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Original Article

Antibacterial effect of sodium hypochlorite gel on Enterococcus faecalis in endodontics: A systematic review

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Abstract

Aim: The aim of this systematic review was to investigate the in vitro antibacterial effect of sodium hypochlorite gel on enterococcus faecalis during root canal treatment.

Methodology: An electronic databases (PubMed, ScienceDirect, Google Scholar, CENTRAL, Health Advance) was performed (last updated was 1st April 2019). The reference lists of the included studies were hand searched. Randomized in vitro studies that compared sodium hypochlorite gel to solution as a root canal irrigant were included. No limitation on publications date.

Results: Out of 20741 articles, only two studies with 116 human teeth were included in this review. The studies showed 50% high risk of bias, and 50% medium risk of bias for the included studies. Both sodium hypochlorite gel and solution had some bacterial growth inhibition over E-faecalis. The heterogeneity in methodology of the included studies and the lack of high evidence led to contradictory results. However, sodium hypochlorite solution was better in enhancing the antibacterial effect according to the robust study.

Conclusions: There is insufficient reliable evidence about the antibacterial effect of sodium hypochlorite gel on enterococcus faecalis. Although the robust study included in this review revealed that sodium hypochlorite gel is less efficient than the solution as a root canal irrigant, further studies are needed to consider the most effective type, concentration, duration and treatment protocols in enhancing the antibacterial effect of sodium hypochlorite.

Keywords: Sodium hypochlorite gel, Enterococcus Faecalis, root canal treatment

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Introduction

Successful endodontic treatment depends on the ability to disinfect the root canal by cleaning and shaping (1). Disinfecting a root canal includes removal of necrotic tissues, accumulated debris and eliminating the number of bacteria (2). Upon infections, Enterococcus faecalis has been considered the main and most resistant microorganism in root canal system (3). Mechanical preparation alone is insufficient to clean the root canal system (4). Maciel showed that about 50% of root canal walls remain uninsulated during instrumentation (5). Thus, irrigation allows cleaning all

isthmus, fins, ramifications, and all lateral canals that are not accessible after using endodontic file systems (6, 7).

Sodium hypochlorite is the most common irrigant in endodontics due to its antibacterial effect and the ability to dissolve soft tissues (8, 9). Disadvantage of sodium hypochlorite is its high toxicity when it is extruded beyond the apex (10, 11). A study reported that 42% of endodontic clinicians had at least one occurrence of sodium hypochlorite extrusion beyond the apex during their career (12). Extrusion beyond the apex can cause severe pain, swelling, inflammation, ecchymosis, ulcerations, damage and destruction of endothelial and fibroblasts cells resulting in emphysema, and sensor motor defects (13, 14). Sodium hypochlorite gel has been introduced in endodontics as a more controllable and a safer irrigant that can reduce the possibility of the apical extrusion (15).

There is no evidence that summarizes systematically the efficacy of each type of sodium hypochlorite over the other. Hence, the aim of this systematic review is to investigate the in vitro effectiveness of sodium hypochlorite gel on E. faecalis.

Materials and Methods

Protocol:

A pilot search through PubMed was conducted, and one eligible study was assessed before writing the protocol. This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.16

Review question:

Is sodium hypochlorite gel as a root canal irrigant as effective as the solution in eliminating E-faecalis in human teeth?

Information sources and search strategy:

This review included all publications in English language about the antibacterial effect of sodium hypochlorite gel on Enterococcus faecalis in Endodontics in extracted human teeth. No predetermined restrictions on year of publications or publication status.

All editorials, personal opinions, reviews, guidelines, abstracts, conferences, and commentary articles were excluded.

This study question was conducted according to the population, intervention, comparison, and outcome (PICO) study design (Table 1).

Table 1. PICO format.

PICO study design	
Population	Eligibility Criteria: Human extracted teeth Exclusion Criteria: Animal teeth
Intervention	Irrigation of sodium hypochlorite gel in different concentrations and different time exposure during root canal treatment
Comparison	Sodium hypochlorite gel vs. sodium hypochlorite solution
Outcome	Changes in the measurements of E faecalis counts using agar plate count method
Study design	Eligibility criteria: In-Vitro randomized studies. Studies in English Language. Exclusion Criteria: Animal studies. In-Vivo human studies, In-Vitro studies on animal teeth. Case reports, case series. Editorials, personal opinions, retrospective studies, reviews, Guidelines, abstracts, conferences, commentary articles

The study search began from 1st January 2019 in different electronic databases (Medline via PubMed, ScienceDirect, Google Scholar, Cochrane Databases, Health Advance) through advanced search using the following keyword: (Antibacterial effect) Or (Antimicrobial efficacy) or (Bactericidal Activity) AND (Sodium hypochlorite gel) And (Enterococcus Faecalis) or (E-faecalis) And (Endodontics) OR (Root canal Treatment). The full search strategy is presented in (Table 2). A hand search of the reference list of selected articles were also screened for possible related studies that were not discovered by electronic search for additional relevant publications.

Table 2. Electronic database and search strategy (last search was on 1st April 2019)

Database	Site	Search strategy	Search builder	limits	items	Items involoved after excluding irrelavent articles.
PubMed	https://www.ncbi.nlm.nih.gov/pubmed	(Antibacterial effect) Or (Antibacterial efficacy) Or (Bactericidal Activity) AND(Sodium hypochlorite gel) And (Enterococcus Feacalis) or (E-faecalis) And (Endodontics) OR (Root canal Treatment)	All fields	English language	17799	133
SienceDirect	https://www.sciencedirect.com/	(Antibacterial effect) Or (Antibacterial efficacy) Or (Bactericidal Activity) AND(Sodium hypochlorite gel) And (Enterococcus Feacalis) or (E-faecalis) And (Endodontics) OR (Root canal Treatment)	Title, Abstract, keyword	-	190	8
Google Scholar	https://scholar.google.com/	(Antibacterial effect) Or (Antibacterial efficacy) Or (Bactericidal Activity) AND(Sodium hypochlorite gel) And (Enterococcus Feacalis) or (E-faecalis) And (Endodontics) OR (Root canal Treatment	-	English language	2630	120
CENTRAL (Cochrane library)	https://www.cochranelibrary.com/	(Antibacterial effect) Or (Antibacterial efficacy) Or (Bactericidal Activity) AND(Sodium hypochlorite gel) And (Enterococcus Feacalis) or (E-faecalis) And (Endodontics) OR (Root canal Treatment	Title, Abstract, keyword	Trials	1	0
Health Advance	https://www.healthadvance.com/	Antibacterial effect) Or (Antibacterial efficacy) Or (Bactericidal Activity) AND(Sodium hypochlorite gel) And (Enterococcus Feacalis) or (E-faecalis) And (Endodontics) OR (Root canal Treatment	All content	-	119	3
Hand search through reference list					2	0
Overall					20741	264

Study selection and data extraction:

Study selection was performed according to PICO format, and the obtained articles were assessed independently by two authors (SA) and (NB) to clear the inclusion and exclusion of criteria.

Inclusion criteria for the study were:

In-Vitro randomized studies, studies in English Language. Studies comparing sodium hypochlorite gel to solution during endodontic treatment.
Assessing the antibacterial effect on E-faecalis using CFU counts method.

Exclusion Criteria:

Animal studies, In-Vivo human studies, In-Vitro studies on animal teeth, case reports, case series, editorials, personal opinions, retrospective studies, reviews, guidelines, abstracts, conferences, and commentary articles.

Papers were excluded when they did not fulfill one or more of the inclusion criteria. After excluding the irrelevant articles, all abstracts of the remaining articles were screened to eliminate articles from data obtained through abstract. Finally, a full text assessment was done to confirm the acceptability of articles based on the inclusion and exclusion criteria.

Data was extracted by the same two authors (SA) and (NB) independently. Disagreement between the two authors were resolved through discussion with a third author (OA).Data was arranged by the general information (name of author, year of publication), study characteristics (study design, treatment comparison), study sample (sample size, type of teeth), root canal irrigation (instrumentation, type of needle, volume, duration, concentration of NaOCl), microbiology procedure (type of E-faecalis, incubation time and temperature) and outcomes (measurements of E faecalis counts using agar plate count method, and evaluation of antibacterial activity).

Assessment of methodology quality:

The risk of bias of all included studies was assessed independently by the same two authors (SN) and (NB) based on a previous study,[17] some modifications have been added to the assessment. Disagreement between the two authors were resolved through discussion with a third author (OA).

It consists of 7-point rating checklist that describes the methodological aspect of each study, such as description of randomization of teeth, use of teeth free of caries and cracks sample size calculation, endodontic treatment, and irrigation done by the same operator, blinding of observer, presence of control, maturity of biofilms.

If the parameter was reported, the paper had a Y (Yes) on this specific parameter, and if the parameter was not reported or information was not possible to find, the paper had a N (No) on this specific parameter. Studies reported 1-3 items are considered high risk of bias, while studies with 4-5 items are medium risk of bias, and studies with 6-7 items are low risk of bias.

Statistical Analysis

Variables from relevant data related to previous studies were collected and organized in tables. No meta-analysis was performed due to the heterogeneity of treatment protocols.

Results

Study selection:

Data extraction of this review was performed according to PRISMA flow diagram.

Initial searching identified a total of 20741 articles. Screening of article titles revealed a 264 potential studies. Screening of each independent abstract results in including 5 publications for possible inclusion. Applying of inclusion and exclusion criteria after full text assessment identified 2 articles that met the criteria of this systematic review.

The PRISMA flow diagram (Fig.1) shows the search methodology and results.

Exclusion of studies:

The reason for excluding three studies after full text assessment was:

Assessing the antibacterial effect of sodium hypochlorite gel against number of species without including Enterococcus faecalis (n=1) (18).

Antibacterial test was performed on E.faecalis by saturating a paper disk in each tested irrigant and placing them onto culture agar-plates pre-adsorbed with bacterial cells (No endodontic treatment done on human teeth) (n=2) (19, 20).

Quality assessment:

The quality assessment of included studies revealed medium risk of bias for one of the included studies21 and high risk of bias for the other (15). Lack

of blinding of observer, no sample size calculation, and not reporting performance of endodontic treatment by the same operator was the main problematic field for both studies, and not using mature biofilm made one of the studies at high risk of bias. The overall risk of bias is concluded in (Table 3). Study characteristics:

The studies were compared regarding sample size, and treatment protocol of irrigating samples. The two included studies were published between 2016-2017. They involved 116 human teeth, and the inclusion criteria were mature single rooted teeth with straight root canal. All teeth exhibited no resorption, calcification, anomalies, caries, crakes or fractures. All teeth were decoronated to standardize the working length of all samples.

Instrumentation done for all samples 1 mm shorter than the working length. However, different instrumentation techniques were used in the two studies.

Teeth in both studies were sterilized in the autoclave for 20 min. Brain Heart Infusion (BHI) Broth was prepared with optical density 1.5 × 108 CFU/ml.

Difference in incubation time in BHI broth was seen, also differences in concentration, volume, duration of NaOCl were also reported. Although the two studies reported the same manner in evaluating growth of E. faecalis, the way of taking samples from root canal, and the evaluating times were different. The characteristics of the included studies are illustrated in (Table 4A and 4B).

Figure 1. Flowchart diagram for the number of articles obtained through the study.

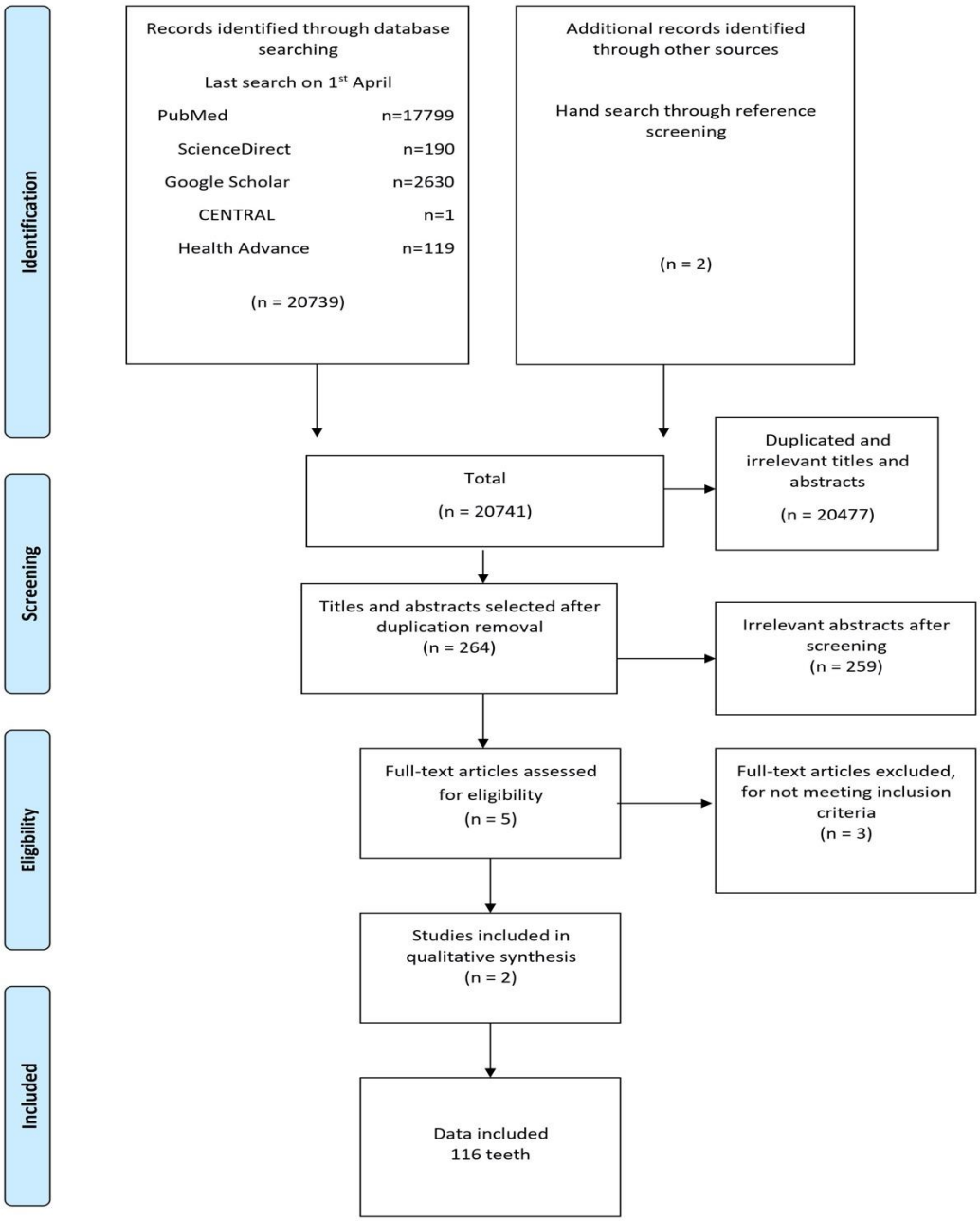

Table 3. Assessment of risk of bias of included studies.

	Zand and others 2016 (21)	Shamsi and others 2017 (15)
Teeth randomization	Y	Y
Teeth free of caries and cracks	Y	Y
Sample size calculation	N	N
Endodontic treatment done by the same operator	N	N
Blinding of observer	N	N
Presence of control	Y	Y
Maturity of biofilms	Y	N
Risk of bias	Medium	High

Results of individual studies:

The two included studies compared different volume, duration, and concentration of sodium hypochlorite gel to solution and to a control group.

Shamsi et al15 showed no E-faecalis growth in 5.25% sodium hypochlorite gel group, 5.25% sodium hypochlorite solution. Results showed no significant difference between sodium hypochlorite gel and solution.

Zand et al. showed no E. faecalis growth in NaOCl solution at both concentrations 2.5% and 5.25%, and significant difference was observed between 2.5% sodium hypochlorite gel and sodium hypochlorite solution at both concentrations (2.5% and 5.25%) (21). However, no significant difference was seen between sodium hypochlorite solution at both concentrations (2.5% and 5.25%). Results are illustrated in (Table 5).

Table 4A. Characteristics of the included studies

Study	Methods			
	Study characteristics			
	Study design	Treatment comparison	Sample size	Type of teeth
Zand et al 2016	Randomized in vitro study. E. faecalis growth between, saline, control (infected with no irrigation), NaOCl gel, and solution		60	single rooted human mature teeth with single canal
Shamsi et al 2017	Randomized in vitro study E. faecalis growth between, saline, control (infected with no irrigation) or (sterilized only with no irrigation), NaOCl gel, and solution		56	single rooted human mature teeth with single canal

Table 4B. Characteristics of the studies

Intervention							
Root canal preparation & irrigation			Microbiology procedure			Measurement of outcome	Antibacterial activity evaluation
Instrumentaion	Volume, duration, concentration of NaOCl	Type of needle	E. faeacalis	Evaluation Method	Incubation time and temperature		
K files up to 35# with crown down technique in two coronal thirds Gates Glidden (4#,3, 2, 1) 1 mm short of apex	5 ml for 30 min 2.5% NaOCl gel 2.5% NaOCl solution 5.25% NaOCl solution	Not reported	ATCC29212	CFU/ml cultured on BHI agar plates	6 weeks at 37°C	10µg of dentin shavings through Gates-Glidden drills 1 mm short of the apex	Incubation at 37° for 48 hours
Protaper (S1, S2, F1, F2, F3) 1 mm short of apex	1 ml for 1 min 5,25% NaOCl gel 5.25% NaOCl solution	30 gauge	ATCC29212	CFU/ml cultured on BHI agar plates	48 hours at 37°C	Sterile paper points 1 mm short of the apex remained for 1 min inside the canal	Incubation at 37° for 4 days

Table 5. Summary of the results of the included studies.

Study	Sample size in each group	Outcome	Mean	Standard deviation (SD)	P-value	Results
Shamsi et al	56 teeth in 4 groups (14 per group)	Colony-forming units (CFU)	G1 5.25% NaOCl solution =0 G2 5.25% NaOCl gel =0	_____	p<0.05	No significant difference was seen between: G1 and G2
Zand et al	60 teeth in 4 groups (15 per group)	Colony-forming units (CFU)	G1 2.5% NaOCl gel =21 G2 2.5% NaOCl solution =0 G3 5.25% NaOCl solution=0	G1 2.5% NaOCl gel =91.3 G2 2.5% NaOCl solution=0 G3 5.25% NaOCl solution=0	P<0.05	Significant difference was seen between: G1 and G2 G1 and G3 No significant difference was seen between: G2 and G3

Discussion

In this review, antibacterial effect of sodium hypochlorite gel was evaluated against Enterococcus faecalis. E. faecalis is the most common bacteria isolated in resistant infections and failed root canal treatments, with prevalence ranging from 24% to 77% (22).

It is highly resistant towards antimicrobial regimens in endodontic treatment, besides it has the ability to survive in harsh aerobic or anaerobic environment where nutrients are scarce (23). E. faecalis can invade the dentinal tubules, adhere to them, and proliferate at wide temperature variations (24).

Sodium hypochlorite gel has been introduced to endodontics as a more controllable and safer irrigant than solution (15), thus reducing the risk of apical extrusion from root canal and with the same efficacy of smear layer removal between 2.5% sodium hypochlorite gel and solution at the same concentrations (25).

This systematic review revealed different results between the two included studies and this might be attributed to the significant heterogeneity in methodologies. Different concentrations of NaOCl were used, in addition to the differences among irrigation protocols including volumes and durations, also the incubation time of E-faecalis in BHI broth and time evaluation of antibacterial activity were also disparate.

In Shamsi et al study, 1 min irrigation of 5.25% sodium hypochlorite gel was as effective as 5.25% solution with no significant difference.15 In this study, incubation time of E-faecalis was 48 hours in BHI broth. Zand demonstrates effective antibacterial activity of 2.5% sodium hypochlorite gel. However, it was significantly less efficient when compared to 2.5% and 5.25% sodium hypochlorite solution. Incubation time in this study was 6 weeks.21 This difference in results could be attributed to the high concentration of NaOCl used in Shamsi et al study.15 According to a review published by Cochrane collaboration library, the higher concentration of sodium hypochlorite, the more reduction in the proportion of samples with positive bacterial cultures (26). This difference in results could also be referred to the short incubation time of E-faecalis in Shamsi et al study. 15 Depending on immature biofilm in this study made it under high risk of bias, since incubating bacteria for only 48 hours make them susceptible for any antibacterial agent. A study conducted by Kishen showed that E- faecalis covered the whole dentin surface after 4 weeks of incubation, and after 6 weeks, the mature biofilm exhibited a highly organized honey-comb like structure with signs of mineralization (27). Studies demonstrate that the biofilm formation of E- faecalis is more difficult to remove than its planktonic form, and more resistant to antibacterial factors (28, 29).

The limitations of this systematic review are related primarily to the lack of high-level evidence from randomized in-vitro studies, the methodological heterogeneity between the two included studies, and the limited number of studies published on this topic.

Conclusions

There is currently insufficient reliable evidence about the antibacterial effect of sodium hypochlorite gel on enterococcus faecalis. According to the robust study included in this review, sodium hypochlorite solution was more effective than its gel form towards E.faecalis. Further studies are needed to consider which type, concentration, duration, and treatment protocols of sodium hypochlorite are most effective.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception- S.A.N.; Design- S.A.N., O.A; Supervision- O.A., N.B.; Materials- S.A.N., N.B.; Data Collection and/or Processing- N.B., O.A.; Analysis and/or Interpretation- S.A.N.; Literature Review- S.A.N., N.B., Writer- S.A.N.; Critical Review- N.B., O.A.

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