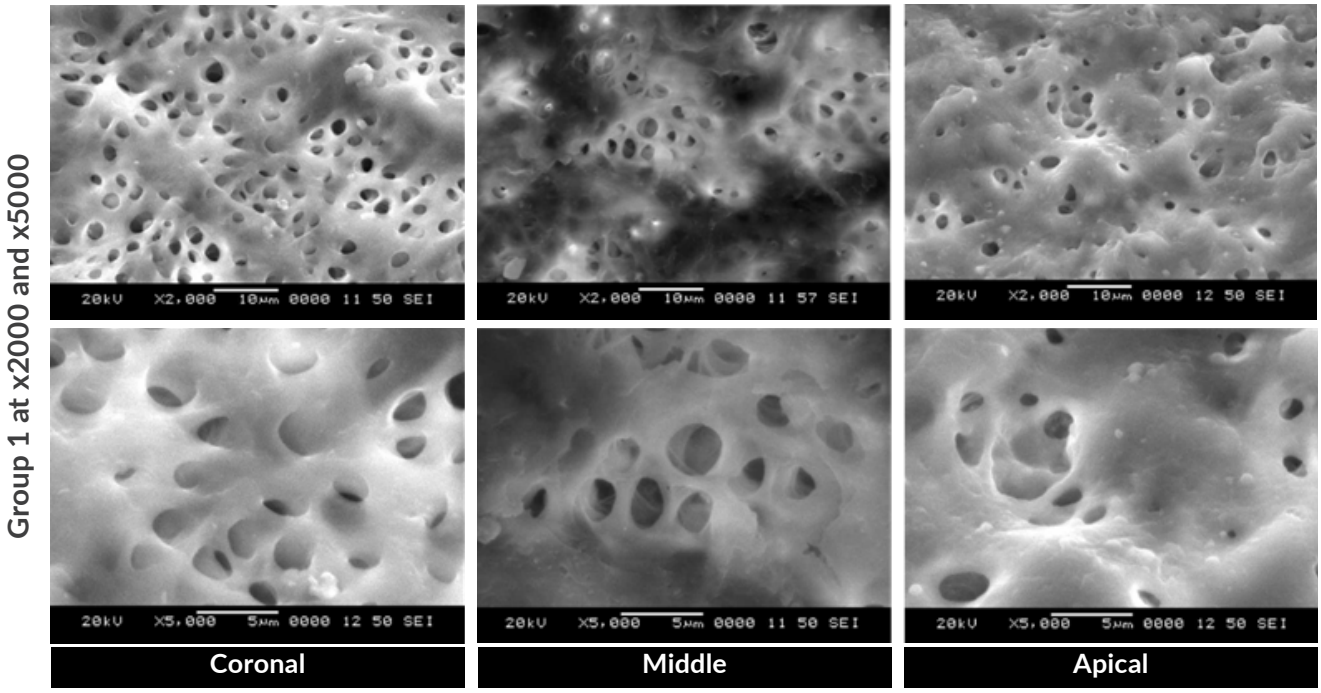


Figure 2. Representative scanning electron microscope images of coronal, middle and apical thirds, Group 1 (local anaesthetic solution and saline) at x2000 and x5000. Group 1 showed patent dentinal tubules with no precipitate formed in the coronal, middle or apical third of the specimen.



ISSN Print 0719-2460 - ISSN Online 0719-2479.

Figure 3. Representative scanning electron microscope images of coronal, middle and apical thirds, Group 2 (local anaesthetic solution and NaOCl) at magnification x2000 and x5000. Group 2 shows a fewer number of patent dentinal tubules with precipitate occluding the tubules in the middle and apical third.

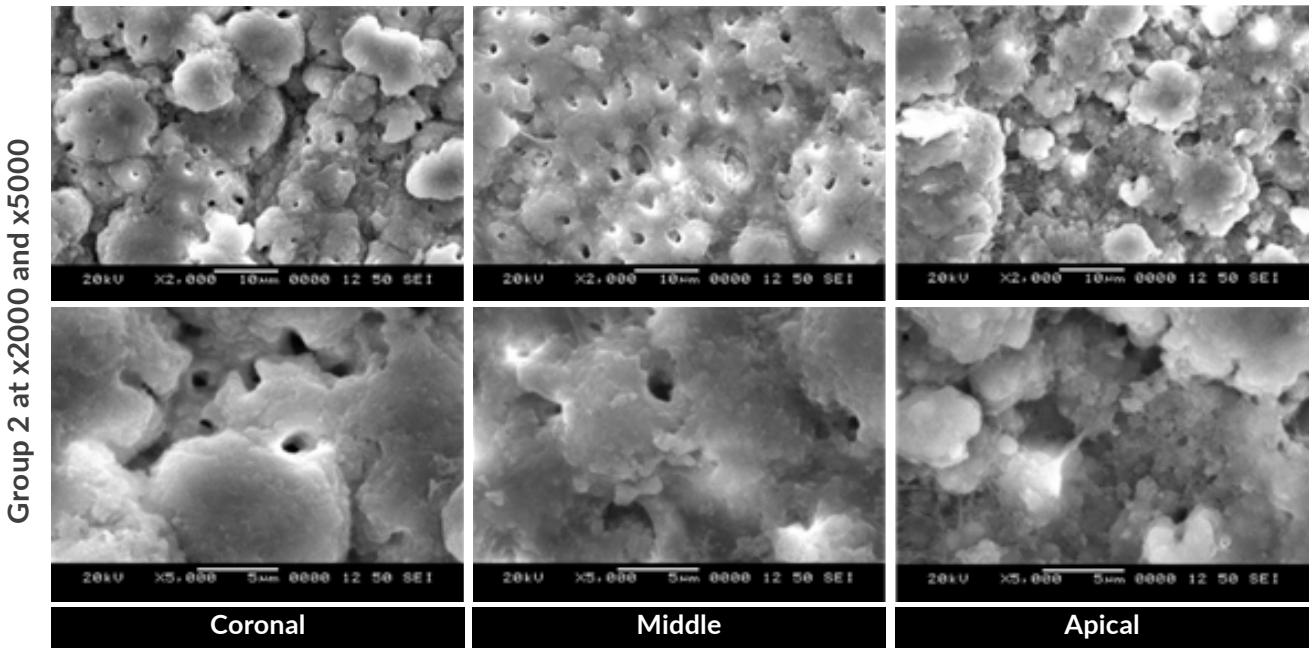


Figure 4. Representative scanning electron microscope images of coronal, middle and apical thirds, Group 3 (local anaesthetic solution and 0.2% chitosan nanoparticles) at magnification x2000 and x5000. Group 3 shows patent dentinal tubules with no precipitate formed in the coronal, middle and apical third of the specimens.

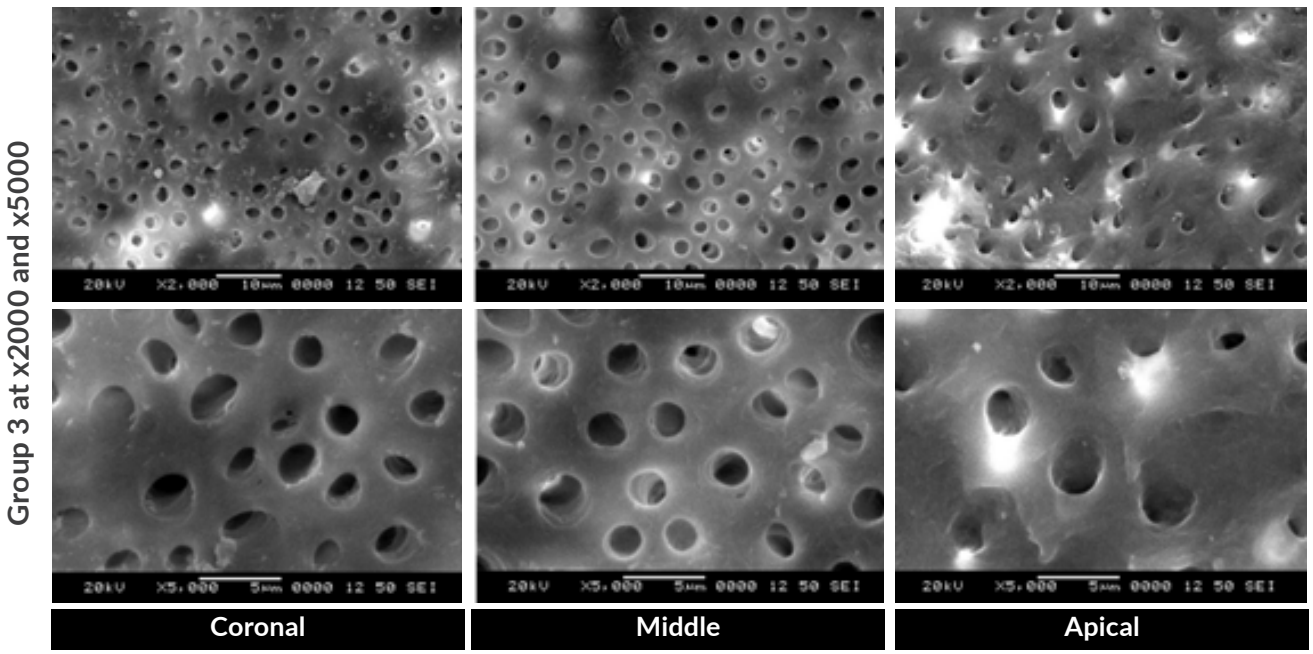


Figure 5. Representative scanning electron microscope images of coronal, middle and apical thirds of teeth treated similar to Group 2 followed by instrumentation at magnification x2000 and x5000; it was observed that even after sequential instrumentation and irrigation with NaOCl the precipitate formed could not be entirely removed, but the amount of precipitate was reduced in all three sections.

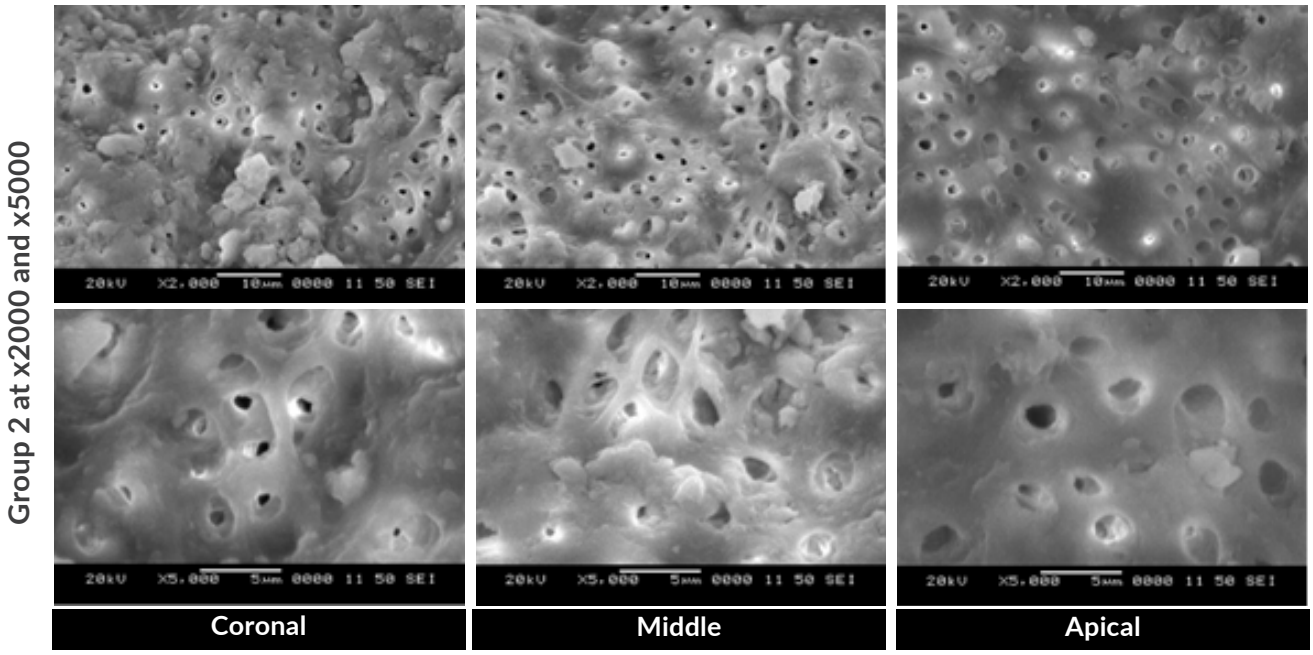


Table 1. Comparison of outcome between the study groups: Group 1 was irrigated with sterile water; Group 2 was irrigated with 2.5% NaOCl; Group 3 was irrigated with 0.2% chitosan nanoparticles.

	Group		Total	Group		Total	Group		Total
	1	2		2	3		1	3	
No	17	2	19	2	20	22	17	20	37
Percentage (%)	85.0	10.0	47.5	10.0	100.0	55.0	85.0	100.0	92.5
Yes	3	18	21	18	0	18	3	0	3
Percentage (%)	15.0	90.0	52.5	90.0	0.0	45.0	15.0	0.0	7.5
Total	**20**	**20**	**40**	**20**	**20**	**40**	**20**	**20**	**40**
Percentage (%)	100	100	100	100	100	100	100	100	100

#:Chi Square Test. ***:** $p<0.05$ Statistically Significant; $p>0.05$ Non-Significant (NS).

ISSN Print 0719-2460 - ISSN Online 0719-2479.

Table 2. Comparison of outcome before and after instrumentation in Group 2 (irrigated with 2.5% NaOCl).

	Post No	Pre Yes	Total
No	1	0	1
Percentage (%)	100.0	0.0	10.0
Yes	0	9	9
Percentage (%)	0.0	100.0	90.0
Total	**1**	**9**	**10**
Percentage (%)	100.0	100.0	100.0
McNemar Test		$p = 1.00$(NS)	

#:Chi Square Test. ***:** $p<0.05$ Statistically Significant; $p>0.05$ Non-Significant (NS).

RESULTS.

Scanning electron microscopy analysis showed differences in presence and amount of precipitate between groups. (Figures 2 to 5)

The difference in precipitate formation between Group 1 and Group 2 and between Group 2 and Group 3 is statistically significant, whereas that between Group 1 and Group 3 is statistically insignificant (Table 1).

Table 2 compares the removal of precipitate in the 10 teeth that followed a similar irrigation protocol as Group 2, but additional cleaning and shaping of canals was performed. The amount of precipitate removed after instrumentation in Group 2 is statistically not significant.

DISCUSSION.

Various researchers have studied the interaction between routinely used irrigants. Basrani et al stated that on interaction, sodium hypochlorite and chlorhexidine (CHX) form parachloroaniline, whereas Orhan *et al*,.[25] observed the formation of a brown precipitate.[26] Rasimick *et al*,.[27] stated that a nontoxic white precipitate formed on mixing 17% EDTA and 1% chlorhexidine (CHX).

Vidhya et al. was the first to study the interaction between lidocaine hydrochloride and NaOCl, CHX, EDTA. They reported the formation of a precipitate on interaction between NaOCl and lidocaine, whereas no precipitate was formed on interaction between EDTA or CHX with lidocaine.[20]

A study by Saravanakarthikeyan et al., found similar results where 2% lidocaine and 2.5% NaOCl formed a precipitate that covered the entire dentin surface leaving very few patent dentinal tubules.

Even after instrumentation, there was presence of precipitate at all root thirds, inferring that complete removal of the precipitate does not occur even after cleaning and shaping.[28] The precipitate formed, as discussed by Vidhya *et al*,.[20] is toxic and may slowly leach into the periapical tissues. Blockage of the dentine tubules may also hamper the penetration of intracanal medicaments, irrigants and sealers. Hence, it may negatively affect the seal of the root canal. Chitosan, on the other hand, did not show any precipitate formation and resulted in patent dentinal tubules.

Overproduced inflammatory prostaglandins derived from COX-2 contribute to the inflammatory pathophysiology. Chou *et al*.,[29] demonstrated that

chitosan significantly inhibits the formation of Prostaglandin E2 and COX-2 protein expression. Chitosan also inhibits the formation of pro-inflammatory cytokines tumor necrosis factor-a and interleukin-1b but increases the generation of anti-inflammatory cytokine interleukin-10.

This suggests that chitosan has a beneficial wound healing and anti-inflammatory effect.Thus the application of chitosan in an inflamed pulp should result in resolution of inflammation to some extent and aid in action of local anesthetic solution that is injected into the pulp chamber.

Chitosan causes minimal chelation and produces cleaner dentinal walls with minimal erosion of intraradicular dentin and removes smear layer efficiently.[30] While evaluating the effect of chitosan root dentine surface, it was found it causes significantly less surface alteration and energy-dispersive X-ray analysis showed that the Ca/P ratio of root dentine was higher.[31] Furthermore, studies have reported chitosan to have potential to remineralize the demineralized dentin coated with it. Treatment with CNPs resulted in significantly lower biofilm formation and resistance to bacterial adherence.[32]

In a confocal laser scanning microscopy study by Kesim *et al.*,[33] comparing the effectiveness of final irrigation with chitosan, EDTA, and citric acid on a resin-based sealer, they found that chitosan and EDTA showed increased mean values of sealer penetration into dentinal tubules.

Given its anti-inflammatory and hemostatic properties, ability to remove smear layer, the deeper penetration of nanoparticles and its antimicrobial benefits, Chitosan can be used as an alternative root canal irrigant. Flushing out the LA before administration of NaOCl, it may avoid the interaction between LA and NaOCl, without compromising the coronal seal of post endodontic restoration, since it does not form any precipitate. Also, due to its anti-inflammatory and hemostatic properties, it may help reduce inflammation of the pulp and alleviate pain, aiding in the extirpation of pulp for root canal procedures.

CONCLUSION.

Intrapulpal administration of LA and NaOCl results in precipitate formation that occludes dentinal tubules and cannot be removed by conventional chemomechanical preparation of root canal space. Hence, the combination of local anesthetic solution and sodium hypochlorite form a precipitate on root canal walls which is not removed by chemomechanical instrumentation. Whereas the combination of local anesthetic solution and chitosan nanoparticles does not form any precipitate on root canal walls.

The effect of chitosan for flushing out residual lidocaine hydrochloride before using NaOCl, and its effect in inflamed pulp should be further evaluated. Additional research into the effect of 2,6-xylidine on dental and periapical tissues should be carried out.

ISSN Print 0719-2460 - ISSN Online 0719-2479.

Conflict of interests:

Authors report no conflicts of interest in connection with this article.

Ethics approval:

The study was approved by A. B. Shetty Memorial Institute of Dental Sciences Institutional Ethical Committee (certificate No. ABSM/EC/12/2020).

Funding:

Self financed

Authors' contributions:

Dr. Gurmeen Kaur: Research concept and design, Data curation, Formal analysis, Writing the article-original draft.

Dr. Chitharanjan Shetty: Conceptualization, Data analysis and interpretation, Critical revision of article, Final approval.

Dr. Shalin Ann Saji: Resources, Writing the article-review and editing.

Dr. Sunheri Bajpe: Resources, Writing the article-review and editing.

Acknowledgements:

None.

REFERENCES.

1. Hargreaves KM, Keiser K. Local anesthetic failure in endodontics: mechanisms and management. Endod Topics. 2002;(1)2002:26–39. doi:10.1034/J.1601-1546.2002.10103.X
2. Claffey E, Reader A, Nusstein J, Beck M, Weaver J. Anesthetic efficacy of articaine for inferior alveolar nerve blocks in patients with irreversible pulpitis. J Endod. 2004;30(8):568-71. doi: 10.1097/01.don.0000125317.21892.8f. PMID: 1527 3637.
3. Aggarwal V, Jain A, Kabi D. Anesthetic efficacy of supplemental buccal and lingual infiltrations of articaine and lidocaine after an inferior alveolar nerve block in patients with irreversible pulpitis. J Endod. 2009;35(7):925-9. doi: 10.1016/j.joen.2009.04.012. PMID: 19567309.
4. Claffey E, Reader A, Nusstein J, Beck M, Weaver J. Anesthetic efficacy of articaine for inferior alveolar nerve blocks in patients with irreversible pulpitis. J Endod. 2004;30(8):568-71. doi: 10.1097/01.don.0000125317.21892.8f. PMID: 15273637.
5. Fowler S, Drum M, Reader A, Beck M. Anesthetic Success of an Inferior Alveolar Nerve Block and Supplemental Articaine Buccal Infiltration for Molars and Premolars in Patients with Symptomatic Irreversible Pulpitis. J Endod. 2016;42(3):390-2. doi:10.1016/j.joen.2015.12.025. PMID: 26831048.
6. Zanjir M, Lighvan NL, Yarascavitch C, Beyene J, Shah PS, Azarpazhooh A. Efficacy and Safety of Pulpal Anesthesia Strategies during Endodontic Treatment of Permanent Mandibular Molars with Symptomatic Irreversible Pulpitis: A Systematic Review and Network Meta-analysis. J Endod. 2019;45(12):1435-1464.e10. doi: 10.1016/j.joen.2019.09.002. PMID: 31601433.
7. Nagendrababu V, Pulikkotil SJ, Suresh A, Veettil SK, Bhatia S, Setzer FC. Efficacy of local anaesthetic solutions on the success of inferior alveolar nerve block in patients with irreversible pulpitis: a systematic review and network meta-analysis of randomized clinical trials. Int Endod J. 2019;52(6):779-789. doi: 10.1111/iej.13072. PMID: 30638269.
8. Lin JY, Cheng YC, Chen JY, Chien CC, Lin SC, Wen YR, Tsou TS, Ling QD. Increased spinal prodynorphin gene expression in reinflammation-associated hyperalgesia after neonatal inflammatory insult. BMC Neurosci. 2010;11:139. doi: 10.1186/1471-2202-11-139. PMID: 20973986; PMCID: PMC2978219.
9. Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014;13(7):533-48. doi: 10.1038/nrd4334. PMID: 24948120; PMCID: PMC4228377.
10. Jänig W. Sympathetic nervous system and inflammation: a conceptual view. Auton Neurosci. 2014;182:4-14. doi: 10.1016/j.autneu.2014.01.004. PMID: 24525016.
11. Honda K, Kitagawa J, Sessle BJ, Kondo M, Tsuboi Y, Yonehara Y, Iwata K. Mechanisms involved in an increment of multimodal excitability of medullary and upper cervical dorsal horn neurons following cutaneous capsaicin treatment. Mol Pain. 2008;4:59. doi: 10.1186/1744-8069-4-59. PMID: 19019214; PMCID: PMC2661045.
12. Chaudhary P, Martenson ME, Baumann TK. Vanilloid receptor expression and capsaicin excitation of rat dental primary afferent neurons. J Dent Res. 2001;80(6):1518-23. doi: 10.1177/00220345010800060801. PMID: 11499505.
13. Willoughby DA, Moore AR, Colville-Nash PR. COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. Lancet. 2000;355(9204):646-8. doi: 10.1016/S0140-6736(99)12031-2. PMID: 10696997.
14. Willoughby DA, Moore AR, Colville-Nash PR. COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. Lancet. 2000;355(9204):646-8. doi: 10.1016/S0140-6736(99)12031-2. PMID: 10696997.
15. Nakanishi T, Shimizu H, Hosokawa Y, Matsuo T. An immunohistological study on cyclooxygenase-2 in human dental pulp. J Endod. 2001;27(6):385-8. doi: 10.1097/00004770-200106000-00003. PMID: 11487130.
16. Chang YC, Yang SF, Huang FM, Liu CM, Tai KW, Hsieh YS. Proinflammatory cytokines induce cyclooxygenase-2 mRNA and protein expression in human pulp cell cultures. J Endod. 2003;29(3):201-4. doi: 10.1097/00004770-200303000-00009. PMID: 12669881.
17. Boopathi T, Sebeena M, Sivakumar K, Harikaran J, Karthick K, Raj A. Supplemental pulpal anesthesia for mandibular teeth. J Pharm Bioallied Sci. 2013;5(Suppl 1):S103-8. doi: 10.4103/0975-7406.113307. PMID: 23946560.
18. Birchfield J, Rosenberg PA. Role of the anesthetic solution in intrapulpal anesthesia. J Endod. 1975;1(1):26-7. doi: 10.1016/S0099-2399(75)80246-9. PMID: 1061766.
19. Zehnder M. Root canal irrigants. J Endod. 2006;32(5):389-98. doi: 10.1016/j.joen.2005.09.014. PMID: 16631834.
20. Vidhya N, Karthikeyan BS, Velmurugan N, Abarajithan M, Nithyanandan S. Interaction between lidocaine hydrochloride (with and without adrenaline) and various irrigants: A nuclear magnetic resonance analysis. Dent Res J (Isfahan). 2014;11(3):395-9. PMID: 25097652; PMCID: PMC4119375.
21. Kmiec M, Pighinelli L, Tedesco MF, Silva MM, Reis V. Chitosan-properties and applications in dentistry. Adv Tissue Eng Regen Med Open Access. 2017;2(4):205–211. doi: 10.15406/atroa.2017.02.00035
22. Dai T, Tanaka M, Huang YY, Hamblin MR. Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. Expert Rev Anti Infect Ther. 2011;9(7):857-79. doi: 10.1586/eri.11.59. Erratum in: Expert Rev Anti Infect Ther. 2013;11(8):866. PMID: 21810057; PMCID: PMC3188448.
23. Hosseini S, Kassaee MZ, Elahi SH, Bolhari B. A New Nano-Chitosan Irrigant with Superior Smear Layer Removal and Penetration. Nanochem Res, 2016; 1(2): 150-156. doi: 10.7508/ncr.2016.02.002
24. Silva PV, Guedes DF, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int Endod J. 2013;46(4):332-8. doi: 10.1111/j.1365-2591.2012.02119.x. PMID: 22970844.

ISSN Print 0719-2460 - ISSN Online 0719-2479.

25. Basrani BR, Manek S, Sodhi RN, Fillery E, Manzur A. Interaction between sodium hypochlorite and chlorhexidine gluconate. J Endod. 2007;33(8):966-9. doi: 10.1016/j.joen.2007.04.001. PMID: 17878084.
26. Rasimick BJ, Nekich M, Hladek MM, Musikant BL, Deutsch AS. Interaction between chlorhexidine digluconate and EDTA. J Endod. 2008;34(12):1521-3. doi: 10.1016/j.joen.2008.08.039. PMID: 19026886.
27. Rasimick BJ, Nekich M, Hladek MM, Musikant BL, Deutsch AS. Interaction between chlorhexidine digluconate and EDTA. J Endod. 2008;34(12):1521-3. doi: 10.1016/j.joen.2008.08.039. Epub 2008 Oct 11. PMID: 19026886.
28. Saravanakarthikeyan B, Devarajan S, Sankeerthana K, Sujatha V, Mahalaxmi S. In vitro assessment of interaction between lidocaine hydrochloride and sodium hypochlorite on root canal dentin before and after chemomechanical instrumentation procedures. J Conserv Dent. 2019;22(3):255-259. doi: 10.4103/JCD.JCD_565_18. PMID: 31367109; PMCID: PMC6632634.
29. Chou TC, Fu E, Shen EC. Chitosan inhibits prostaglandin E2 formation and cyclooxygenase-2 induction in lipopolysaccharide-treated RAW 264.7 macrophages. Biochem Biophys Res Commun. 2003;308(2):403-7. doi: 10.1016/s0006-291x(03)01407-4. PMID: 12901883.
30. Kamble AB, Abraham S, Kakde DD, Shashidhar C, Mehta DL. Scanning Electron Microscopic Evaluation of Efficacy of 17% Ethylenediaminetetraacetic Acid and Chitosan for Smear Layer Removal with Ultrasonics: An In vitro Study. Contemp Clin Dent. 2017;8(4):621-626. doi: 10.4103/ccd.ccd_745_17. PMID: 29326515; PMCID: PMC5754985.
31. Mathew SP, Pai VS, Usha G, Nadig RR. Comparative evaluation of smear layer removal by chitosan and ethylenediaminetetraacetic acid when used as irrigant and its effect on root dentine: An in vitro atomic force microscopic and energy-dispersive X-ray analysis. J Conserv Dent. 2017;20(4):245-250. doi: 10.4103/JCD.JCD_269_16. PMID: 29259361; PMCID: PMC5721506.
32. Kishen A, Shi Z, Shrestha A, Neoh KG. An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. J Endod. 2008;34(12):1515-20. doi: 10.1016/j.joen.2008.08.035. PMID: 19026885.
33. Kesim B, Burak AK, Ustun Y, Delikan E, Gungor A. Effect of chitosan on sealer penetration into the dentinal tubules. Niger J Clin Pract. 2018;21(10):1284-1290. doi: 10.4103/njcp.njcp_127_18. PMID: 30297560.