INTRODUCTION

The most commonly employed anaesthetic solution in endodontics is 2% lignocaine hydrochloride with adrenaline (LA) (1). The success of pulpal anaesthesia resulting from conventional mandibular inferior alveolar nerve block (IANB) and maxillary infiltration techniques in symptomatic irreversible pulpitis (SIP) cases is reported to be only 28-47% (2-4) and 54-70% (5, 6) respectively. Though, various supplemental methods such as intra-ligamentary injection, intra-osseous injection techniques are available, it is wise to anticipate that an intrapulpal injection (IPI) is sometimes required to obtain total pulpal analgesia and for...
effective canal debridement, especially in teeth with acutely inflamed/hot pulp (7).

Following access cavity preparation, during cleaning and shaping, sodium hypochlorite (NaOCl) in various concentrations (0.5 % to 5.25%) is the commonly employed primary endodontic irrigant due to its excellent pulp tissue dissolution and dentine disinfecting capabilities (8, 9). NaOCl also possess strong antibacterial effect against a wide spectrum of micro-organisms, in addition to its therapeutic lubricating action (10, 11). Free active chlorine (FAC) content in NaOCl is mainly responsible for the dissolution of the organic tissue by breaking down proteins into amino acids; a process explained by chloramination (12). FAC also oxidizes the metabolic enzymes irreversibly and blocks the bacterial metabolism, contributing to its antimicrobial activity.

However, the interaction between local anaesthetic solution (administered for IPI) and the subsequently used NaOCl is often overlooked in many clinical instances (13). Vidhya et al. (14) reported that a chemical interaction between lignocaine hydrochloride (with and without adrenaline) and NaOCl occurs, forming a precipitate (2,6-xylidine), which is a known carcinogen. Such potential interactions might arise within the pulp chamber and root canal space, when NaOCl is subsequently employed for pulp tissue dissolution or as a hemostatic agent following IPI (15, 16). There are various reports in literature addressing the interaction between various endodontic irrigants and NaOCl with the resultant reduction in FAC (17, 18). These findings are considered to be clinically significant because the therapeutic properties of NaOCl will be compromised, if there is a significant fall in its FAC content. A careful review of literature revealed that there is only very limited information on the significant interaction between LA and NaOCl, and its effect on the FAC content. Hence, the aim of the present in vitro study was to evaluate the available FAC of 3% NaOCl after admixing with 2% lignocaine HCl with adrenaline (LA) in varying proportions, i.e., 9:1, 7:3 and 1:1 w/v. Our proposed null hypothesis was that, there were no variations in the FAC of NaOCl admixed with different proportions of LA.

MATERIALS AND METHODS
3% w/v of NaOCl (Vensons India, Bengaluru, India), 2% lignocaine hydrochloride containing 1:80,000 adrenaline (LA) (Astra Zeneca Pharma India, Ltd) and demineralised water (DW) (Well Thought Chemicals, Karnataka, India) were used in the present study. Three different volumes (27 mL, 21 mL and 15 mL) of NaOCl without any additive taken in independent aliquots served as the effective concentrate (undiluted NaOCl control group). This group indicated the actual FAC content of undiluted NaOCl. The experimental solutions (NaOCl-LA group) were made by diluting above volumes of NaOCl and LA in 9:1, 7:3 and 5:5 ratios (i.e., 27 mL: 3 mL, 21 mL: 9 mL and 15 mL: 15 mL) respectively. Similar admixed ratios of NaOCl and DM in the above proportions served as NaOCl-DM group. This group provided a measure of reduction in the FAC content of NaOCl as a result of dilution alone. FAC in each group was then calculated by iodometric titration method which was repeated five times for each test group. Independent assessments were made for each of the three different proportions for the respective test solutions and averaged.

**Titration procedure**
Iodometric titration was performed and active chlorine concentration \( C_{_{\text{NaOCl}}} \) was evaluated immediately after mixing for all combinations of NaOCl-DM and NaOCl-LA. 5 mL of the sample solution from each group \( V_s \) was carefully pipetted into a 100 mL conical flask (Tarsons, Delhi, India). Then, the analytical solutions were treated with iodide ion \( I^- \) (Chenchems, Chennai, India) a weak reducing agent, which later undergo oxidation to an elemental iodine \( I_2^- \). This elemental iodine combines with any excess iodide present to produce \( I_2^- \), a species that forms a bluish black colored complex with starch. This was then titrated with 0.1 mol/L concentration \( C_{_I^-} \) of standardized thiosulphate \( (S_2O_3^-) \) (Chenchems, Chennai, India) till the bluish black solution changed colourless and the total volume of thiosulphate \( V_T \) added was noted. This colour change was due to the reduction of elemental iodine back to iodide ion \( I^- \). These chemical processes are generally redox reactions and hence, this analysis is referred to as a redox titration.

The concentration of FAC was then calculated using the following formula:

\[
C_{_{\text{FAC}}} = \frac{V_s \times C_{_I^-} \times M_{_{\text{Cl}}} \times (g/mol)}{V_T}
\]

Where, \( V_s \)=Volume of the standardised thiosulfate used for the titration (in mL), \( C_{_I^-} \)=Concentration of standardized thiosulfate (0.1 mol), \( V_T \)=Volume of respective experimental and control solutions (5mL) and \( M_{_{\text{Cl}}} \)=Molar atomic mass of chlorine (35.4527 g/mol).

**Statistical analysis**
Statistical analysis was performed with IBMSPSS Statistics Version 22.0 (IBM Corp, Armonk, NY, USA) using one-way analysis of variance (ANOVA) and post hoc tukey test to compare the mean FAC between the control and experimental groups and
amongst the individual admixtures of NaOCl-DM and NaOCl-LA groups assessed in three different proportions. The level of significance was set at P<0.05 (Fig. 1).

RESULTS
The mean FAC values of all the test groups (control and experimental groups) are illustrated in Figure 1. Dilution of NaOCl with demineralised water produced arithmetic reduction in active chlorine content. Inter group comparisons revealed statistically significant differences (P<0.05) between all the test groups. FAC reduction was also observed amongst each of the proportional admixtures of NaOCl-DM as well as NaOCl-LA group. An intra-group analysis revealed statistical differences (P<0.05) in NaOCl-LA, whereas no statistical differences (P>0.05) were reported in NaOCl-DM. The mean FAC of NaOCl solutions admixed with LA showed the greatest reduction when compared with its similar counter proportions of NaOCl-DM admixtures. Further, there was a dose dependent reduction in FAC content in both the experimental groups, with lowest FAC values were observed in 1:1 solution admixtures followed by 7:3 and 9:1 proportions.

DISCUSSION
The success rate of maxillary anaesthesia varies from 54%-70% in SIP (5, 6). With this range of success rates, there is always a probable chance that the patient experiences pain and discomfort during endodontic treatment, thereby necessitating, supplementary anaesthetic techniques like intraligamentary, or intra-osseous, supplemental buccal and/or lingual anaesthesia as well as block anaesthesia and IPI to effectively perform the treatment. The most common attributed reasons are decreased tissue pH, altered resting nerve membrane potentials as well as upregulated anaesthetic-resistant sodium channels that are encountered in inflammatory conditions such as SIP (19). In addition, individual variations in bone density, tooth anatomy and its position are other significant contributing factors (20). In a clinical scenario, the success of local anaesthesia varies due to several reasons, including operator factors, where proper guidelines for achieving adequate anaesthesia are not quite duly followed. For instance, the waiting time period after IANB and maxillary infiltration technique administration is 15 mins and 10 mins respectively, which may be overlooked by the operator (21). Furthermore, despite the increased overall success of anaesthesia of supplemental buccal and lingual infiltration as well as the use of blocks, these are not routinely performed in SIP (22). Also, the teeth diagnosed with SIP often requires increased volume of anaesthetic administration and possibly premedication (23-25). Failure in adhering to the aforesaid anaesthetic guidelines often predisposes the patient to discomfort during access opening and pulp extration, requiring supplemental IPI (13).

IPI in SIP possess several advantages such as, immediate onset of action coupled with effective pulpal anaesthesia and does not require any special delivery devices (as in case of intra-osseous technique) (13, 16). Further, IPI technique can also be administered in case of systemic diseases, where premedication or other supplemental intra-ligamentary/intra-osseous methods are not usually recommended (26, 27). Intra-ligamentary injection has been reported to cause post-opera-
tive pain for 2 days along with the feeling of the tooth being “high” (28). Intraosseous technique is also quite painful with additional drawbacks such as post-operative swelling, paraesthesia, greater bone penetration and increased heart rate in susceptible individuals (29-31). Moreover, the success of intraosseous injection is reported to be 88% with 2% lignocaine and 86% with 4% articaine respectively, which potentiates the need for an additional IPI in hot tooth cases (32, 33). Despite the administration of proper anaesthetic techniques with all the aforementioned precautionary measures, there arise few clinical situations where pulp extirpation in one or two canals in multirooted teeth results in excruciating pain, restricting access to the coronal third of the canal. IPI will be more useful in these clinical situations to effectively continue the treatment. Topical 20% benzocaine gel along with hyaluronidase application can be considered to minimize the pain discomfort during IPI administration (34).

Sometimes, IPI needs to be also administered into the root canal orifices to achieve a snug fit of the needle when it is not possible to give IPI within the pulp chamber under pressure (16). Following this, NaOCl will be used for achieving pulp tissue dissolution, and/or for hemostasis in certain cases and also during routine cleaning and shaping procedures (35). Studies have reported that approximately 35-40% of canal walls remain uninstrumented due to anatomic complexities and the role of NaOCl to dissolve the tissue remnants and in disinfection is indispensable (12, 35). FAC content in NaOCl is mainly responsible for its pulp tissue dissolution and antimicrobial properties (11-13). Hence, it is not uncommon that the two solutions, i.e., NaOCl and lignocaine HCl (employed for IPI) to come into contact with each other. However, the effect of this interaction on the active chlorine content of NaOCl has not been explored previously and hence, the present investigation would be the first of its kind to evaluate the same.

NaOCl is an oxidizing agent that exists in solution exhibiting a dynamic balance, as shown by the following reaction:

\[ \text{NaOCl + H}_2\text{O} \rightarrow \text{NaOH} + \text{HOCl} \rightarrow \text{Na}^+ + \text{OH}^{-} + \text{H}^+ + \text{OCl}^- \]

FAC refers to the free and unbound chlorine which is readily available to react, and exists in the form of hypochlorous acid (HOCl) and hypochlorite ions (OCl-) (13). It can be stated that only 9:1 (NaOCl/LA) ratio rather other proportions is more clinically relevant. However, this small proportion of LA may not be sufficient enough to assess the effect of interaction between NaOCl and lignocaine HCl on the FAC content. Moreover, endodontic treatment in SIP tooth associated with multi-rooted canals may require additional IPI in each canal orifice to ensure patient’s maximum comfort. Hence, the other two proportions (i.e., 7:3 and 1:1) were also considered in the present study to effectively assess the changes in FAC. It can also be argued that, the remaining lignocaine HCl after an IPI can be easily removed with the introduction of fresh volume of NaOCl irrigant. However, the interaction between NaOCl and LA resulted in a precipitate formation on the radicular dentin which was difficult to remove completely following chemo-mechanical instrumentation (36). This leads to a possible speculation that LA remnants may be present in minute quantities; however, this needs further investigation. In addition, IPI is generally
used in acutely inflamed vital teeth where enormous pulp tissue will be present, thus requiring abundance of NaOCl for their removal.

Based on the results of the present study, it can be inferred that the admixed NaOCl solutions with either DM or LA resulted in a reduction in the active chlorine content. Additionally, there was a further significant reduction in FAC when the dilution of NaOCl was with LA. It is also quite evident that mixtures containing even small proportions of LA showed significant loss in FAC, thereby rejecting the null hypothesis tested. In general, the prime reasons attributed for any reduction in FAC content of NaOCl will be the result of a chemical reaction and/or dilution effect (11, 35, 37, 38). For instance, when NaOCl was used in conjunction with EDTA, a chemical interaction between alkaline NaOCl and the acidic hydrogens of EDTA through acid-base neutralization reaction occurs leading to chlorine gas evaporation and FAC reduction (36).

Dilution of NaOCl with demineralized water in this study produced the expected arithmetic reduction in active chlorine content which is in accordance with that of Clarkson et al. (17). This clearly indicates that mean FAC reduction in NaOCl-DM group is dependent on the effect of dilution alone, whereas in NaOCl-LA group, the mean FAC reduction is dependent on the chemical reaction, in addition to the dilution effect. According to the International Union of Pure and Applied Chemistry, the chemical name of lignocaine-HCl is 2-(diethylamino)-N-(2.6-dimethylphenyl) acetamide; hydrochloride. When NaOCl interacts with LA, it liberates hypochlorous acid (main source of FAC) that combines with the carbon atoms present in lignocaine HCl structure. This results in further disruption of the molecule with subsequent cleavage of the double bond present in lignocaine HCl (14). On further hydrolysis, it forms the precipitate 2.6-xylidine. This invariably explains the FAC loss in solution admixtures containing NaOCl-LA group, attributing to the consumption of HOCl in the chemical interaction, as depicted in Figure 2.

In addition to its effect on the FAC content, the clinical significance of the resultant precipitate is that it may act as a potential barrier for penetration of intra-canal irrigants/medicaments, and may compromise the seal of the root canal. It may also hinder with the coronal seal of the post endodontic restoration if the precipitate is not removed completely from the pulp chamber walls (37). Further studies are underway to explore the effect of the NaOCl-LA precipitate on the coronal and radicular dentin. Since, the beneficial effects of NaOCl solutions are totally dependent on the levels of FAC, adequate care should be taken to avoid such potential NaOCl-LA interactions. Further, following IPI, copious rinse with distilled water should be advocated to remove the remnants of the anaesthetic solution from the pulpal space before NaOCl use. Also, since the anaesthetic effect of the intra-pulpal anaesthesia is mainly due to the back-pressure of the solution rather independent of the solution injected (15), it may be advisable to use 0.9% normal saline rather LA for IPI.

The limitation of this in vitro study is that it does not simulate the clinical factors such as the buffering capacity of dentin as well as the instability of NaOCl solution during its chair side manipulation. Hence, the FAC values are generally lower than that calculated by iodometric titration method, as stated in previous studies (39, 40). In addition, the inclusion of chlorate, chlorite, perchlorate, and bromate ions in FAC measurements in the titration analysis makes it less accurate compared with FAC measurements using liquid chromatography and tandem mass chromatography (40). Hence, further validation is required to extrapolate our findings.

The results of the present study implicate the importance of avoiding interaction between NaOCl and LA, as there is a considerable reduction in FAC content of NaOCl even with low proportions of LA (9:1 or 7:3 w/v).

CONCLUSION

Within the limitations of this in vitro study, it can be concluded that the available FAC content of NaOCl was reduced, as dilution increased. In addition, when NaOCl was mixed with LA, there was a further significant reduction in the FAC content which may directly affect its clinical applications.

Disclosures

Conflict of interest: The authors report no potential conflict of interest.

Ethics Committee Approval: Ethics Committee approval has been sought for this in vitro study form Institutional Ethical Committee, SRM Dental College, Ramapuram, India (IRB Approval Number is SRMU/M&HS/SRMDC/2020/S/014)

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