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Scanning electron microscopic analysis of interaction between 2% lidocaine hydrochloride (with adrenaline) with 3% sodium hypochlorite and 2% chlorhexidine on root canal dentin after Chemomechanical instrumentation: An *in vitro* study

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Abstract
Introduction: To investigate the effect of resultant precipitate formed on interaction between 2% lidocaine hydrochloride (with adrenaline) (LA) with 3% sodium hypochlorite (NaOCL) and 2% chlorhexidine (CHX) on root canal dentin after chemomechanical preparation using scanning electron microscopy (SEM).
Methods: Thirty six mandibular premolars were decoronated, and the root length was standardized. All specimens were randomly distributed into the following three groups:
Group I (control): 2% LA mixed with sterile water.
Group II: 2% LA with 3% NaOCL,
Group III: 2% LA with 3% CHX. In all groups mechanical instrumentation with rotary files was done. Teeth samples were sectioned into three parts, split and SEM analysis of root canal wall was done at cervical. Middle, and apical root thirds.
Results: SEM images revealed patent dentinal tubules with no precipitate occlusion in group I and group III, whereas there was occlusion of dentinal tubules with a precipitate in group II at all the three root levels studied.
Conclusions: The precipitate formed on the interaction between 2% LA solution with 3% NaOCL tends to occlude the dentinal tubules at the coronal, middle, and apical root thirds. The chemomechanical rotary instrumentation procedure did not effectively remove the precipitate from all the three root levels of the specimens studied. LA/sterile water group and LA/CHX group did not result in any precipitate formation on root canal dentin.

Keywords: Intrapulpal anesthesia, lidocaine hydrochloride, precipitate, sodium hypochlorite, chlorhexidine

Introduction
The ability to effectively clean the endodontic space is dependent on both instrumentation and irrigation via chemomechanical means [1-3]. Irrigants used during cleaning and shaping of the root canal system play an essential role in the successful debridement and disinfection [4-9].
The ideal irrigant used for root canal therapy should possess adequate tissue dissolving property with lubricating action, prolonged antimicrobial effect, be non-toxic, non-allergenic, and be an effective germicide and fungicide [10]. No single irrigant can perform all the desired actions and hence, usually a combination of irrigants is employed during root canal therapy [9].
The most commonly used irrigant during root canal therapy is sodium hypochlorite (NaOCl) in concentrations ranging from 0.5% to 6%. It is an excellent tissue solvent, and antibacterial agent. Ethylene diamine tetra-acetic acid (EDTA) is a chelating agent and it removes calcium ions from tooth structure [11]. EDTA solutions are usually used in concentrations ranging from 10% to 17% for smear layer removal [10]. However, 17% EDTA has minimal tissue dissolution capacity compared to that of sodium hypochlorite [12].

Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent, active against Gram-positive and Gram-negative bacteria including yeast cells [13]. It is commonly used in 2% concentration for root canal disinfection. It also exhibits substantivity resulting in prolonged antimicrobial effect [14].

Basrani *et al*., reported that the interaction between NaOCl and CHX results in the formation of para-chloroaniline precipitate [15], a known carcinogen [16]. In a similar manner, Grande *et al*., reported the interaction between EDTA and NaOCl by NMR analysis, and concluded that EDTA reduces the free available chlorine content of NaOCl, and hence, its tissue-dissolving capability [17].

The most commonly used local anesthetic solution in endodontics is 2% lidocaine hydrochloride with adrenaline (LA) in 1:100,000 concentration because of its improved efficacy at low concentrations and decreased allergenic characteristics [18].

Inferior alveolar nerve block (IANB) is the primary standard technique to achieve mandibular anesthesia, whereas local infiltration is used to anesthetize the maxillary teeth [19].

Many factors such as the individual variations in response to the drug administered, operator differences, and anatomical variations, apart from decreased pH, altered membrane excitability of peripheral nociceptors, and increased tetrodotoxin‑ resistant sodium channels are responsible for the reduced anesthetic effect in clinical cases of inflamed teeth [10, 20, 21, 22, 23, 24].

Following the failure of these conventional methods, adjuvant anesthetic techniques such as supplemental injection with 4% articaine hydrochloride with adrenaline, intraligamentary/ intraosseous methods, and/or intrapulpal injections are usually employed to ensure profound anesthetic effect [23].

The intrapulpal injection technique (IPI) is one of the most commonly employed supplemental anesthetic techniques. In general, administration of LA directly into the pulp chamber provides complete analgesia for effective pulp extirpation and root canal instrumentation. After pulp deroofing procedure, IPI is further administered into the root canal orifices with adequate back pressure to facilitate complete removal of pulp remnants from the canal. Following IPI, sodium hypochlorite (NaOCl) (in concentrations ranging from 0.5% to 5.25%) is usually employed in routine cleaning and shaping procedures which is considered the gold standard irrigant for pulp tissue dissolution in endodontics [9].

However, Vidhya *et al* [25], evaluated the chemical interaction between LA and NaOCl using nuclear magnetic resonance(NMR) spectroscopy and reported the formation of a precipitate, 2, 6‑ xylidine, which is a known carcinogen.

The aim of the present *in vitro* study was to evaluate the effect of the combination of 2% LA with 3% NaOCL and 2% CHX and the resultant precipitate on root canal dentin after chemomechanical rotary instrumentation procedure using Scanning electron microscopic (SEM) evaluation. The null hypotheses tested were: (1) LA/NaOCl combination does not result in any precipitate formation on root canal walls and (2) conventional chemomechanical instrumentation will completely remove the precipitate formed (if any) following LA/NaOCl use from the coronal, middle, and apical-thirds of the root canal.

Method

Specimen Preparation

36 freshly extracted mandibular premolars with straight roots were used in this study. Radiographs of tooth were obtained to confirm the presence of a single canal and mature root apex. Teeth with caries, cracks, fractures, resorption, previous restorations and root dilacerations were excluded from this study. The teeth were cleaned carefully of debris and calculus and stored in normal saline solution (Goa Formulations Ltd, Mumbai, India).All the teeth were then decoronated at or near the cementoenamel junction using a high-speed diamond disc (axiss Dental, Indore, M.P, India) to obtain a standardized root length of 14 mm. The foraminal opening was sealed with resin composite (Ivoclar vivadent, Schaan, Liechtenstein) to prevent the extrusion of experimental solutions from the apical foramen. A glide path was established using #10-size k-file(mani, japan). All specimens were initially rinsed with 5 ml of 17% EDTA (Avuprep, Dental avenue, Mumbai, India) for 1 min. After that 0.5 ml of 2% LA (Indoco Remedies Ltd, Mumbai, India) was administered by inserting a 27-gauge stainless steel beveled needle into the orifices of the root specimens and the solution was injected into the pulpal space under pressure.

Grouping

The specimens were then randomly distributed into three groups based on the test solutions employed. After LA administration, Group I, II and III were irrigated with each 2 ml of sterile water (Park Benz Laboratories, Raisen), 3% NaOCL (Neelkanth, Apexicon Dental Product, Kerala, India) and 2% CHX (Neelkanth, Apexicon Dental Product, Kerala, India) for 1 min respectively. Following irrigation, working length (WL) was determined by inserting 10 size k file into each canal of all the groups, until it was just visible at the apical foramen and then reducing 1 mm from the recorded length. The canals of all groups were prepared with rotary endodontic instruments (DENTSPLY Maillefer, Ballaigues) in a sequential manner upto F2. Irrigation was performed with 2 ml of sterile water, 2 ml of 3% NaOCL, 2 ml of 2% CHX in group I, II and III for 1 min between each instrument change. The final irrigation sequence involved the use of 5 ml of 17% EDTA for 1 min. Irrigation was performed using 30 gauge stainless steel side vented needle and the needle tip was inserted to 1 mm short of WL. Final irrigation was done with 5 ml of distilled water. The canals were later dried with sterile absorbent paper points.

Scanning electron microscopy evaluation

The specimens of all the three groups were grooved buccolingually along the entire length, with the help of a 168-L high-speed bur, without perforating into the root canal space. The roots were then split carefully along the length of the groove with an enamel chisel. One half of the split root was randomly selected, sputter coated and subjected to SEM evaluation. Root samples were scanned at the cervical, middle and apical third levels using SEM (Zeiss) and observed under x5000.

Results

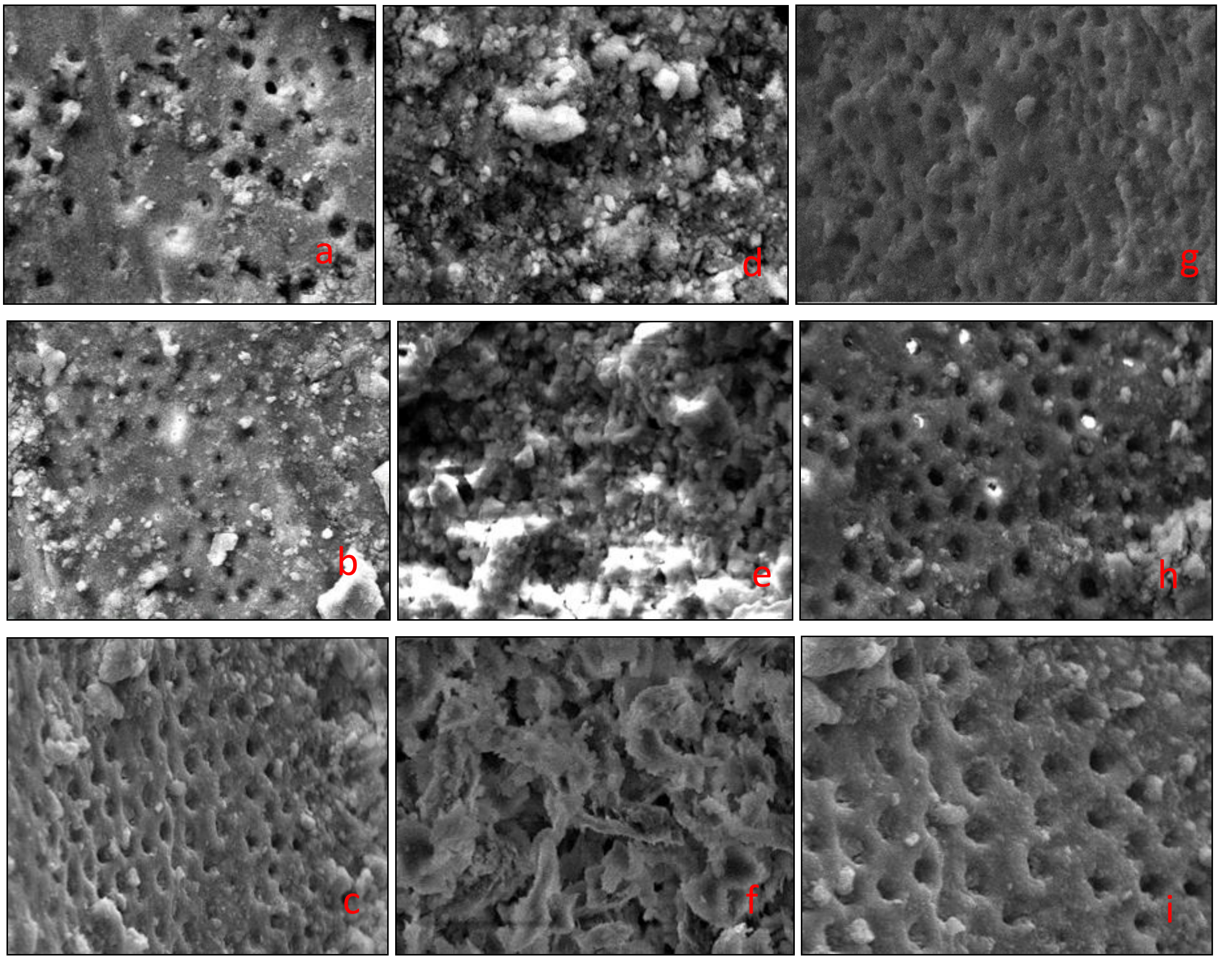

Fig 1: Illustrate the representative SEM images of the coronal, middle and apical thirds of Group I, Group II and Group III at x 5000. Group I (i.e LA/sterile water) revealed patent dentinal tubules with no precipitate formation throughout the entire extent of the root canal surface, whereas specimens in Group II (i.e LA/NaOCL) revealed precipitate occlusion at all the three root thirds of the specimens studied. Group III (i.e LA/CHX) revealed patent dentinal tubules with no precipitate formation throughout the entire extent of the root canal surface.

Discussion

Mechanical shaping and cleaning greatly remove the majority of the inflamed pulp remnants and infected dentin from the root canal system. However, owing to its anatomical complexities, organic and inorganic residues including bacteria cannot be completely removed from the canal and do often persist [26, 27]. Hence, chemical debridement in the form of various irrigants is required in addition to mechanical preparation [14].

The most commonly employed irrigants in endodontics such as NaOCl, EDTA, and chlorhexidine **(**CHX) are always used in conjunction to achieve the desired therapeutic effects [9] As a result, these irrigants routinely come into contact with each other during the root canal therapy.

Basrani *et al.* [15] in an earlier study reported that the interaction between NaOCl and CHX resulted in the formation of parachloroaniline (PCA) precipitate, which is a known carcinogen. Although Orhan *et al.* [28] in a recent study proved that the precipitate did not contain free PCA, the authors claimed that there was a definite formation of a brown precipitate on mixing NaOCl and CHX. Rasimick *et al.* [29] also reported the formation of a white precipitate when 17% EDTA and 1% CHX were mixed, but this was reported to be nontoxic.

Although the interactions between various endodontic irrigants are quite well known, the interaction between LA solution (employed for IPI) and subsequently used irrigants are often overlooked in a clinical scenario.

Vidhya *et al.* [25] evaluated the interaction between lidocaine hydrochloride (with or without adrenaline) and commonly used irrigants such as NaOCl, CHX, and EDTA using NMR spectroscopy.

The authors proved that NaOCl and LA combination resulted in the formation of a precipitate, whereas EDTA and CHX, when admixed with LA solution, did not result in any precipitate formation [25].

In the present study, 17% EDTA was used as initial rinse for effective removal of smear layer and resulting in demineralization of peritubular and intertubular dentin for enhanced visualization of open dentinal tubules.

SEM evaluation of the root canal walls also provides ultrastructural assessment of the cleanliness of the dentin surface following different irrigation and cleaning methods, as employed in previous studies. Our results showed that all root canal specimens in group I (LA/sterile water) and group III (LA/CHX) revealed almost complete removal of smear layer in coronal, middle and apical third. In group 11, the entire dentin surface was covered with a precipitate with very few patent dentinal tubules at all the three levels. Thus, both the proposed null hypotheses were rejected.

As stated by Vidhya *et al.*, [25] there is an acid hydrolytic reaction between NaOCl and LA, thereby releasing hypochlorous acid which combines with carbon atoms present in lidocaine HCl molecule, leading to its disruption with subsequent cleavage of the double bond. On further hydrolysis, 2,6‑xylidine (a known metabolite of lidocaine HCl) precipitate was formed. A major concern about this precipitate is that 2,6‑xylidine was reported to be a toxic compound, as reported in the literature [30] This is a clinically significant study because the IPI technique is a routinely used procedure after establishing glide path in teeth where conventional anesthetic techniques have failed, with an added advantage of causing negligible systemic effects. It may be of concern that this toxic precipitate will attach to the root canal surface and slowly leach into the periapical tissues. It may also causes penetration of intracanal irrigants/medicaments and compromise the seal of the root canal. It also hinders the coronal seal of the postendodontic restoration if the resultant precipitate is not removed completely from the pulp chamber walls. Hence, it is advisable to avoid the immediate use of NaOCl following IPI with LA solution to avoid such detrimental effects.

Since the anesthetic effect of the intrapulpal anesthesia is mainly due to the backpressure of the solution independent of the solution injected, as stated by Birchfield and Rosenberg, [31] it may be advisable to use 0.9% normal saline rather LA for IPI. The limitation of the study was that the intrapulpal anesthesia is usually employed in the pulp chamber and its entry into the root canal is minimal. So the precipitate formed will be also in negligible amounts that may be removed during the subsequent cleaning and shaping procedures. Future investigations are warranted to determine the possible effects of the resultant precipitate on the mechanical properties of root dentin and also the effect of this precipitate on the sealing ability of root canal obturation and postendodontic coronal restoration also has to be explored.

Conclusions

Within the limitations of this *in vitro* SEM study, it can be concluded that: (1) intrapulpal injection with LA into the pulpal space followed by subsequent irrigation with NaOCl forms a precipitate which occludes the dentinal tubules at the coronal‑, middle‑, and apical‑thirds of the root canal; (2) conventional chemomechanical rotary instrumentation does not completely remove this precipitate; and (3) the combined use of LA/sterile water and LA/CHX revealed patent dentinal tubules with no precipitate formation.

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