Effects of Combining Sodium or Calcium Hypochlorite with Other Root Canal Irrigants- A Systematic Review

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Abstract

The root canal system is a very complex structure. Mere instrumentation with hand files or rotary instruments does not clean the canals of debris and microbes. This makes irrigation a vital step in root canal treatment. Irrigants are chemicals that interact with each other when used in combination. A thorough understanding of the chemical nature of the irrigants is necessary to prevent any undesired effects during or after root canal therapy.

An automated search was conducted for articles and published data on the interaction between sodium or calcium hypochlorite and other root canal irrigants. References of the selected articles were also searched manually for related articles. The present study focused on root canal irrigants and the byproducts of their interaction, published from 2012 to 2022.

In the present review, ten studies were included. These studies were based on the interaction between sodium or calcium hypochlorite and other root canal irrigants and the byproducts formed in the process. Six of the ten studies were performed on extracted teeth, and four were conducted in test tubes. It was observed that NaOCl and CHX formed precipitates with other irrigants. These precipitates block the dentinal tubules, and precipitates like PCA have been proven to be carcinogenic. Thus, intermediate irrigants like distilled water must be used to flush out one irrigant before using another.

It is necessary to follow a suggested irrigation protocol to avoid the precipitation of harmful chemicals in the root canals that may also interfere with the bonding of root canal sealers.

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Introduction

The success of root canal treatment relies on the endodontic triad, i.e., cleaning and shaping, disinfection and obturation of root canals.[1]Cleaning and shaping of root canals is usually achieved by bio-mechanical preparation and copious irrigation with irrigants such as sodium hypochlorite, chlorhexidine, EDTA, normal saline, MTAD and QMiX.[2-4]Considering the complex anatomy and morphology of the root canals, irrigants make cleansing the canals easier by eliminating the organic residues, bacteria, and smear layer.[5]

The irrigants are ideally required to have a prolonged antibacterial effect and have no adverse impact on the periapical tissues. However, there is no ideal endodontic irrigant, as not all irrigants meet the requirements of a perfect endodontic root canal irrigant. As a result, it is advised to use a combination of irrigants for more diverse effects, such as the dissolution of organic debris and removal of the smear layer, both of which are important for the disinfection of the canals.[6] Table 1 describes the root canal irrigants used during endodontic treatment.

It is reported and advised to use multiple irrigants during endodontic treatment to enhance antimicrobial efficacy.[19] NaOCl is recommended to dissolve the organic component of the debris, and EDTA (17%) is used to eliminate the smear layer by demineralization. Finally, the remaining bacteria are killed by thoroughly rinsing the canals with antibiotic agents such as CHX, Q-Mix, MTAD or NaOCl.[20] These solutions encounter each other during irrigation, and a chemical reaction occurs between them.[21] This chemical reaction causes solid precipitate formation as a

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byproduct which does not get flushed out quickly. As a result, this residue acts as a barrier between the root canal sealers and the root canal wall causing coronal or apical microleakage and failure of endodontic treatment. These byproducts could also be carcinogenic and have detrimental effects on periapical tissue.[22, 23]

The interaction between NaOCl, which is alkaline by nature, and CHX, a dicationic acid, is reported to enhance the antibacterial properties of NaOCl. Still, a brown precipitate is formed due to the reaction that forms a chemical smear layer blocking the dentinal tubules.[24]Precipitate formation with NaOCl is directly proportional to the concentration of NaOCl and can be noticed at a concentration as low as 0.19%. In contrast, colour change can be observed at a concentration of 0.023%. [25] Basrani et al. reported the presence of parachloroanaline (PCA), a potent toxin and carcinogen, in the brown residue. [25] This PCA has been reported to induce Methemoglobinemia, Haemolytic anaemia and carcinoma in rats. [26]

Thus, to prevent the formation of PCA and avoid complications, it has been suggested to rinse the root canal thoroughly after irrigation with NaOCl before using the next irrigant. Irrigation can be done with normal saline or citric acid. [27, 28]

The interaction between NaOCl and EDTA was studied by Grawehr et al. in 2003, and they concluded that EDTA undesirably affected NaOCl. When NaOCl encounters EDTA, one of the antagonistic reactions is the loss of free chlorine ions, which lowers the tissue dissolving capability and the antibacterial activity.[17]Thus, it was reported that after using EDTA or NaOCl, the canals must be thoroughly rinsed with normal saline or distilled water before using the next irrigant. [29]

When other irrigants such as EDTA and CHX interacted, milky- a white precipitate was formed due to an acid-base reaction called the salting-out process, as reported by Rasimick et al. [30]

Thus, considering the above studies, the purpose of the present study was to conduct a systematic review of all the in-vitro, ex-vivo and chemical analytic studies conducted regarding the effects of the combination of Sodium or Calcium Hypochlorite with other root canal irrigants.

Materials and methods

A systematic literature search was conducted in English and with time constraints, i.e., in-vitro, ex-vivo studies and chemical analytic studies on the chemical interaction between sodium/calcium hypochlorite and other endodontic irrigants published from 2012 to 2022 across the electronic databases such as PubMed, Scopus and Google India http://www.google.co.in., using chlorhexidine, citric acid, EDTA, endodontic irrigant, interaction, M-TAD, Q-MiX, precipitate, root canal irrigants, sodium hypochlorite as keywords. Supplementary records were obtained by searching through the bibliography of the included studies. Medical Subject Headings (MeSH) and keywords were formed during the search. The search was mainly focused on systematic reviews (level Ia evidence) and experimental trials (evidence levels IIb). The Oxford Center for Evidence-Based Medicine (http://www.cebm.net/levels of evidence. asp) guidelines were used to categorize the level of evidence for articles. [31] The research question was, "What are the effects of combining Sodium or Calcium Hypochlorite with other root canal irrigants?" The inclusion and exclusion criteria are mentioned in Table -2.

All references were combined and uploaded to EndNote v.X9.3.3 (Clarivate Analytics, PA, USA) to exclude duplicate articles. Two reviewers set aside all the titles and abstracts of the studies selected for full-text review. Two reviewers individually assessed the full text of the reference chosen articles differing from the intended inclusion criteria. In the final systematic review, the search results were detailed and abridged in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Table-3). [32]

Quality Assessment

The quality assessment of each study included in the review was evaluated by a set of questionnaires which examined whether a focused research question had been addressed in each study if pertinent keywords were used and the precision and validity of the outcomes and results. The questionnaire was based on the guidelines for reporting pre-clinical in vitro studies on dental materials.[33] (Table 4)

Results

A total of 115 studies were first collected using our retrieval search approach with the help of the reference manager End Note v.X9.3.3. As a result, 60 duplicate records were deleted from this set. Twenty were considered ineligible by the automation tool, and five articles were rejected for other reasons. After reading the titles and abstracts of the remaining 30 articles, another ten unrelated research were deleted. Of the remaining 20 studies, ten were eventually chosen for the systematic review based on full-text screening—many of the studies were eliminated due to multiple exclusion criteria. Table 3 depicts a flow chart of the studies screened, identified, evaluated for eligibility, included, and eliminated in this systematic review.

Characteristics of the included studies

A total of 10 studies were selected and analyzed in the present study. (Table 5) All the studies were published between 2012 to 2022. Six of the ten studies assessing the interaction between irrigants were performed on extracted teeth [34, 36, 37, 39,42,43], and four were conducted in test tubes. [35, 38, 40, 41] In the studies where teeth were used, the dentin from those teeth was cut into blocks, and the degree of dentinal tubule blockage by the residues was studied. In other studies, the tooth's root was split open, and canal blockage by the precipitate was studied in the coronal third, middle third and apical third. The interaction and byproducts of the interaction were analysed with the help of various instruments and devices like SEM, Light microscope, Electrospray ionization quadrupole time-of-flight mass spectrometry, Time-of-flight secondary ion mass spectrometry (TOF-SIMS) etc.

Discussion

Irrigation of the root canals during RCT is a crucial step as it flushes out the debris, the necrotic tissue, and the dentinal shavings from the canal. Irrigation during instrumentation of the root canals reduces the endotoxins in the canal.[44] Over 35% of the canal surface is untouched during instrumentation by hand or rotary instruments. Thus, these surfaces are cleaned by irrigants acting as antimicrobial agents to eliminate the organic and inorganic fragments in the root canal. [45]

Many irrigants have been used in endodontics. (Table -1) It is necessary to understand the chemical nature of these irrigants as multiple irrigants are used during root canal therapy to enhance the cleaning effect on the canals. These irritants may interact with each other and produce precipitates which may obliterate the canal walls and interfere wit h bonding between the root canal sealer and canal walls. The residues may interact with dental or periapical tissues and cause adverse reactions. Thus, the present literature review highlights the necessity for the dentist to understand the chemical nature of irrigants and their strength to prevent any untoward incidents during the treatment. [46]

In the present review, all the selected studies discussed the interaction between NaOCl and CHX. Both the irrigants, when tested individually, display good antimicrobial properties. But when combined, the antibacterial property was found to be augmented.[47] The main problem occurred when the root canals were irrigated with these irrigants one after the other. An insoluble precipitate formation was observed in this case. This residue was brown and contained Para-Chloro- Aniline, a cytotoxic carcinogen.[25]

This brown precipitate was examined meticulously in a study conducted by Prado et al. [35] , which assessed the byproducts formed during the interaction between the irrigants regularly used in endodontic treatment, represented in (Table 5). In this study, the association of NaOCl at 1%–5.25% concentrations with 2% CHX solution and gel caused the formation of orange-brown precipitates. The mass spectrometry analyses confirmed the presence of other byproducts of chlorination from NaOCl. The orange-brown colour of the residue could be associated with the guanidine oxidation of CHX. These findings were similar to those of the studies conducted by Nowicki et al. and Thomas et al., who could not trace the existence of para-chloroaniline using nuclear magnetic resonance.[48, 49] Nevertheless, these results differed from the results of Basrani et al., who found PCA using x-ray photon spectroscopy and TOF secondary ion mass spectrometry. [25]The difference in outcomes may be due to the use of different techniques. TOF secondary ion mass spectrometry deals with surface analyses like x-ray photon spectroscopy and has a different process of transferring molecules or aggregates

into the mass spectrometer. On the other hand, using nuclear magnetic resonance or ESI-QTOF-MS, the residue is all dissolved in a solvent, and the solution is analysed afterwards. [25, 35]

In the present review, few selected studies used dentin blocks to observe the blockage of dentinal tubules by the orange-brown precipitate (Table -3). Bui, Baumgartner, & Mitchell et al. mentioned [50] that the precipitate significantly affected the patency of dentinal tubules—similarly, Gasic. J et al.[33] confirmed that the precipitate obliterated the dentinal tubules. Sekiba et al. [43] found that the residue blocked the dentinal tubules in the canals' coronal, middle and apical thirds. More precipitate was in the apical third. [42, 43] It can be assumed that this obliteration may affect the bonding of root canal sealers with the canal walls, and prognosis may be affected.

In another study by Buyukozer Ozkan et al., photon-induced photoacoustic streaming (PIPS) activated irrigants were used to irrigate the root canals. Still, it was observed that PIPS activation didn't affect the oxidizing agents, and the precipitate was formed when PIPS-activated NaOCl was used with CHX.[41]

Another commonly used root canal irrigant during endodontic treatment is EDTA; the properties are mentioned in Table -1. In the present review, five studies examined EDTA with other irrigants for precipitate formation. According to Prado et al., a white precipitate was formed when 17% EDTA was combined with 2% CHX. [34] This was confirmed by Ashutosh N et al. and Tatjana K et al. [42]According to Rasimick et al., the precipitate formed after mixing 17% EDTA with 2% or 20% CHX was found to be EDTA or CHX salt, as examined using reversed-phase high-performance liquid chromatography.[30] On the other hand, when EDTA was mixed with NaOCl, it was observed that EDTA retained its calcium-complexing ability. Still, it reduced NaOCl's tissue-dissolving capacity, and no free chlorine was noticed in the combinations. This was because NaOCl lost the available chlorine and its tissue-dissolving effectiveness when EDTA was added. [51] This was proved by Prado et al. and Ashutosh et al., who observed bubble formation when the two irrigants were mixed. [35,40] QMiX is a new irrigant introduced in 2011. Its properties are mentioned in table 1. It is advised to be used as a last rinse following NaOCl irrigation at the end of instrumentation. According to the patent, QMiX contains a CHX analogue, Triclosan, and EDTA acts as a decalcifying agent and is designed to be both an antibacterial irrigant and a tool for removing debris from canal walls.[52]Additionally, it is included as another example of the decalcifying agent due to its unique characteristics, which have the potential for contact with the three primary irrigants in endodontics (NaOCl with EDTA+CHX).[53]

It has been observed that immediate colour change, gas release, and precipitate formation occur when mixing 5.25% NaOCl and QMiX. An unidentified inorganic salt precipitate forms within minutes. The precipitate forms a chemical smear layer, which occludes the dentinal tubules and may interfere with the seal of root filling. [54, 55] This was proven by Kolosowski. K et al, Arslan.H et al, Ashutosh N et al. and Tatjana K et al. [36,37,40,42]

A brown solution is formed when MTAD is used as the final irrigant after NaOCl.[56] A similar phenomenon was observed by Ashutosh et al. when 3% NaOCl was combined with MTAD.[40] Reactive Oxygen Species can explain this reaction (ROS) derived from O_2, hydroxyl radical, and H_2O_2 oxidize various tetracyclines to produce quinone derivatives, with reduced antimicrobial potency of hydronaphthacene antibiotics. [57,58] Thus, it is advisable not to use MTAD irrigation immediately after using NaOCl. An intermediate irrigant such as distilled water or normal saline can help wash out traces of NaOCl, after which MTAD can be used.

Dark brown precipitates are formed when 2% CHX and MTAD are mixed. This precipitate formation is due to the reaction of CHX with citric acid present in MTAD.[40] Similarly, when CHX and MTAD are used together, a similar dark brown precipitate occurs because of the reaction between chlorhexidine in the QMix and citric acid in the MTAD.[40] According to Khatod K et al., CHX also reacted with normal saline causing salt precipitation. This was also observed by Prado et al. [35, 38]

Hence, from all the observations mentioned above, it can be determined that a rational irrigation sequence needs to be developed so that irrigants can be appropriately administered to avoid any untoward mishaps during and after the endodontic treatment. Using these chemicals systematically to derive their full potential is also necessary, which is essential for endodontic treatment.[59] Basrani and Haapasalo

presented a suggested irrigation protocol in 2012 which was highly advisable, with one modification: Ethanol as an alternative to saline as an irrigant between CHX & NaOCl. [19, 54]

Conclusions

The dentist should understand the interactions between the numerous chemicals in irrigants since they could reduce one another's effectiveness and lead to the formation of Byproducts that are hazardous to the host. The orange-brown precipitate (PCA) formed by a combination of NaOCl-CHX is most concerning due to its cytotoxicity, resistance to removal, ability to occlude dentine canals, ability to cause microleakage, and failure of the endodontic treatment as a result. Except for PCA generation from the (NaOCl-CHX) reaction, most investigations indicate that intermediate flushes with distilled water seem appropriate to prevent or at least limit the development of the byproducts.

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Declaration of Interest

The authors report no conflict of interest.

Sl. No	Name of Irrigant	Chemical Structure	pH	Type	Mechanism of Action	Concentration of solution	Advantages	Disadvantages
1.	Sodium Hypochlorite[7]	NaOCl	9-12	Cl -releasing agent	1. Antibacterial activity 2. Oxidizing activity 3. Tissue dissolution	0.5 to 15%	1. Tissue solvent 2. Bactericidal 3. Debris cleared with effervescence	1. Toxic in high concentration 2. Tissue irritant 3. Tissue necrosis
2.	Chlorhexidine (CHX)[8]	$C_{22}H_{30}C_{12}N_{10}$	5.5 -7	Bis-guanides	1. Bacteriostatic in low concentration 2. Bactericidal in high concentration	0.12-2%	1. Substantivity 2. Bactericidal	1.Cytotoxic 2.Genotoxic 3. Delays wound healing 4. Incapable of tissue dissolution 5. Discoloration of teeth and tongue
3.	Ethylene Diamine Tetra acetic Acid (EDTA)[8]	$C_{10}H_{16}N_2O_8$	7-8	Polyprotic acid	1.Chelated Calcium ions in calcified canals	10-17%	1.Opens calcified canals 2.Widens narrow canals	1. Cytotoxic 2.Affects macrophage adherence
4.	Q Mix[9]	2% CHX + 17% EDTA	-	Bis-guanides + Polyprotic acid	1.Removal of smear layer 2. Bactericidal	Proprietary Blended formula	1.Removes smear layer from canal walls. 2. Antibacterial agent	1. Cytotoxic in higher concentrations 2. precipitation with other irrigants
5.	BIO-PURE™ M-TAD[10]	Doxycycline (3%) + Citric Acid (4.25%) + Tween 80 (0.5%)	2.15	Tetracycline containing irritant	1.Citric acid removes smear layer 2. Doxycycline is antibacterial agent	Proprietary Blended formula	1.Removal of Smear Layer 2.Antibacterial Agent	1.Discolouration of teeth 2.Contraindicated in pregnancy 3.Expensive 4.Short shelf life
6.	Chitosan[11]	$C_{56}H_{103}N_9O_{39}$	3.5	Natural polysacchride	1.Chelating agent 2.Antibacterial agent	0.2%	1. Chelating agent 2.Anti- fungal agent 3.Bio-compatiable	1. Very expensive
7.	Tetra Clean[12]	1.Doxycycline hyclate 2.Polypropylene glycol 3.Citric acid and cetrimide	-	Tetracycline containing irrigant	1.Antimicrobial agent	Proprietary Blended formula	1.Active against anaerobic bacteria and facultative bacteria 2.More potent than MTAD	1. Expensive
8.	Citric acid (CA)[13]	$C_6H_8O_7$	1.8	Acidic irrigant	1.efficient smear layer removal	10%- 20%	1.Biocompatiable	1.Mild degree of cytotoxicity
9.	Hydroxyethylidene bisphosphonate (HEBP) (Etidronic acid)[14]	$C_2H_8O_7P_2$	7	Soft chelating agent	1.Mild chelating agent	18%	1.No erosive effect on dentin 2. doesn't interact with NaOCl	1. No interaction or precipitatewith other
10.	Maleic Acid[15]	$C_4H_4O_4$	1.04	Mild organic acid	1.Removes smear layer in apical third	7%	1.Removes smear layer in apical third 2.Increase wettability of canal walls	1.Highly acidic
11.	Chlorine dioxide[16]	ClO_2	4.67	Cl-releasing agent	1.Powerful oxidizing agent 2.Bactericidal, virucidal, fungicidal	13.8%	1.Bactericidal 2.Kills enveloped and non-enveloped bacteria	1.Damages RBCs of it seeps beyond apex.
12.	Silver Diamine Fluoride (SDF)[17]	$Ag(NH_3)_2F$	8-9	Reducing agent	1.Powerful protein coagulating agent 2.Bactericidal	3.8%	1.Bactericidal agent 2. Occludes the dentinal canals	1. contact dermatitis/stomatitis 2. to be used with rubber dam
13.	Triclosan and Gantrez®[18]	S-97 BF PVM/MA copolymer $C_{12}H_7Cl_3O_2$	6.7	Broad Spectrum Antibiotic	1.Bactericidal, Fungicidal and Viricidal	-	1. Broad spectrum antibiotic	-
14.	Ozonated Water[19]	H_2O_4	7	Oxidizing agent	1.Bactericidal, Fungicidal and viricidal	0.5-4mg/L	1. Bactericidal, Fungicidal, Viricidal	1. Contradictory results about antibacterial activity

Table 1. Various irrigants used in endodontics.

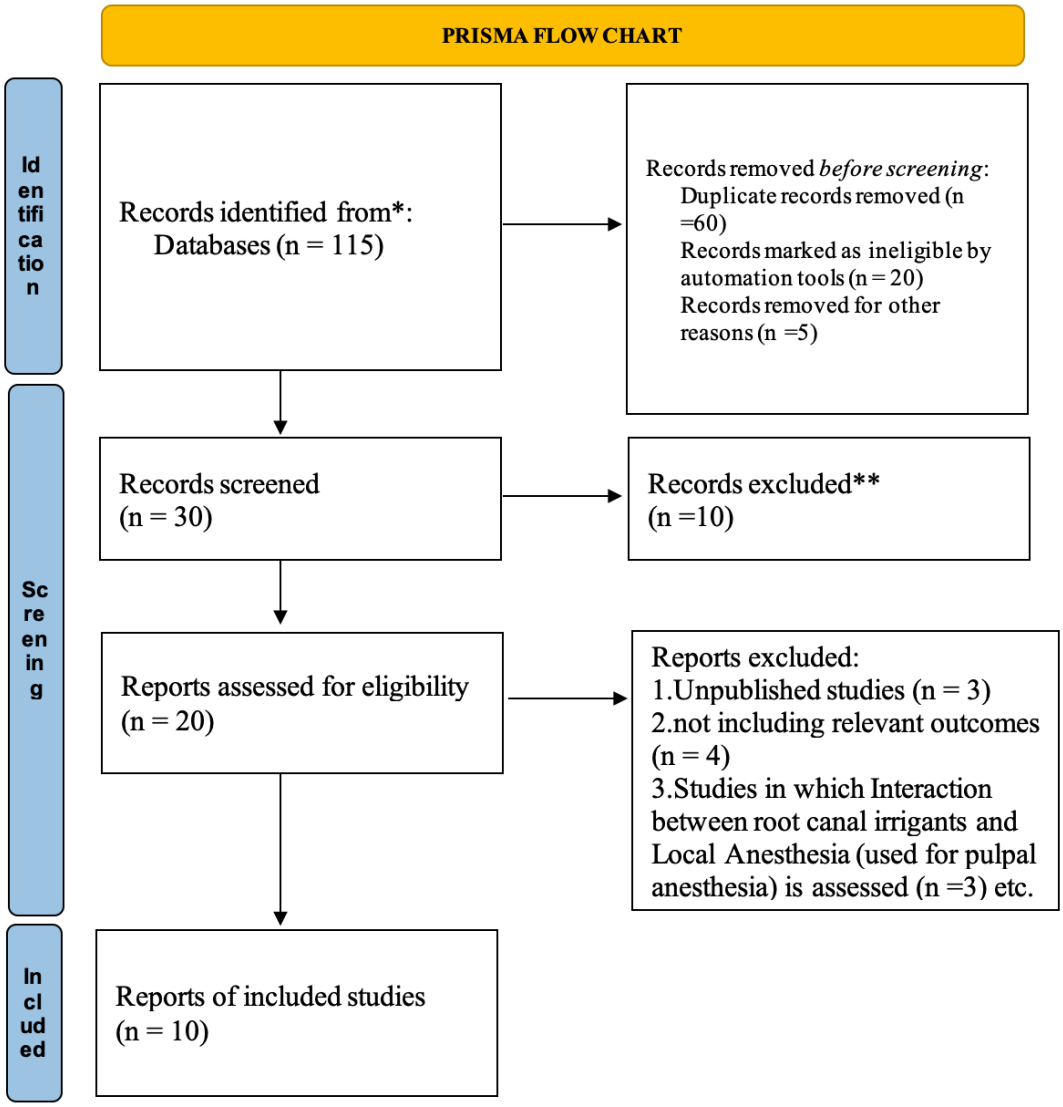

Table 2. Prisma Flow chart.

	Gasic. J et al.[33]	Prado. M et al.[34]	Kolosowski. K et al.[35]	Arslan.H et al.[36]	Khatod K. et al.[37]	Alegre. O et al.[38]	Ashutosh N et al.[39]	Buyukozer Ozkan et al.[40]	Tatjana K et al.[41]	Sekiba et al.[42]
Did the study include keywords that identify areas covered in the study?	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Did the study address a clearly focused question?	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Are the aims and purpose of the study clearly stated?	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Are the details of the technique sufficiently given to allow its transferability to other clinical settings?	yes	yes	Yes	no	Yes	Yes	no	Yes	no	Yes
Are the outcomes of the studies clearly stated?	yes	yes	no	Yes	no	Yes	Yes	Yes	Yes	no
Are the outcomes measured using a defined method?	yes	yes	yes	yes	Yes	yes	yes	Yes	Yes	Yes
Are the methods of collecting data clearly described?	yes	yes	Yes	no	Yes	Yes	no	Yes	no	Yes
Is the case clearly described?	yes	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Are the results credible and relevant for practice?	Yes	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Are the conclusions drawn justified by the results?	yes	yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Quality assessment score	10/10	10/10	9/10	8/10	9/10	10/10	8/10	10/10	8/10	9/10

Table 3. Quality assess ment table.

Sl no.	First author Country Year	Reference citation	Type of study	Irrigants tested	Control	Was the chemical reaction tested in tooth sample?	Tests for analysis of the byproduct	Byproducts	Undesired results
1.	Gasic. J et al.[34] Serbia 2012	Gasic J. et.al Ultrastructural analysis of the root canal walls after simultaneous irrigation of different sodium hypochlorite concentration and 0.2% chlorhexidine gluconate. Microsc Res Tech. 2012;75(8):1099-103.	Ex-vivo study	1. NaOCl (at different concentrations) 2. CHX (at different concentrations)	5% NaOCl alone 5% CHX alone	Yes. 35 single-rooted teeth	1. SEM 2. Light microscope	1. Yellow-brown solution	1. The precipitate obliterated the dentinal canal walls
2.	Prado. M et al.[35] Brazil 2013	Prado M. et.al Interactions between irrigants commonly used in endodontic practice: a chemical analysis. J Endod. 2013;39(4):505-10.	Chemical analysis	1. NaOCl solutions at 0.16%, 1%, 2.5%, and 5.25% 2. 2% CHX solution and gel 3. 17% EDTA, 10% citric acid 4. 37% phosphoric acid 5. Saline solution (0.9% sodium chloride) 6. Ethanol	Distilled water	No	1. Electrospray ionization quadrupole time-of-flight mass spectrometry	1. CHX produced an orange-brown precipitate when associated with NaOCl (1%–5.25%) 2. CHX produced Orange-white precipitate when associated with 0.16% NaOCl. 3. When associated with EDTA, CHX produced a white milky precipitate 4. When CHX was associated with saline solution and ethanol, a salt precipitation was produced.	1.Precipate formation was observed with CHX.
3.	Kolosowski. K et al.[36] Canada 2014	Kolosowski KP. et. Al Qualitative analysis of precipitate formation on the surface and in the tubules of dentin irrigated with sodium hypochlorite and a final rinse of chlorhexidine or QMIX. J Endod. 2014;40(12):2036-40.	In-vitro study	1.2.5% NaOCl 2. Q-MIX 3. 17% EDTA 4. 2% CHX	Saline	Yes. Dentin blocks obtained from 4 maxillary molars	1.Time-of-flight secondary ion mass spectrometry (TOF-SIMS)	1. Brown precipitate was found in all dentin blocks that were treated with Q-MiX immediately after treatment with NaOCl	1. PCA comprising precipitate was formed on the surface of the dentin and in the tubules of dentin, irrigated with NaOCl followed by CHX. 2. No PCA was detected in the ppt in dentinal tubules irrigated with NaOCl and Q-MIX possibly due to low concentration of CHX in Q-MIX.
4.	Arslan.H et al.[37] Turkey 2014	Arslan H. et.al Evaluation of orange-brown precipitate formed in root canals after irrigation with chlorhexidine and QMix and spectroscopic analysis of precipitates produced by a mixture of chlorhexidine/NaOCl and QMix/NaOCl. Int Endod J. 2015;48(12):1199-203.	Ex-vivo study	1.NaOCl(at different concentrations) 2. CHX(at different concentrations) 3. Q-MIX	Distilled water	Yes. 57 single rooted anterior teeth	1.Stereomicroscope 2. ^{1}H NMR spectra	1. Orange – Brown precipitate was formed in root canals irrigated with NaOCl followed by CHX 2. precipitate formation was observed in root canals irrigated with NaOCl followed by Q-MIX.	1.Orange – Brown precipitate was found to be Para chloro aniline 2. The precipitate with irrigation of NaOCl and Q-MIX was not found to be Para chloro aniline.
5.	Khatod K. et al.[38] India 2015	Kaustubh Khatod, Ajay Saxena, Manoj Chandak et al. Chemical Interactions between Different Irrigating Solutions: A Spectrometric Study. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2015; 14 (4) :69-74.	Chemical analysis	1.NaOCl (0.16%, 1%, 2.5% and 5.25%) 2.CHX solution 3.10% citric acid 4.37% phosphoric acid 5.7% maleic acid 6.saline solution 7.ethanol	Distilled water	No	1.Hybrid Quadrupole-Orbital Mass Spectrometer	1. Orange- Brown precipitate with 2% CHX is mixed with (1-5.25 %) NaOCl 2. Salt precipitation when 2% CHX is mixed with Saline solution 3. Bubble formation when 5.25 % NaOCl is mixed with 10% Citric Acid 4. Yellow solution with bubble formation when 5.25 % NaOCl is mixed with 37% Phosphoric Acid	1. The interaction of NaOCl and CHX forms orange-brown precipitate due to oxidizing action NaOCl. 2. The precipitate formed with ethanol and saline solution was due to lower solubility and salting-out process respectively. 3. NaOCl solution on reacting with citric acid and phosphoric acid lead to chlorine formation
6.	Alegre. O et al.[39] Chile 2017	Alegre O. et. Al. Determination of residual parachloroaniline produced by endodontic treatment after the use of 5% sodium hypochlorite and 2% chlorhexidine combined: an ex-vivo study. Revista Clínica de Periodoncia, Implantología y Rehabilitación Oral [Internet]. 2017;10(3):145-148.	Ex-vivo study	1. 5% NaOCl 2. 0.9% NaCl 3. 17% EDTA 4. 2% CHX	Nil	Yes. Twenty premolars	1. UV visible Spectrophotometer	1. PCA formation was detected at the least concentration of CHX and NaOCl.	1. CHX hydrolyzes to PCA spontaneously in the presence of heat, light and alkaline environments even at the least concentration
7	Ashutosh N et al.[40] India 2018	Ashutosh N, Gupta AK, Sharma V. Interaction between Irrigants commonly used in Endodontic Practice and their Characterization using 1H NMR Spectra. Int J Res Health Allied Sci 2018; 4(3):15-19.	Chemical analysis	1. 3% NaOCl 2. 17% EDTA 3. 2% CHX 4. Q- MIX 5. MTAD 6. Distilled water	Distilled water	No	1. H NMR	1.3% NaOCl +17% EDTA =Bubble formation 2. 3% NaOCl 2% CHX =Orange brown ppt 3. 3% NaOCl + MTAD = Mud Colour ppt 4. 17% EDTA + 2% CHX =Milky-White ppt 5. 2% CHX + Q-MIX = Milky White ppt 6. 2% CHX + MTAD = Dark brown ppt 7. Q-MIX+ MTAD = Dark brown ppt	1. CHX must be used very cautiously during irrigation of root canals. 2. Combination of CHX with other alkaline irrigants must be avoided. 3. Combination of Q MiX and MTAD must be avoided
8	Buyukozer Ozkan et al.[41] Turkey 2019	Buyukozer Ozkan H, Terlemez A, Orhan EO. Proton Nuclear Magnetic Resonance Spectroscopy Analysis of Mixtures of Chlorhexidine with Different Oxidizing Agents Activated by Photon-Induced Photoacoustic Streaming for Root Canal Irrigation. Photobiomodul Photomed Laser Surg. 2020;38(6):374-379	Chemical analysis	1. 98% PCA 2. 2% CHX 3. 5.25% NaOCl 4. 5.25% NaOCl +30 sec PIPS 5. 5.25% NaOCl +60 sec PIPS 6. 3.5% ClO2 7. 3.5% ClO2 + 30 sec PIPS 8.3.5% ClO2 + 60 sec PIPS	Distilled water. 0.5% NaOCl	No	1.Proton nuclear magnetic resonance (HNMR) spectroscopy)	1. Interaction between NaOCl or PIPS activated NaOCl and CHX causes formation of brown precipitate. 2. Interaction between ClO_2 or PIPS activated ClO_2 and CHX causes no ppt formation, but it changes the colour of the solution.	1.PIPS activation does not cause changes in reactions of oxidizing agents.
9	Tatjana K et al.[42] Serbia 2019	Tatjana K*, Ljiljana B, Dajana Nogo Ž, Bojan K, Irena T. Formation of Precipitates on the Surface of Root Dentin After Various Final Irrigation Protocols. Biomed J Sci & Tech Res 2019;14(2): 10493- 10501.	Ex-vivo study	1.CHX(at different concentrations) 2. NaOCl(at different concentrations) 3.EDTA 4.Q-MiX 5. MTAD	Distilled water	Yes. 90 maxillary anterior teeth.	1. Scanning Electron Microscope	1.Precipitate was formed in all thirds of the roots irrigated with CHX as final irrigant. 2. Lowest Precipitate was formed in MTAD and Q -MiX group	1.Higher amount of precipitate is formed when CHX is the final irrigant compared to MTAD and QMIX.
10	Sekiba et al.[43] India 2021	Bharath Makonahalli Jaganath, Sahadev Chickmagaravalli Krishnegowda, Sandeep Rudranaik et al. Comparative Evaluation of the Precipitate Formation on Interaction of Calcium Hypochlorite with 17% Edta, 20% Citric Acid and 2% Chlorhexidine: An In Vitro Study. WJPMR 2021;7(5):239 – 243.	Ex-vivo study	1.Calcium Hypochlorite (at different concentrations) 2. CHX (at different concentrations) 3. EDTA 4. Citric acid	Nil	Yes. 30 Single -rooted teeth	1.Stereomicroscope	1. Orange brown ppt was formed when Calcium Hypochlorite reacted with CHX 2. Chlorine gas was released when Calcium Hypochlorite reacted EDTA 3.Chlorine gas was released when Calcium Hypochlorite reacted with citric acid	1.Highest amount of ppt was formed when CHX reacted with Calcium hypochlorite in all the coronal, middle and apical thirds of the canal.

Table 4. Studies conducted on interaction between endodontic irrigants.

References

1. Musani I, Goyal V, Singh A, Bhat C. Evaluation and comparison of biological cleaning efficacy of two endo files and irrigants as judged by microbial quantification in primary teeth - an in vivo study. Int J Clin Pediatr Dent. 2009 ;2(3):15-22. doi: 10.5005/jp-journals-10005-1013. Epub 2009 Dec 26. PMID: 25206117; PMCID: PMC4086577.
2. Walker A. Definite and Dependable therapy for pulpless teeth. *J AM Dent Assoc* . 1936;23:14–18.
3. Lekshmy DS, Kamath PM. Antimicrobial efficacy of 0.2 and 2% Chlorhexidine and sodium hypochlorite as root canal irrigant: An Invivo study. *Endodontology.* 2001;13:57–62
4. Nogo-Zivanovic D., Bjelovic L., Ivanovic V., Kanjevac T., Tanaskovic I.. Consideration of the Therapeutic Potential of Irrigants in Endodontic Therapy. Experimental and Applied Biomedical Research (EABR). 2018;19(2): 103-112. https://doi.org/10.1515/sjecr-2016-0095
5. Gutmann JL, Dumsha TC, Lovdahl PE. Problem Solving in Endodontics. 4th ed. St Louis, MO: Elsevier; 2006.
6. Baumgartner JC, Brown CM, Mader CL, et al. A scanning electron microscopic evaluation of root canal debridement using saline, sodium hypochlorite, and citric acid. J Endod 1984;10:525–30.
7. Sodium Hypochlorite In Endodontics – The Bench Mark Irrigant: A Review. European Journal of Molecular & Clinical Medicine, 2020; 7(5): 1235-1239.
8. Agrawal Vineet S, Rajesh M, Sonali K and Mukesh P. A Contemporary Overview of Endodontic Irrigants – A Review. J Dent App. 2014;1(6): 105-115.
9. AlKahtani et al.: Cytotoxicity of QMix™ endodontic irrigating solution on human bone marrow mesenchymal stem cells. BMC Oral Health 2014 14:27.
10. Srikumar GP, Sekhar KS, Nischith KG. Mixture tetracycline citric acid and detergent - A root canal irrigant. A review. J Oral Biol Craniofac Res. 2013;3(1):31-5. doi: 10.1016/j.jobcr.2012.09.001. Epub 2012 Sep 14. PMID: 25737877; PMCID: PMC3941632.
11. 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. Epub 2012 Sep 13. PMID: 22970844.
12. Agrawal Vineet S, Rajesh M, Sonali K and Mukesh P. A Contemporary Overview of Endodontic Irritants – A Review. J Dent App. 2014;1(6): 105-115. ISSN:2381-9049
13. Yamaguchi M, Yoshida K, Suzuki R, Nakamura H. Root canal irrigation with citric acid solution. J Endod. 1996;22(1):27-9. doi: 10.1016/S0099-2399(96)80232-9. PMID: 8618082.
14. Tartari T, de Almeida Rodrigues Silva E Souza P, Vila Nova de Almeida B, Carrera Silva Júnior JO, Facíola Pessoa O, Silva E Souza Junior MH. A new weak chelator in endodontics: effects of different irrigation regimens with etidronate on root dentin microhardness. Int J Dent. 2013; 2013:743018. doi: 10.1155/2013/743018. Epub 2013 Jul 25. PMID: 23983692; PMCID: PMC3745862.
15. Ballal NV, Ferrer-Luque CM, Sona M, Prabhu KN, Arias-Moliz T, Baca P. Evaluation of final irrigation regimens with maleic acid for smear layer removal and wettability of root canal sealer. Acta Odontol Scand. 2018;76(3):199-203. doi: 10.1080/00016357.2017.1402208. Epub 2017 Nov 10. PMID: 29126370.
16. Lundstrom JR, Williamson AE, Villhauer AL, Dawson DV, Drake DR. Bactericidal activity of stabilized chlorine dioxide as an endodontic irrigant in a polymicrobial biofilm tooth model system. J Endod. 2010;36(11):1874-8. doi: 10.1016/j.joen.2010.08.032. Epub 2010 Sep 19. PMID: 20951304.

17. Shah S, Bhaskar V, Venkatraghavan K, Choudhary P, M. G, Trivedi K. Silver Diamine Fluoride: A Review and Current Applications. Journal of Advanced Oral Research. 2014;5(1):25-35. doi:10.1177/2229411220140106
18. Alpan, A. L. , Bakar, O. . Ozone in Dentistry. In: Derco, J. , Koman, M. , editors. Ozone in Nature and Practice [Internet]. London: IntechOpen; 2018 [cited 2022 Nov 23]. Available from: https://www.intechopen.com/chapters/60693 doi: 10.5772/intechopen.75829
19. Basrani B, Haapasalo M. Update on endodontic irrigating solutions. Endod Topics. 2012;27(1):74-102
20. Rôças IN, Siqueira JF Jr. Comparison of the in vivo antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as root canal irrigants: a molecular microbiology study. J Endod. 2011;37(2):143-50. doi: 10.1016/j.joen.2010.11.006. PMID: 21238793.
21. Tatjana K*, Ljiljana B, Dajana Nogo Ž, Bojan K, Irena T. Formation of Precipitates on the Surface of Root Dentin After Various Final Irrigation Protocols. Biomed J Sci & Tech Res 2019; 14(2). BJSTR. MS.ID.002512
22. Akisue E, Tomita VS, Gavini G, Poli de Figueiredo JA. Effect of the combination of sodium hypochlorite and chlorhexidine on dentinal permeability and scanning electron microscopy precipitate observation. J Endod 2010; 36:847–50.
23. Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridement. J Endod 1987;13:47–51.
24. Rossi-Fedele G, Doğramaci EJ, Guastalli AR, Steier L, de Figueiredo JA. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. J Endod 2012; 38(4): 426-31. [http://dx.doi.org/10.1016/j.joen.2012.01.006] [PMID: 22414823]
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.
26. Chhabra RS, Huff JE, Haseman JK, Elwell MR, Peters AC. Carcinogenicity of p- chloroaniline in rats and mice. Food Chem Toxicol. 1991;29(2):119-24.
27. Nowicki JB, Sem DS. An In Vitro Spectroscopic Analysis to Determine the Chemical Composition of the Precipitate Formed by Mixing Sodium Hypochlorite and Chlorhexidine. Journal of Endodontics. 2011;37(7):983-8.
28. Krishnamurthy S, Sudhakaran S. Evaluation and Prevention of the Precipitate Formed on Interaction between Sodium Hypochlorite and Chlorhexidine. J Endod. 2010;36(7):1154-7.
29. Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J. 2003;36(6):411-7. doi: 10.1046/j.1365-2591.2003.00670.x. PMID: 12801288.
30. 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.
31. Phillips B, Ball C, Sackett D, et al. Oxford Centre for Evidence-based Medicine-Levels of Evidence. Since November 1998. Updated by Jeremy Howick March 2009.
32. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1. doi: 10.1186/2046-4053-4-1. PMID: 25554246; PMCID: PMC4320440.
33. Faggion CM Jr. Guidelines for reporting pre-clinical in vitro studies on dental materials. J Evid Based Dent Pract. 2012 Dec;12(4):182-9. doi: 10.1016/j.jebdp.2012.10.001. PMID: 23177493.
34. Gasic J, Popovic J, Zivković S, Petrovic A, Barac R, Nikolic M. Ultrastructural analysis of the root canal walls after simultaneous irrigation of different sodium hypochlorite concentration and 0.2% chlorhexidine gluconate. Microsc Res Tech. 2012;75(8):1099-103. doi: 10.1002/jemt.22036. Epub 2012 Mar 15. PMID: 22419366.
35. Prado M, Santos Júnior HM, Rezende CM, Pinto AC, Faria RB, Simão RA, Gomes BP. Interactions between irrigants commonly used in endodontic practice: a chemical analysis. J Endod. 2013;39(4):505-10. doi: 10.1016/j.joen.2012.11.050. Epub 2013 Jan 30. PMID: 23522546.
36. Kolosowski KP, Sodhi RN, Kishen A, Basrani BR. Qualitative analysis of precipitate formation on the surface and in the tubules of dentin irrigated with sodium hypochlorite and a final rinse of chlorhexidine or QMiX. J Endod. 2014;40(12):2036-40. doi: 10.1016/j.joen.2014.08.017. Epub 2014 Oct 8. PMID: 25305239.
37. Arslan H, Uygun AD, Keskin A, Karatas E, Seçkin F, Yıldırım A. Evaluation of orange-brown precipitate formed in root canals after irrigation with chlorhexidine and QMix and spectroscopic analysis of precipitates produced by a mixture of chlorhexidine/NaOCl and QMix/NaOCl. Int Endod J. 2015;48(12):1199-203. doi: 10.1111/iej.12427. Epub 2015 Jan 17. PMID: 25557836.
38. Kaustubh Khatod, Ajay Saxena, Manoj Chandak et al. Chemical Interactions between Different Irrigating Solutions: A Spectrometric Study. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2015; 14 (4) :69-74.
39. Alegre O, Yévenes I, Parada J, Maggiolo S, , Fernández E. Determination of residual parachloroaniline produced by endodontic treatment after the use of 5% sodium hypochlorite and 2% chlorhexidine combined: an ex-vivo study. Revista Clínica de Periodoncia, Implantología y Rehabilitación Oral [Internet]. 2017;10(3):145-148.
40. Ashutosh N, Gupta AK, Sharma V. Interaction between Irrigants commonly used in Endodontic Practice and their Characterization using 1H NMR Spectra. Int J Res Health Allied Sci 2018; 4(3):15-19.
41. Buyukozer Ozkan H, Terlemez A, Orhan EO. Proton Nuclear Magnetic Resonance Spectroscopy Analysis of Mixtures of Chlorhexidine with Different Oxidizing Agents Activated by Photon-Induced Photoacoustic Streaming for Root Canal Irrigation. Photobiomodul Photomed Laser Surg. 2020;38(6):374-379. doi: 10.1089/photob.2019.4734. Epub 2020 Mar 2. PMID: 32119810.
42. Tatjana K*, Ljiljana B, Dajana Nogo Ž, Bojan K, Irena T. Formation of Precipitates on the Surface of Root Dentin After Various Final Irrigation Protocols. Biomed J Sci & Tech Res 2019;14(2): 10493- 10501.
43. Bharath Makonahalli Jaganath, Sahadev Chickmagaravalli Krishnegowda, Sandeep Rudranaik et al. Comparative Evaluation of the Precipitate Formation on Interaction of Calcium Hypochlorite with 17% Edta, 20% Citric Acid and 2% Chlorhexidine: An In Vitro Study. WJPMR 2021;7(5):239 – 243.
44. Boreak N. Effect of Bio Mechanical Preparation on Endotoxin Levels in Patients with Chronic Apical Periodontitis. Journal of International Dental and Medical Research. 2022;15(1):158-64.
45. Peters OA, Schönenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. Int Endod J. 2001;34(3):221-30. doi: 10.1046/j.1365-2591.2001.00373.x. PMID: 12193268.
46. Mortenson D, Sadilek M, Flake NM, Paranjpe A, Heling I, Johnson JD, Cohenca N. The effect of using an alternative irrigant between sodium hypochlorite and chlorhexidine to prevent the formation of para-chloroaniline within the root canal system. Int Endod J. 2012;45(9):878-82. doi: 10.1111/j.1365-2591.2012.02048.x. Epub 2012 Apr 6. PMID: 22486894.
47. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod. 1998;24(7):472-6. doi: 10.1016/S0099-2399(98)80049-6. PMID: 9693573.
48. Nowicki J.B., Sem D.S. An in vitro spectroscopic analysis to determine the chemical composition of the precipitate formed by mixing sodium hypochlorite and chlorhexidine. J. Endod. 2011; 37:983–988. doi: 10.1016/j.joen.2011.03.033.

49. Thomas JE, Sem DS. An in vitro spectroscopic analysis to determine whether para-chloroaniline is produced from mixing sodium hypochlorite and chlorhexidine. J Endod. 2010;36(2):315-7. doi: 10.1016/j.joen.2009.10.028. Epub 2009 Dec 14. PMID: 20113799; PMCID: PMC2818400.
50. Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the Interaction between Sodium Hypochlorite and Chlorhexidine Gluconate and its Effect on Root Dentin. Journal of endodontics. 2008;34(2):181-185. doi: 10.1016/j.joen.2007.11.006
51. Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J. 2003;36(6):411-7. doi: 10.1046/j.1365-2591.2003.00670. x. PMID: 12801288.
52. Haapasalo M, inventor: The University of British Columbia, assignee. Composition and method for irrigation of a prepared dental root canal. USA, 2008.
53. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. Int Endod J. 2012;45(4):363-71. doi: 10.1111/j.1365-2591.2011.01985. x. Epub 2011 Nov 16. PMID: 23134158.
54. Vivacqua-Gomes N, Ferraz CC, Gomes BP, et al. Influence of irrigants on the coronal microleakage of laterally condensed gutta-percha root fillings. Int Endod J 2002;35: 791–5
55. Krishnamurthy S, Sudhakaran S. Evaluation and prevention of the precipitate formed on interaction between sodium hypochlorite and chlorhexidine. J Endod 2010;36: 1154–7.
56. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endod 2003; 29:233–9.
57. Lau C, Lu J, Kai M. Chemiluminescence determination of tetracycline based on radical production in a basic acetonitrile-hydrogen peroxide reaction. Anal Chim Acta. 2004; 503:235–9.
58. Miskoski S, Sanchez E, Garavano M, Lopez M, Soltermann AT, Garcia NA. Single molecular oxygen-mediated photo-oxidation of tetracyclines: kinetics, mechanism and microbiological implications. J Photochem Photobiol B Biol 1998; 43:164 –71.
59. Boreak.N. Effect of Bio Mechanical Preparation on Endotoxin Levels in Patients with Chronic Apical Periodontitis. Journal of International Dental & Medical Research. 2022; 15 (1):158-164.