revista de ciências da saúde NOVA ESPERANÇA
VOLUME 23 - NÚMERO 1
ISSN ELETRÔNICO 2317-7160

CHELATING ACTIVITY OF EDTA AND CHITOSAN IN ENDODONTIC TREATMENT: A SYSTEMATIC REVIEW AND META-ANALYSIS

ATIVIDADE QUELANTE DO EDTA E QUITOSANA EM TRATAMENTO ENDODÔNTICO: UMA REVISÃO SISTEMÁTICA E METANÁLISE

[I]sabela Alcântara Farias, [II]Matheus de Andrade Rodrigues, [III]Helene Soares Moura,
[IV] Larissa Chaves Morais de Lima, [V]Morgana Maria Souza Gadêlha de Carvalho, [*VI]Rodrigo Barros Esteves Lins.

Abstract. This systematic review and meta-analysis (MA) aimed to compare the efficacy between ethylenediaminetetraacetic acid (EDTA) (15-17%) and chitosan (CH) (0.04-1%) solution in the final irrigation of endodontic treatment in *in vitro* studies. The electronic search was performed on six scientific platforms, using MeSH terms, supplementary concepts, and keywords. The PICOS search strategy was: Population (P): human teeth undergoing endodontic treatment; Intervention (I): CH solution; Comparison (C): EDTA-based irrigating solution; Outcome (O): level of chelating activity; and Study Type (S): *in vitro* studies. The risk of bias was assessed in 14 parameters and the MA by the RevMan Software. According to a random effect model, the mean, standard deviation, and total samples were used to calculate the mean and standard difference at a 95% confidence interval. The Index I² assessed heterogeneity. 22 studies of the 2,568 articles were evaluated, 7 of which were submitted to MA. The risk of bias was considered low. Three MAs evaluated the concentration of calcium ions, cement penetration into the dentinal tubules, and Knoop microhardness with no significant differences. Final irrigation of endodontic treatment with EDTA solution has a chelating activity similar to CH, based on *in vitro* studies with a low risk of bias.

Keywords: Chitosan; Edetic acid; Systematic review.

Resumo. Esta revisão sistemática e metanálise (MA) objetivou comparar a eficácia entre a solução do ácido etilenodiaminotetracético (EDTA) (15-17%) e quitosana (CH) (0,04-1%) na irrigação final do tratamento endodôntico em estudos in vitro. A busca eletrônica foi realizada em seis plataformas científicas, através de uma chave de busca contendo termos MeSH, conceitos suplementares e termos livres. A estratégia de busca PICOS foi: População (P): dentes humanos submetidos ao tratamento endodôntico; Intervenção (I): solução de CH; Comparação (C): solução irrigante à base de EDTA; Desfecho (O): nível de atividade quelante; e Tipo de Estudo (S): estudos in vitro. O risco de viés foi avaliado em 14 parâmetros e as MA pelo Software RevMan. A média, desvio padrão e o número total de amostras foram usadas para calcular a diferença média padrão em um intervalo de confiança de 95%, de acordo com um modelo de efeito randômico. A heterogeneidade foi avaliada pelo índice I2. Assim foram avaliados 22 estudos dos 2.568 artigos selecionados, sendo 7 submetidos à MA. O risco de viés foi considerado baixo para todos os estudos. Três MA avaliaram a concentração de íons cálcio, penetração do cimento nos túbulos dentinários e microdureza Knoop, para todas não foi observada diferença significativa entre as soluções de irrigação final. De acordo com esta revisão sistemática e MA, a irrigação final do tratamento endodôntico com solução de EDTA apresenta atividade quelante similar à CH, baseado em estudos in vitro com baixo risco de viés.

Palavras-chave: Quitosana; Ácido edético; Revisão sistemática.

[I]Cirurgiã-Dentista, Universidade Estadual da Paraíba
CEP: 58233-000, Araruna, Paraíba, Brasil
orcid.org/0000-0003-4525-025X.

[II]Cirurgião-Dentista, Universidade Estadual da Paraíba
CEP: 58233-000, Araruna, Paraíba, Brasil
orcid.org/0000-0003-2501-6546.

[III]Cirurgiã-Dentista, Mestra, Universidade Estadual da Paraíba
CEP: 58233-000, Araruna, Paraíba, Brasil
orcid.org/0000-0001-8134-4566.

[IV]Cirurgiã-Dentista, Doutora, Universidade Estadual da Paraíba
CEP: 58233-000, Araruna, Paraíba, Brasil
orcid.org/0000-0002-9351-5438.

[V]Bióloga, Doutora, Universidade Estadual da Paraíba
CEP: 58233-000, Araruna, Paraíba, Brasil
orcid.org/0000-0001-5001-4580.

[*VI]Cirurgião-Dentista, Doutor, Faculdade de Odontologia, Universidade Federal de Alagoas
CEP:57072-900, Maceió, Alagoas, Brasil
E-mail:rodrigo.lins@foufal.ufal.br,

orcid.org/0000-0002-8224-6578.

English translation by Matheus de Almeida Barbosa
Submissão:03/07/2024 Aceite:28/04/2025
DOI: 10.17695/rcsne.vol23.n1.p105-119

INTRODUCTION

One of the functions of using chelating solutions for the final irrigation of endodontic treatment (FIET) is the removal of the smear layer.1 Thus, studies recommend that this removal begins by irrigating the root canal with sodium hypochlorite (NaOCl), followed by final irrigation with ethylenediaminetetraacetic acid (EDTA). However, this association of NaOCl and EDTA reduces microhardness and promotes dentin erosion.2 On the other hand, less irritating and more biocompatible chelating solutions have been proposed for this purpose, considering that EDTA can cause changes in the biomechanical properties of dentin, altering the proportion of organic and inorganic components and, consequently, affecting the permeability, hardness, and dentin solubility.3

Currently, an ideal irrigating solution is yet to be available. At the same time, nothing has tissue dissolution activity, antimicrobial capacity, low toxicity, and can remove the smear layer.4 Therefore, the auxiliary chemical substance most frequently used is NaOCl in different concentrations.5 However, NaOCl alone is insufficient for a complete chemical-mechanical preparation (CMP), requiring chelating substances for the final irrigation of root canals in endodontic treatment.6

EDTA is the most common decalcifying agent used as a final irrigant in endodontic treatment.7 However, it has the disadvantages of erosion and changes in dentin's mechanical and biological properties,8 in addition to causing difficulty in adapting the filling material to the root canal wall.9 Furthermore, this substance is considered an emerging pollutant since it was not initially found in nature.10

Chitosan (CH) is a cationic biopolymer obtained from the deacetylation of chitin in an alkaline medium; it is a natural polysaccharide used in dentistry because it is non-toxic, biocompatible, bioadhesive, and biodegradable, in addition to presenting broad-spectrum chelator and antimicrobial properties.11,12

CH can unclog the dentinal tubules, removing the smear layer without causing significant dentin erosion.13 Therefore, several authors have treated this substance as an alternative solution to EDTA. 14 Thus, FIET with CH can remove the smear layer and inhibit bacterial recolonization.6 However, studies on CH's chelating capacity for smear layer removal are still scarce in the literature.

Due to the aggravating factors presented by EDTA and the emergence of CH as a less harmful alternative to the tooth structure, a systematic review and meta-analysis of evidence from *in vitro* studies on the comparison between EDTA and CH in FIET may be essential to guide future research. Moreover, practices involving the dynamics of chelating substances. Therefore, this systematic review and meta-analysis aimed to evaluate the chelating activity of an EDTA solution in FIET compared to a CH solution. The null possibility was that EDTA and CH solutions would show the same efficacy regarding chelating activity when used in FIET.

MATERIALS AND METHODS

<u>Study and registration protocol</u>

This study was registered on the Open Science Framework (OSF) platform and was conducted according to preferred reporting items for systematic reviews and meta-nalyses (PRISMA) 15. The following question to be answered was to define the search strategies to be applied in the scientific bases of the literature: Does the use of CH solution present better chelating activity compared to the EDTA solution in the final irrigation of endodontic treatment?

<u>Search strategy</u>

The electronic search was performed in April 2023 in the following databases: PubMed, Scopus, Virtual Health Library (VHL), Cochrane Library, Embase, and Web of Science.

The articles were selected according to the PICOS research question model, following the following parameters: Population (P - population): human teeth undergoing endodontic treatment; Intervention (I - intervention): CH-based irrigating solution; Comparison (C - solution comparison): EDTA-based irrigating solution; Outcome (O - stage): level of chelating activity (depth of penetration promoted in the dentinal tubules, calcium concentration and Knoop microhardness).; Type of study (S – study design): *in vitro* studies. In the search, a combination of MeSH terms, additional terms, synonyms, and accessible terms was used, thus creating a search key for the search strategy, as shown in Table 1.

TABLE 1. Search strategy used in electronic databases.

Database	Search strategy
Pubmed (368)	(((((((((((((((((Tooth[MeSH Terms]) OR (Root Canal Preparation[MeSH Terms])) OR (Root Canal Therapy[MeSH Terms])) OR (Root Canal Irrigants[MeSH Terms])) OR (Teeth[Title/Abstract])) OR (Canal Preparat*, Root[Title/Abstract])) OR (Canal Therap*,Root[Title/Abstract])) OR (Canal Irrigants, Root[Title/Abstract])) OR (Human tooth[Title/Abstract])) OR (Root canal[Title/Abstract])) OR (Endodontic[Title/Abstract])) OR (Endodontic treatment[Title/Abstract])) OR (Tooth[Title/Abstract])) OR (Root Canal Preparation[Title/Abstract])) OR (Root Canal Therapy[Title/Abstract])) OR (Root Canal Irrigants[Title/Abstract])) AND ((((((((Chitosan[MeSH Terms]) OR (Chelating Agents[MeSH Terms])) OR (Chelating Agents[Title/Abstract])) OR (Chitosan[Title/Abstract])) OR (Poliglusam[Title/Abstract])) OR (Chelators[Title/Abstract])) OR (nanoparticle[Title/Abstract])) OR (Endodontic irrigation solution[Title/Abstract]))) AND (((((((((Edetic Acid[MeSH Terms]) OR (Chelating Agents[MeSH Terms])) OR (Chelating Agents[Title/Abstract])) OR (Edetic Acid[Title/Abstract])) OR (EDTA[Title/Abstract])) OR (Ethylenedinitrilotetraacetic Acid[Title/Abstract])) OR (Edathamil[Title/Abstract])) OR (Chelators[Title/Abstract])) OR (nanoparticle[Title/Abstract])) OR (Endodontic irrigation solution[Title/Abstract]))) AND (((((((((In Vitro Techniques[MeSH Terms]) OR (Laboratories[MeSH Terms])) OR (In Vitro Techniq*[Title/Abstract])) OR (In Vitro as Topic[Title/Abstract])) OR (In Vitro[Title/Abstract])) OR (Laborator*[Title/Abstract])) OR (Microscopy[Title/Abstract])) OR (Microscope[Title/Abstract])) OR (In vitro studies[Title/Abstract])) OR (Laboratories[Title/Abstract]))
BVS – LILACS (109)	#1 [Tooth] explode all trees #2 MeSH descriptor: [Root Canal Preparation] explode all trees #3 MeSH descriptor: [Root Canal Therapy] explode all trees #4 MeSH descriptor: [Root Canal Irrigants] explode all trees #5 (Teeth):ti,ab,kw OR (Canal Preparat*, Root):ti,ab,kw OR (Canal Therap*,Root):ti,ab,kw OR (Canal Irrigants, Root): ti,ab,kw OR (Human tooth):ti,ab,kw #6 (Root canal):ti,ab,kw OR (Endodontic):ti,ab,kw OR (Endodontic treatment):ti,ab,kw OR (Tooth):ti,ab,kw OR (Root Canal Preparation):ti,ab,kw #7 (Root Canal Therapy):ti,ab,kw OR (Root Canal Irrigants):ti,ab,kw #8 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 #9 MeSH descriptor: [Chitosan] explode all trees #10 MeSH descriptor: [Chelating Agents] explode all trees #11 (Poliglusam):ti,ab,kw OR (Chelators):ti,ab,kw OR (nanoparticle):ti,ab,kw OR (Endodontic irrigation solution):ti,ab,kw OR (Chitosan):ti,ab,kw #12 (Chelating Agents):ti,ab,kw #13 #9 OR #10 OR #11 OR #12 #14 MeSH descriptor: [Edetic Acid] explode all trees #15 MeSH descriptor: [Chelating Agents] explode all trees #16 (EDTA):ti,ab,kw OR (Ethylenedinitrilotetraacetic Acid):ti,ab,kw OR (Edathamil):ti,ab,kw OR (Chelators):ti,ab,kw OR (nanoparticle):ti,ab,kw #17 (Endodontic irrigation solution):ti,ab,kw OR (Edetic Acid):ti,ab,kw OR (Chelating Agents):ti,ab,kw #18 #14 OR #15 OR #16 OR #17 #19 MeSH descriptor: [In Vitro Techniques] explode all trees #20 MeSH descriptor: [Laboratories] explode all trees #21 (In Vitro Techniq*):ti,ab,kw OR (In Vitro as Topic):ti,ab,kw OR (In Vitro):ti,ab,kw OR (Laborator*):ti,ab,kw OR (Microscopy):ti,ab,kw #22 (Microscope):ti,ab,kw OR (In vitro studies):ti,ab,kw OR (Laboratories):ti,ab,kw 13774 #23 #19 OR #20 OR #21 OR #22 #24 #8 AND #13 AND #18 AND #23
COCHRANE LIBRARY (93)	#1 [Tooth] explode all trees #2 MeSH descriptor: [Root Canal Preparation] explode all trees #3 MeSH descriptor: [Root Canal Therapy] explode all trees #4 MeSH descriptor: [Root Canal Irrigants] explode all trees #5 (Teeth):ti,ab,kw OR (Canal Preparat*, Root):ti,ab,kw OR (Canal Therap*,Root):ti,ab,kw OR (Canal Irrigants, Root):ti,ab,kw OR (Human tooth):ti,ab,kw #6 (Root canal):ti,ab,kw OR (Endodontic):ti,ab,kw OR (Endodontic treatment):ti,ab,kw OR (Tooth):ti,ab,kw OR (Root Canal Preparation):ti,ab,kw #7 (Root Canal Therapy):ti,ab,kw OR (Root Canal Irrigants):ti,ab,kw #8 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 #9 MeSH descriptor: [Chitosan] explode all trees #10 MeSH descriptor: [Chelating Agents] explode all trees #11 (Poliglusam):ti,ab,kw OR (Chelators):ti,ab,kw OR (nanoparticle):ti,ab,kw OR (Endodontic irrigation solution):ti,ab,kw OR (Chitosan):ti,ab,kw #12 (Chelating Agents):ti,ab,kw #13 #9 OR #10 OR #11 OR #12 #14 MeSH descriptor: [Edetic Acid] explode all trees #15 MeSH descriptor: [Chelating Agents] explode all trees #16 (EDTA):ti,ab,kw OR (Ethylenedinitrilotetraacetic Acid):ti,ab,kw OR (Edathamil):ti,ab,kw OR (Chelators):ti,ab,kw OR (nanoparticle):ti,ab,kw

	#17(Endodontic irrigation solution):ti,ab,kw OR (Edetic Acid):ti,ab,kw OR (Chelating Agents):ti,ab,kw #18 #14 OR #15 OR #16 OR #17 #19 MeSH descriptor: [In Vitro Techniques] explode all trees #20 MeSH descriptor: [Laboratories] explode all trees #21 (In Vitro Techniq*):ti,ab,kw OR (In Vitro as Topic):ti,ab,kw OR (In Vitro):ti,ab,kw OR (Laborator*):ti,ab,kw OR (Microscopy):ti,ab,kw #22 (Microscope):ti,ab,kw OR (In vitro studies):ti,ab,kw OR (Laboratories):ti,ab,kw 13774 #23 #19 OR #20 OR #21 OR #22 #24 #8 AND #13 AND #18 AND #23
EMBASE (142)	#1- tooth:ti,ab,kw OR 'root canal preparation':ti,ab,kw OR 'root canal therapy':ti,ab,kw OR 'root canal irrigants':ti,ab,kw OR teeth:ti,ab,kw OR 'canal preparat*, root':ti,ab,kw OR 'canal therap*,root':ti,ab,kw OR 'canal irrigants, root':ti,ab,kw OR 'human tooth':ti,ab,kw OR 'root canal':ti,ab,kw OR endodontic:ti,ab,kw OR 'endodontic treatment':ti,ab,kw #2- chitosan:ti,ab,kw OR 'chelating agents':ti,ab,kw OR poliglusam:ti,ab,kw OR chelators:ti,ab,kw OR nanoparticle:ti,ab,kw OR 'endodontic irrigation solution':ti,ab,kw #3- 'edetic acid':ti,ab,kw OR 'chelating agents':ti,ab,kw OR edta:ti,ab,kw OR 'ethylenedinitrilotetraacetic acid':ti,ab,kw OR edathamil:ti,ab,kw OR chelators:ti,ab,kw OR nanoparticle:ti,ab,kw OR 'endodontic irrigation solution':ti,ab,kw #4- 'in vitro techniques':ti,ab,kw OR laboratories:ti,ab,kw OR 'in vitro techniq*':ti,ab,kw OR 'in vitro as topic':ti,ab,kw OR 'in vitro':ti,ab,kw OR laborator*:ti,ab,kw OR microscopy:ti,ab,kw OR microscope:ti,ab,kw
Scopus (1339)	#1- (TITLE-ABS-KEY (tooth) OR TITLE-ABS-KEY (root AND canal AND preparation) OR TITLE-ABS-KEY (root AND canal AND therapy) OR TITLE-ABS-KEY (root AND canal AND irrigants) OR TITLE-ABS-KEY (teeth) OR TITLE-ABS-KEY (canal AND preparat*, AND root) OR TITLE-ABS-KEY (canal AND therap*,root) OR TITLE-ABS-KEY (canal AND irrigants, AND root) OR TITLE-ABS-KEY (human AND tooth) OR TITLE-ABS-KEY (root AND canal) OR TITLE-ABS-KEY (endodontic) OR TITLE-ABS-KEY (endodontic AND treatment)) #2- (TITLE-ABS-KEY (chitosan) OR TITLE-ABS-KEY (chelating AND agents) OR TITLE-ABS-KEY (poliglusam) OR TITLE-ABS-KEY (chelators) OR TITLE-ABS-KEY (nanoparticle) OR TITLE-ABS-KEY (endodontic AND irrigation AND solution)) #3- (TITLE-ABS-KEY (edetic AND acid) OR TITLE-ABS-KEY (chelating AND agents) OR TITLE-ABS-KEY (edta) OR TITLE-ABS-KEY (ethylenedinitrilotetraacetic AND acid) OR TITLE-ABS-KEY (edathamil) OR TITLE-ABS-KEY (chelators) OR TITLE-ABS-KEY (nanoparticle) OR TITLE-ABS-KEY (endodontic AND irrigation AND solution)) #4- (TITLE-ABS-KEY (in AND vitro) OR TITLE-ABS-KEY (techniques AND laboratories) OR TITLE-ABS-KEY (in AND vitro AND techniq*) OR TITLE-ABS-KEY (in AND vitro AND as AND topic) OR TITLE-ABS-KEY (in AND vitro) OR TITLE-ABS-KEY (laborator*) OR TITLE-ABS-KEY (microscopy) OR TITLE-ABS-KEY (microscope))
Web of science (517)	#1- TS: (Tooth) OR TS: (Root Canal Preparation) OR TS: (Root Canal Therapy) OR TS: (Root Canal Irrigants) OR TS: (Teeth) OR TS: (Canal Preparat*, Root) OR TS: (Canal Therap*,Root) OR TS: (Canal Irrigants, Root) OR TS: (Human tooth) OR TS: (Root canal) OR TS: (Endodontic) OR TS: (Endodontic treatment) #2- TS: (Chitosan) OR TS: (Chelating Agents) OR TS: (Poliglusam) OR TS: (Chelators) OR TS: (nanoparticle) OR TS: (Endodontic irrigation solution) #3- TS: (Edetic Acid) OR TS: (Chelating Agents) OR TS: (EDTA) OR TS: (Ethylenedinitrilotetraacetic Acid) OR TS: (Edathamil) OR TS: (Chelators) OR TS: (nanoparticle) OR TS: (Endodontic irrigation solution) #4- TS: (In Vitro Techniques) OR TS: (Laboratories) OR TS: (In Vitro Techniq*) OR TS: (In Vitro as Topic) OR TS: (In Vitro) OR TS: (Laborator*) OR TS: (Microscopy) OR TS: (Microscope)

Selection of articles

All articles found in the selected databases were transferred to the Mendeley software (Mendeley Software, London, UK). Duplicate studies were automatically excluded by the program. Then, two independent researchers (XXX and YYY) carried out the selection of studies, the first screening being carried out from the title and abstract, based on the inclusion criteria: being related to the theme in the area of endodontics, carrying out an approach on EDTA and CH and be an *in vitro* study. The study was automatically excluded from the systematic review if the title and abstract presented any exclusion criteria.

In this context, the exclusion criteria were clinical trials; *in situ* or animal studies; systematic, scoping, or integrative literature reviews; conference abstracts; not evaluating the chelating activity of EDTA or CH-based solutions; or both substances that have not been used as FIET. In the selection of articles, there was no restriction regarding the year of publication or the language used. After analyzing the title and abstract, the eligible articles were identified, and a more careful selection was made by reading the full text. A third investigator (ZZZ) was requested if there was a disagreement between the two principal investigators.

If the researchers did not have full access to the selected article, the corresponding authors of each article were contacted via email so we could have total access to the texts. After three unsuccessful attempts, the article was considered a loss.

Data collection and extraction

After selecting the articles that met the eligibility criteria for this systematic review, they had their data extracted according to the information of interest for this study: study details (authors, year, location, and design of the study), the concentration of substances used in final irrigation (EDTA and CH), a method used in CMP, result evaluated (Knoop Microhardness; penetration of cement into dentinal tubules; push-out bond strength; failure mode; concentration of calcium ions) and statistical analysis used.

Risk of Bias

The risk of bias was evaluated by two researchers (XXX and YYY), using the study instrument: Guidelines for Reporting Preclinical *In Vitro* Studies on Dental Materials, by Faggion16 whose evaluation criteria follow 14 parameters, in which the studies should present: 1- Structured summary of the trial design, methods, results and conclusions; 2a- Scientific basis; 2b- Specificity in objectives and hypotheses; 3- methodology with detailed intervention for each group; 4- Results with primary and secondary measures completely defined and pre-specified; 5- Sample calculation with detailed report; 6- Specification of the method used to generate the random allocation sequence; 7- Use of masking mechanism during randomized allocation; 8- Determination of who generated the random allocation sequence; 9- Blinding in the study; 10- Statistical method used; 11- Accurately detailed results; 12- Discussion presenting the limitations of the study; 13- Information on funding sources; and 14- Indication of access platform to the assay protocol. Furthermore, as demonstrated by the risk of bias items for *in vitro* studies of dental materials, if the authors reported the parameter to be evaluated, the article would present a ' 'Yes" on that specific parameter; if the information could not be found, the article would receive a "No." Articles that reported only one to three positive parameters in their structure were classified as having a high risk of bias (eleven to thirteen negative parameters), four or five positive parameters as medium risk of bias (nine or ten negative parameters), and six or seven parameters positive as low risk of bias (seven or eight negative parameters).

Meta-analysis

Revman 5.3 software (Review Manager v. 5, The Cochrane Collaboration, Copenhagen, Denmark) was used for data analysis and graph construction. Three separate meta-analyses were performed to analyze the chelating activity of the substances: 1- Calcium concentration; 2- Depth of penetration of the cement in the dentinal tubules; 3- Knoop microhardness. Standard deviation and mean difference were calculated with a 95% confidence interval (CI), and heterogeneity was tested using the I^2 Index.

RESULTS

Selection of studies

After an electronic search in the selected databases, 2,568 articles were found: 368 articles from PubMed, 1,339 from Scopus, 109 from VHL, 93 from Cochrane Library, 142 from Embase, and 517 from Web of Science. Of these, 1,397 duplicate articles were automatically removed, leaving 1,171 articles, of which 1,130 were excluded after the first screening, evaluating title and abstract, and then 41 studies were viewed in full and analyzed for eligibility criteria, being excluded: 7 articles that did not address the chelating activity of substances; 10 articles that did not compare the substances studied (EDTA and CH); 1 article that did not use these substances in the FIET, and 1 article in which it was not possible to obtain access to the full text, even after repeated contact attempts. With this screening and selection carried out, in the end, 22 articles were selected, 7 of which were submitted for meta-analysis (Figure 1).

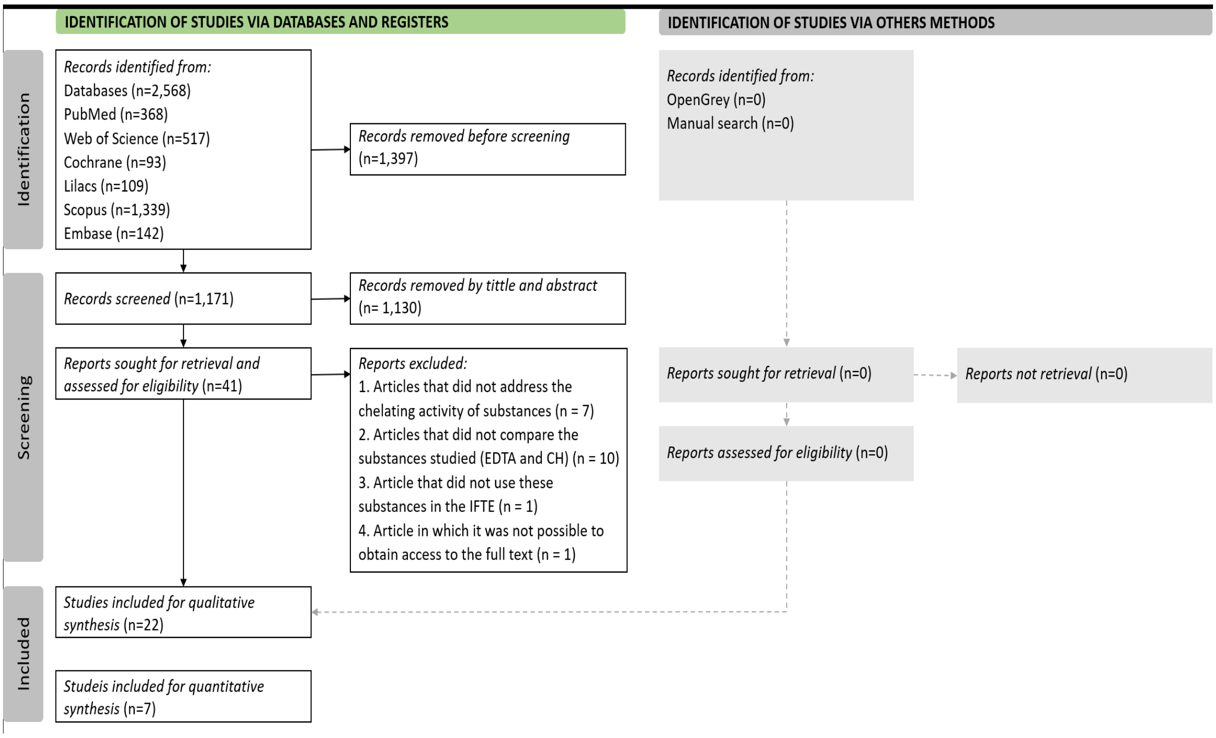

Figure 1: Flowchart of the searched and included studies in this systematic review and meta-analysis.

<u>Characteristics of the selected studies</u>

The characteristics of the studies included in this systematic review are shown in Table 2. The selected articles were developed in Brazil, India, Turkey, Taiwan, Indonesia, Syria, Germany, and Bulgaria and published between the years 2012 and 2021. There was a variation with the concentration of the chelating solutions used, with EDTA presenting a variation of 15 to 17% and CH a variation of 0.04 to 1%; however, the higher concentration used was 0.2%, being used in 16 studies[10,17-32]. For most of the samples, an endodontic treatment with CMP and irrigation with NaOCl and/or distilled water were used in the biomechanical preparation. Only in two studies was this methodology not used for using dentin slices as a sample[6,26]. As an evaluation criterion, nine studies evaluated the removal of the smear layer;[6,18-21,27,30,33,34] three studies evaluated Knoop Microhardness[10,17,26], and four studies evaluated cement penetration into dentinal tubules[10,12,23,25]. There were also other specific evaluations; however, in general, all studies aimed to evaluate the chelating action of substances.

TABLE 2. Data extraction of the included studies.

Author, year, country, and study design	Canal irrigation		Biomechanical preparation	Results	Statistical analysis
	EDTA	CH			
ANTUNES et al., 2019, Brazil, *in vitro*	15% EDTA (5 ml)	0.2% CH solution (5ml)	#R50 reciproval system + 1% NaOCl	Knoop microhardness; sealant penetration; push-out bond strength; fracture mode.	Two-way ANOVA and Tukey post hoc.
MATHEW et al., 2017, India, *in vitro*	17% EDTA	0.2% and 0.5% CH solution	#F3 Protaper + 1% NaOCl + deionized water	Smear layer removal; amount of calcium ions, and nanostructural changes	ANOVA and chi-square test
PEDRO et al., 2017, Brazil, *in vitro*	17% EDTA	0.2% CH solution	#K8/ #K15/ #K10 + 1% NaOCl + solution of thymol 4°C	Amount of calcium ions	Kolmogorov-Smirnov, Levene and ANOVA with Tukey post-hoc
KAMBLE et al., 2017, India, *in vitro*	17% EDTA	0.2% CH solution	# iRace files + 3% NaOCl + ultrasonic activation	Smear layer removal	Mann-Whitney test
KESIM et al., 2018, Turkey, *in vitro*	17% EDTA	0.2% CH solution	#ProFile + 2.5% NaOCl	Sealant penetration	Shapiro-Wilk, Kruskal-Wallis Dunn-Bonferroni tests.
THOTA et al., 2017, India, *in vitro*	17% EDTA (5ml)	0.2% CH solution (5ml)	#K-Files + 2.5% NaOCl + distilled water	Sealant penetration	One-way ANOVA and T-test.
MIRANDA et al. 2017, Brazil, *in vitro*	17% EDTA	0.2% CH solution	#K-Files + 2.5% NaOCl + distilled water	Smear layer removal	Qualitative analysis
KAUR et al. 2020, India, *in vitro*.	17% EDTA	1% CH solution	# 15K #35K + 5% NaOCl	Smear layer removal and biofilm eradication	Kruskal-Wallis and Scheffe tests
SAHA et al. 2017, India, *in vitro*	17% EDTA	0.2% CH solution	#K-Files + 3% NaOCl	Knoop microhardness	ANOVA, Tukey and T-test
HUANG et al. 2018, Taiwan, *in vitro*	15% EDTA	0.04% CH soluble fungal	#K3 Nickel-titanium Rotary files + %5 NaOCl	Smear layer removal	T-test
RATIH, ENGGARDIPTA e KARTIKANINGTYAS et al. 2020, Indonesia, *in vitro*	15% EDTA	0.2% CH solution	#F3 Protaper + 2.5% NaOCl + distilled water	Smear layer removal, Knoop microhardness and roughness	ANOVA and Tukey tests.
KAKI et al., 2018, Turkey, *in vitro*	17% EDTA	0.2% CH solution	Mtwo Rotary instruments #40 + 5% NaOCl + distilled water	Radicular dentine alterations, push-out bond strength	ANOVA and Duncan tests.
DEL CARPIO-PEROCHENA et al., 2015, Brazil, *in vitro*	17% EDTA	1,29 mg / mL CH solution	2.5% NaOCl + distilled water	Smear layer removal	Kruskal-Wallis and Dunn tests.
PIMENTA et al., 2012, Brazil, *in vitro*	15% EDTA	0.2% CH solution	Not performed	Knoop microhardness	ANOVA and Tukey-Kramer tests.
SARKEES et al., 2020, Syria, *in vitro*	17% EDTA	0.2% CH solution	2.5% NaOCl + #F2 Protaper + 5 ml of serum	Amount of calcium ions and smear layer removal	Kruskal-Wallis and Mann-Whitney tests.
SILVA et al., 2013, Brazil, *in vitro*	15% EDTA	0.2% CH solution	#10 K-files + Quantec®; SybronEndo Corporation +1% NaOCl + deionized water	Smear layer removal	Kruskal-Wallis and Dunn tests.
OZLEK et al., 2020, Germany, *in vitro*	17% EDTA	0.2% CH solution	#15 K-type file + #F3 Protaper+ 5.25% NaOCl	Effectiveness of root canal irrigation	Kruskal-Wallis test.
AGARWAL et al., 2019, India, *in vitro*	17% EDTA	0.2% CH solution	#30 files (Hyflex CM) + 5.25% NaOCl	Effect of three endodontic chelating agents	Kruskal-Wallis na Mann-Whitney tests.
GYULBENKIYAN et al., 2020, Bulgaria, *in vitro*	17% EDTA	0.6% CH-citrato	K-files #10 e #15 + #F4 Protaper + 0,2% NaOCl	Sealant sealing ability	T-test
SARI et al., 2018, Turkey, *in vitro*	17% EDTA	0.2% CH solution	K-files #15 + OneShape #25 +1% NaOCl	Smear layer removal	Kruskal-Wallis test.
JOSE et al., 2021, India, *in vitro*	17% EDTA	0,2% CH solution	K-files #10 + #F3 Protaper + 5,25% NaOCl	Push-out bond strength	G * Power

Legend: EDTA – Ethylenediaminetetraacetic acid; NaOCl – Sodium hypochlorite.

Table 3 of the twenty-two studies analyzed obtained a structured summary of the trial design, methods, results, and conclusions. At the same time, these studies also presented a scientific basis and detailed justification, as well as specific objectives and/or hypotheses, except for the studies by Huang et al.[33] and Agarwal et al[28]., which did not present specific objectives and/or concrete hypotheses. Furthermore, the intervention for each group, including how and when it was administered, with sufficient detail to allow replication, was correctly observed in all articles. In contrast, nine studies[6,17,18,24,28,30,31,33,35] still needed to obtain fully defined and pre-specified primary and secondary outcome measures, including how and when they were evaluated. In addition, eleven studies still need to define how the sample size was determined[6,17,18,24,26,28-31,33,35]. Finally, of the twenty-two articles analyzed, none presented a method to generate the randomized allocation sequence, neither used a mechanism to implement it nor defined who generated the sequence, identified the teeth, and assigned the teeth for the intervention.

Furthermore, only the study by Ratih et al.[30] blinded the evaluator after the intervention was assigned. Nevertheless, the work by Miranda et al.[21] did not use statistical methods to compare groups between primary and secondary outcomes. Only the study by Pimenta et al.[17] did not define the results of each group for each primary and secondary outcome and the estimated effect size and precision. Limitations of the trial, addressing sources of potential bias, imprecision, and, if relevant, a multiplicity of analyses were not observed in eight articles[17,18,24,29-31,33,35]. Only four studies[6,17,30,33] explained sources of funding and other support, as well as the role of funders. Finally, of the twenty-two articles, six[19,20,22,23,30,32] show where the complete trial protocol can be accessed. In summary, although the studies do not present some domains according to the guidelines (Table 3), most of these domains are affected, contributing to a detailed analysis of the vision risk of each study. In addition, it is important to emphasize that the research sample plan consists of the articles existing in the literature of the databases worked; that is, no studies were found that fulfilled all the domains in Table 3.

TABLE 3: Risk of bias assessment of the *in vitro* studies included in this systematic review with based in Guidelines for Reporting Preclinical *In Vitro* Studies on Dental Materials.

Item	Pimenta et al., 2012	Silva et al., 2013	Del Carpio-Perochena et al., 2015	Miranda et al., 2017	Thota et al., 2017	Pedro et al., 2017	Mathew et al., 2017	SaHa et al., 2017	Kamble et al., 2017	Huang et al., 2018	Kaki et al., 2018	Kesim et al., 2018
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2b	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
5	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✗
6	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
7	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
8	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
9	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
10	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
11	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
13	✓	✗	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗
14	✗	✗	✗	✗	✓	✓	✗	✗	✗	✗	✗	✗

TABLE 3: continuation.

item	Sari et al., 2018	Aydin et al., 2019	Antunes et al., 2019	Agarwal et al., 2019	Gyulbenkiyan et al., 2020	Kaur et al., 2020	Ozlek et al., 2020	Ratih, Enggardipta e Kartikaningtyas, 2020	Sarkees et al., 2020	José et al., 2021
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2a	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2b	✓	✓	✓		✓	✓	✓	✓	✓	✓
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4	✗	✗	✗	✗	✗	✗	✓	✗	✗	✓
5	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓
6	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
7	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
8	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
9	✗	✗	✗	✗	✗	✗	✗	✓	✗	✗
10	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
11	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	✗	✗	✗	✓	✗	✗	✗	✗	✗	✓
13	✗	✗	✗	✓	✗	✗	✗	✓	✗	✗
14	✗	✗	✗	✗	✗	✗	✗	✗	✓	✓

Meta-analysis

The meta-analysis considered all articles selected for the systematic review. However, only 7 of the 22 selected studies were included in the meta-analysis, and the remaining 15 were not included because they did not present methodological compatibility for statistical comparison. Three meta-analyses were performed: 1- four studies compared the concentration of calcium ions[18,22,29,31]; 2- two studies compared cement penetration in dentinal tubules in three root thirds[10,23]; and 3- two studies compared Knoop microhardness[10,17].

Calcium ion concentration

According to the study by Pedro et al.[22] and Ozlek et al.[29], the CH solution presented the highest amount of calcium ions released. However, according to the study by Silva et al.[18] and Sarkees et al.[31], the values were equivalent. Thus, in general, there was no significant difference concerning the standard mean difference for the release of calcium ions ($p>0.05$). However, as observed in the meta-analysis (Figure 2), taking into account all studies that evaluated the concentration of calcium ions, both solutions showed the same behavior in terms of chelating activity ($p = 0.33$). In addition, heterogeneity was high between studies ($p < 0.001$, $I^2 = 92\%$), demonstrating significant variation in confidence intervals.

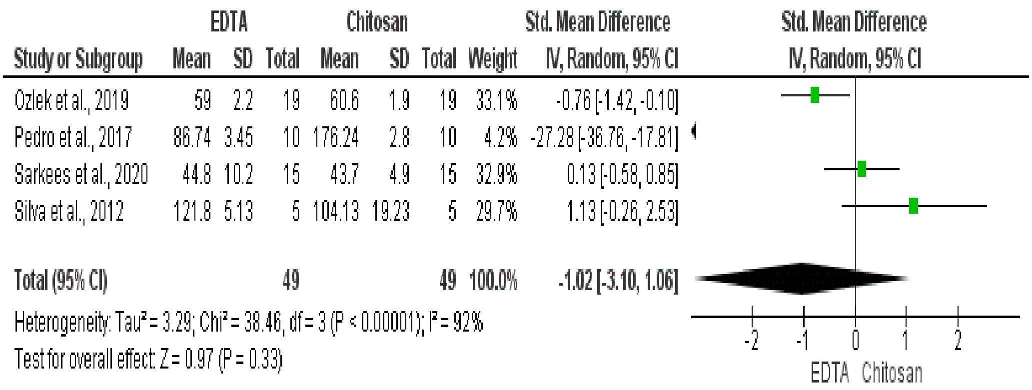

Figure 2: *Forest plot* of the concentration of calcium ions for the studies included in this systematic review and meta-analysis.

Endodontic cement penetration into dentinal tubules

Based on the studies by Thota et al.[23] and Antunes et al.[10], after irrigation of the root canal with EDTA and CH solution, the root region was evaluated in three different thirds: cervical, middle and apical (Figure 3). For the cervical and middle thirds, the mean, the standard difference was not significant in relation to the two solutions ($p = 0.28$ and $p = 0.44$, respectively); however, for the apical third, the mean, and standard difference observed was significant for the EDTA solution ($p = 0.04$). However, in general, no significant difference was observed in the penetration rates of endodontic cement ($p = 0.93$). Therefore, the heterogeneity for the studies was not significant ($p = 0.23$, I2 = 28%).

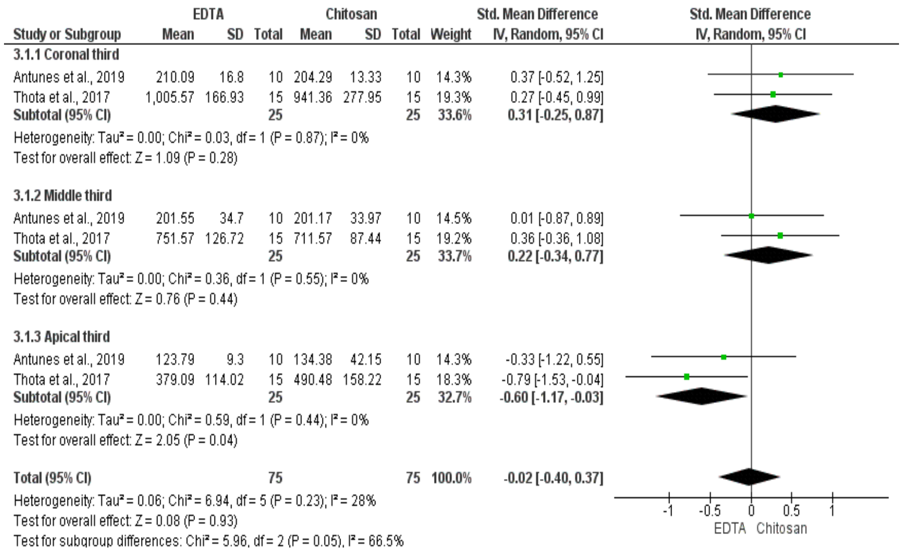

Figure 3: *Forest plot* of the endodontic cement penetration into dentinal tubules for the studies included in this systematic review and meta-analysis.

Knoop microhardness

For the evaluation of Knoop microhardness, the mean and standard deviation values from the studies by Antunes et al.[10] and de Pimenta et al.[17] demonstrated that the standard mean difference was not significant between the two solutions in reducing microhardness ($p = 0.74$) (Figure 4), as well as the heterogeneity was considered low ($p = 0.71$; $I^2 = 0$ %).

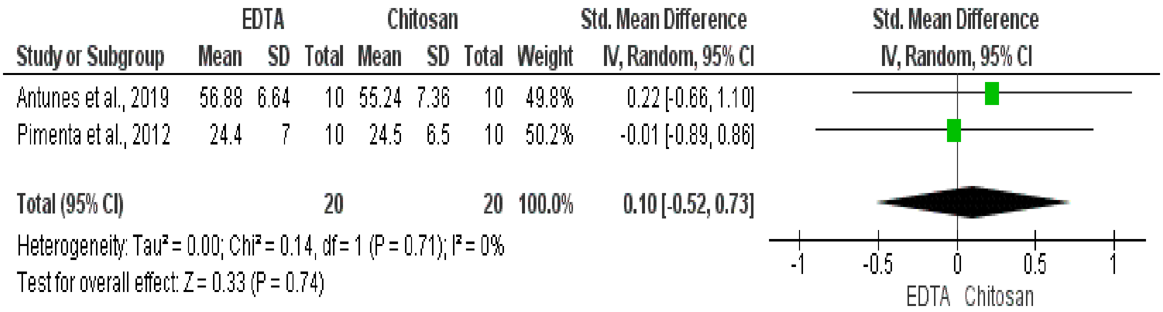

Figure 4: *Forest plot* of the Knoop microhardness for the studies included in this systematic review and meta-analysis.

DISCUSSION

By evaluating the conclusions of the in vitro studies, a quantitative advantage of CH is observed: 11 studies conclude a better chelating activity of this solution[19,20,22,25,27-30,32,34,35], while five studies indicate that there is no significant difference[10,17,26,31,33]. On the other hand, only four studies reported better effectiveness with the EDTA solution[6,12,21,24]; and 2 had conclusions that oscillate between the two solutions[23,30].

When two materials have a similar chelating effect, the solution with the lowest cost-effective concentration should be preferred. It is known that the efficiency of a chelating agent depends on several factors, including application time, pH, concentration, and amount of solution. Furthermore, the relationship between the concentration of the chelating agent and the application of time is necessary, as it was considered that concentrated solutions applied for an extended period cause roughness of the dentin surface[23].

The use of irrigating solutions during CMP provides the effectiveness of eliminating microorganisms from the root canal system, favoring all stages of endodontic treatment. Thus, maximum microbiological eradication is needed beforehand to perform ideal obturation. NaOCl is the antimicrobial irrigating solution used on a large scale due to its high capacity for dissolving organic tissues[36]. However, this solution does not remove the smear layer formed on the root dentin, and it is considered irritating to the periapical tissues, requiring the use of chelating agents such as EDTA and CH in the final irrigation protocol. Its objective is to remove the smear layer, clearing the entrance of the dentinal tubules and, consequently, improving the penetration of the endodontic cement[31].

A chelating agent's effectiveness depends on the material's penetration depth, root canal length, dentin hardness, application time, pH, and material concentration[30]. At the same time, as NaOCl does not entirely remove the inorganic smear layer on the root dentin surface, EDTA can be used for this function. Therefore, a combination of NaOCl and a chelating agent like EDTA is recommended to remove the inorganic smear layer. The use of EDTA, in turn, can change the biomechanical properties of the dentin tissue, affecting the hardness, permeability, and solubility of dentin, considering any change in the calcium phosphate (Ca/P) ratio alters the proportion of organic and inorganic components. In this context, there is a need for more biocompatible agents to perform the chelating function in the final irrigation of endodontic treatment[20].

On the other hand, the CH solution has different characteristics in relation to EDTA, such as being a biocompatible, biodegradable, bioadhesive, and non-toxic solution, in addition to having the ability to remineralize dentin when associated with sodium fluoride[22]. However, most studies selected and included in this systematic review concluded that the CH solution has better chelating activity than EDTA. Nonetheless, it was possible to observe that the solutions were equivalent in the meta-analyses performed.

According to Antunes et al.[10], final root canal irrigation with 15% EDTA or 0.2% CH achieved comparable effects in reducing dentin microhardness, penetration of endodontic cement through the dentinal tubules, and bond strength. In addition, according to the meta-analyses generated in this systematic review, no significant difference was difference was observed between applying an EDTA or CH solution in relation to calcium concentration, surface microhardness, and ability to penetrate the dentinal tubules. According to the studies evaluated, a moderate concentration of 0.2% of CH removes the smear layer more efficiently than 17% of EDTA in the apical third of the root canals[19,27]. On the other hand, CH at 0.2% and EDTA induce a greater penetration of the endodontic cement in the other coronal thirds[25]. After the CMP of the root canals, the filling is started using filling cement. This filling cement is used to fill the spaces between the filling materials and the root canal walls; however, to induce a good adaptation and provide adhesion to the dentin substrate, the smear layer present on the root dentin surface can prevent adequate adhesion by compromising penetration into the dentinal tubules. Taking into account the morphological characteristics of root dentin in the different thirds, it is observed that the penetration depth of the filling cement in the apical third is more remarkable when using the CH solution than the EDTA. However, in the other thirds, this difference is not observed[23]. Thus, CH at 0.2% can be indicated as a better alternative for irrigation of the root canal to EDTA due to its biocompatible, biodegradable, and bioadhesive nature[12,23].

A chelating agent, such as CH, can minimize aggression to dental structures by inducing a release of calcium ions in dentin when in contact. 17% EDTA combined with 5% NaOCl is known to reduce the microhardness of root dentin[22]. According to the study by Mathew et al.[20], the evaluation of roughness parameters showed that the 0.2% and 0.5% CH group generates less surface alteration in root dentin than the 17% EDTA group. Thus, they also found that CH at 0.2% and 0.5% generates less change in the Ca/P ratio in root dentin than 17% EDTA when used as a final irrigant for 1 minute. However, for Pedro et al. [22], the 0.2% CH solution combined with ultrasonic agitation produced the highest amounts of calcium ions released.

This systematic review has a limitation: the lack of methodological compatibility related to laboratory studies, justifying the reduced number of studies included in the meta-analyses. Thus, it was impossible to carry out an approach to removing the smear layer, relating the advantages of CH compared to EDTA. Therefore, studies with greater methodological homogeneity must achieve more complex comparisons of these solutions.

CONCLUSION

Based on the results of this systematic review and meta-analysis, no significant difference was observed between the application of an EDTA or CH solution in relation to calcium concentration, penetration capacity in dentinal tubules, and surface microhardness. However, most *in vitro* studies corroborated the better chelating activity of the CH solution in FIET, as it has a biocompatible, biodegradable, bioadhesive and non-toxic nature, greater capacity to remove the smear layer, greater capacity to induce the release of calcium ions present in the dentin when in contact, less alteration of the root dentin surface and better chelating activity compared to the EDTA solution. Although most of the studies included in this work presented mostly positive criteria in the evaluation of vision risk, the existing studies in the literature present limitations in some domains of this evaluation, requiring the execution of new studies with greater methodological rigor to significantly reduce the vision risk.

REFERENCES

1. Bueno, C. R. E., Cury, M. T. S., Vasques, A. M. V., Sarmiento, J. L., Trizzi JQ, Jacintom, R. C., Sivieri-Araujo, G., & Dezan Júnior, E. Cleaning effectiveness of a nickel-titanium ultrasonic tip in ultrasonically activated irrigation: a SEM study. Braz Oral Res. 2019 Mar 18;33, e017.

2. Demirel, A., Yüksel, B. N., Ziya, M., Gümüş, H., Doğan, S., & Sari, Ş. The effect of different irrigation protocols on smear layer removal in root canals of primary teeth: a SEM study. Acta Odontol Scand. 2019 Jul; 77(5), 380-385.

3. Tuncel, B., Nagas, E., Cehreli, Z., Uyanik, O., Vallittu, P., & Lassila, L. Effect of endodontic chelating solutions on the bond strength of endodontic sealers. Braz Oral Res. 2015; 29, S1806-83242015000100256.

4. Dotto, L., Sarkis Onofre, R., Bacchi, A., & Rocha Pereira, G. K. Effect of Root Canal Irrigants on the Mechanical Properties of Endodontically Treated Teeth: A Scoping Review. J Endod. 2020 May; 46, 596-604.e3.

5. Jardim, P. D. S., Pereira-Cenci, T., Badin, C. F., Ferreira, A. C. D. A., & Jacinto, R. C. The effect of endodontic chemicals on the retention of fiber posts luted using a self-adhesive cement. Appl Adhes Sci. 2014 Aug; 2, 20.

6. Del Carpio-Perochena, A., Bramante, C. M., Duarte, M. A., de Moura, M. R., Aouada, F. A., & Kishen, A. Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restor Dent Endod. 2015 Aug; 40(3), 195-201.

7. Giardino, L., Bidossi, A., Del Fabbro, M., Savadori, P., Maddalone, M., Ferrari, L., Ballal, N. V., Das, S., & Rao, B. S. S. Antimicrobial activity, toxicity and accumulated hard-tissue debris (AHTD) removal efficacy of several chelating agents. Int Endod J. 2020 Aug; 53(8), 1093.

8. Lorenzetti, C. C., Pereira, M. C. D. S., Kuga, M. C., Saad, J. R. C., & Campos, E. A. D. Influence of dentin treatment with EDTA on the bond strength of self-etching adhesive systems. Rev Odontol UNESP. 2019; 48, e20190007.

9. Singh, S., Singh, M., Salgar, A. R., Chandrahari, N., Prathibha, N., & Koppolu, P. Time-Dependent Effect of Various Irrigants for Root Canal on Smear Layer Removal. J Pharm Bioallied Sci. 2019 Feb; 11(1), S51-S58.

10. Antunes, P. V. S., Flamini, L. E. S., Chaves, J. F. M., Silva, R. G., & Cruz Filho, A. M. D. Comparative effects of final canal irrigation with chitosan and EDTA. J Appl Oral Sci. 2019 Nov; 28, e20190005.

11. Roshdy, N. N., Kataia, E. M., & Helmy, N. A. Assessment of antibacterial activity of 2.5% NaOCl, chitosan nano-particles against Enterococcus faecalis contaminating root canals with and without diode laser irradiation: an in vitro study. Acta Odontol Scand. 2019 Jan; 77(1), 39-43.

12. Aydın, Z. U., Özyürek, T., Keskin, B., & Baran, T. Effect of chitosan nanoparticle, QMix, and EDTA on Total-Fill BC sealers' dentinal tubule penetration: a confocal laser scanning microscopy study. Odontology. 2019 Jan; 107(1), 64-71.

13. Zhou, H., Li, Q., Wei, L., Huang, S., & Zhao, S. A comparative scanning electron microscopy evaluation of smear layer removal with chitosan and MTAD. Niger J Clin Pract. 2018 Jan; 21(1), 76-80.

14. Mittal, A., Dadu, S., Yendrembam, B., Abraham, A., Singh, N. S., & Garg, P. Comparison of new irrigating solutions on smear layer removal and calcium ions chelation from the root canal: An in vitro study. Endodontology. 2018 Jun; 30(1), 55-61.

15. Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., McGuinness, L. A., Stewart, L. A., Thomas, J., Tricco, A. C., Welch, V. A., Whiting, P., & Moher, D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 Mar; 372(71), 1.

16. Faggion, C. M. Guidelines for reporting pre-clinical in vitro studies on dental materials. J Evid Based Dent Pract. 2012 Dec; 12(4), 182-189.

17. Pimenta, J. A., Zaparolli, D., Pécora, J. D., Cruz-Filho, A. M. Chitosan: effect of a new chelating agent on the microhardness of root dentin. Braz Dent J. 2012; 23(3), 212-217.

18. Silva, P. V., Guedes, D. F., Nakadi, F. V., Pécora, J. D., Cruz-Filho, A. M. Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int Endod J. 2013 Apr; 46(4), 332-338.

19. Kamble, A. B., Abraham, S., Kakde, D. D., Shashidhar, C., & Mehta, D. L. 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 Dec; 8(4), 621-626.

20. Mathew, S. P., Pai, V. S., Usha, G., & Nadig, R. R. 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 Aug; 20(4), 245-250.

21. Miranda, J. S., Marques, D. A., Landa, F. V., Leite, A. P., & Leite, F. P. Effect of three final irrigation protocols on smear layer removal from the middle third of endodontically treated teeth: a qualitative analysis. Dental Press Endod. 2017 May; 7(2), 72-77.

22. Pedro, F. L. M., Costa, L. M. A. S., Filho, G. S., Guedes, O. A., Pereira, T. M., & Borges, A. H. Assessment of the Amount of Calcium Ions Released after the use of Different Chelating Agents and Agitation Protocols. Open Dent J. 2017 Mar; 11, 133-139.

23. Thota, M. M., Sudha, K., Malini, D. L., & Madhavi, S. B. Effect of Different Irrigating Solutions on Depth of Penetration of Sealer into Dentinal Tubules: A Confocal Microscopic Study. Contemp Clin Dent. 2017 Sep; 8(3), 391-394.

24. Kaki, G. D., & Genç Şen, O. The effects of chelation on the adhesion of two different root canal sealers. J Adhes Sci. 2018 Oct; 32(20), 2195-2203.

25. Kesim, B., Burak, A. K., Ustun, Y., Delikan, E., & Gungor, A. Effect of chitosan on sealer penetration into the dentinal tubules. Niger J Clin Pract. 2018 Oct; 21(10), 1284-1290.

26. Saha, S. G., Sharma, V., Bharadwaj, A., Shrivastava, P., Saha, M. K., Dubey, S. Kala, S., & Gupta, S. Effectiveness of Various Endodontic Irrigants on the Micro-Hardness of the Root Canal Dentin: An in vitro Study. J Clin Diagn Res. 2017 Apr; 11(4), ZC01-ZC04.

27. Sari, F., Tosun, S., Aksoy, F., & Uçar, Z. G. Effect of Combined Use of Photon-Initiated Photoacoustic Streaming and Chitosan on Smear Layer Removal. Eur J Ther. 2018; 24(3), 158-162.

28. Agarwal, S., Raghu, R., Shetty, A., Gautham, P. M., & Souparnika, D. P. An in vitro comparative evaluation of the effect of three endodontic chelating agents (17% ethylenediamine tetraacetic acid, 1% peracetic acid, 0.2% chitosan) on the push out bond strength of gutta percha with a new bioceramic sealer (BioRoot RCS). J Conserv Dent. 2019 Oct; 22(5), 475-478.

29. Ozlek, E., Rath, P. P., Kishen, A., & Neelakantan, P. A chitosan-based irrigant improves the dislocation resistance of a mineral trioxide aggregate-resin hybrid root canal sealer. Clin Oral Investig. 2020 Jan; 24(1), 151-156.

30. Ratih, D. N., Enggardipta, R. A., & Kartikaningtyas, A. T. The effect of chitosan nanoparticle as a final irrigation solution on smear layer removal, microhardness and surface roughness of root canal dentin. Open Dent J. 2020 Feb; 14.

31. Sarkees, M., & Al-Maarrawi, K. Chitosan: A natural substitute of EDTA solution for final irrigation in endodontics treatment. Niger J Clin Pract. 2020 May; 23(5), 697-703.

32. Jose, J. M., Manju Krishna, E. M., Ahamed, S. M., Theruvil, R., Mathew, J., & George, S. Effect of root dentin conditioning using different chelating agents on pushout bond strength of MTA-fillapex and bioroot RCS: An in vitro study. J Conserv Dent. 2021Apr; 24(2), 195-198.

33. Huang, Y., Orhan, K., Celikten, B., Orhan, A. I., Tufenkci, P., & Sevimay, S. Evaluation of the sealing ability of different endodontic cements: a combined SEM and micro-CT study. J Appl Oral Sci. 2018 Jan; 26, e20160584.

34. Kaur, G., Kumar Reddy, T. V., Venkatesh, K. V., & Mahalakshmi, K. Effects of chitosan oligosaccharide and calcium hypochlorite on E. Faecali dentinal biofilm and smear layer removal - SEM analysis. Indian J Dent Res. 2020 Aug; 31(4), 550-556.

35. Gyulbenkiyan, E., Gusiyska, A., Vassileva, R., & Dyulgerova, E. Scanning electron microscopic evaluation of the sealer/dentine interface of two sealers using two protocols of irrigation. J of IMAB 2020 Jan; 26(1), 2887-2891.

36. Brandão-Neto, D. O., Mello, J. V. Z., Marceliano-Alves, M. F. V., Carvalho Coutinho, T. M., Marceliano, E. F. V., Galhardi, M. P. W., Tavares, V. S., Muzy Dias, A. P., & Lins, R. X. Final Endodontic Irrigation with 2% Peracetic Acid: Antimicrobial Activity and Cytotoxicity. Eur J Dent. 2021 Jul;15(3), 533-538.