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REVIEW ARTICLE

Regenerative endodontic treatment in mature teeth: a systematic review and meta-analysis

ABSTRACT

Regenerative endodontic treatment (RET) is an alternative treatment for immature teeth, however, its efficacy on mature teeth is still controversial. This review was aimed to assess the level of evidence of clinical and radiographical outcomes of RET in mature teeth and run a meta-analysis to compare its success rate to conventional root canal treatment (CRCT). The electronic databases PubMed, Science Direct and Web of Science were used to search based on inclusion and exclusion criteria. The Randomized controlled clinical trials (RCTs), case series, and case reports studies of the RET in mature teeth published in the English language from January 2010 till December 2021 were selected. A meta-analysis was performed using the random-effects model on the randomized clinical trials that compare the success rate based on clinical and radiographic outcomes of RET and CRCT. From sixteen articles included in the narrative analysis, two studies were subjected to meta-analysis. Different protocol aspects of RET including disinfection, size of apical preparation, intracanal medications, types of scaffolds, barriers and follow-up periods were described. The meta-analysis showed no significant differences in success rate between CRCT (89.47%) and RET (95.45%) at 12 months (P>0.05), while it showed a significant increase in a positive response to the electrical pulp test of RET (P=0.010). With the limitations, the adopted protocols of RET are comparable to CRCT and could be a potential approach to treat mature teeth with pulp necrosis and/or apical periodontitis. However, providing more evidence is essential to ascertain these findings.

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Introduction

Regenerative endodontic treatment (RET) is a biologically-based procedure aimed to replace damaged structures, including dentin and root structures along with cells of the pulp-dentin complex (1). The modern interest in the RET concept originated from the revascularization capacity of luxated or avulsed immature teeth with open apices providing ideal decontamination conditions (2). The outcome of RET in permanent immature teeth manifested successful restoring of pulp functions and stimulating normal physiological development of the root (3, 4).

Generally, conventional root canal treatment (CRCT) is the standard care for permanent mature teeth with necrotic pulp and apical periodontitis while the incidence of large periapical lesions may require surgical removal. The success rate of CRCT ranged between 68% to 85% in the last 4 to 5 decades (5). The main goal of CRCT is to eliminate clinical signs/symptoms and resolve periapical lesions (6). Evidently, the American Association of Endodontics specified the same goal of CRCT as a primary objective for RET, while increased thickening of the root walls or root length and regained pulp vitality are secondary and tertiary goals respectively (7). Whilst the primary goal is an objective for both endodontic treatments, the secondary goal is beneficial for immature teeth to minimize potential root fractures caused by thin and/or weak instrumented root walls. The tertiary goal could be measured as a desirable goal which is possibly not essential to determine the clinical success of RET due to uncertain response of sensibility tests that may encounter false negative or false positive response (8).

Recently, RET has been investigated to treat permanent mature teeth with necrotic pulps and/or apical periodontitis (9, 10). Unlike CRCT, the apical third is commonly over instrumented and apical foramen is enlarged to remove apical ramifications and bacterial load within root canals (11). Subsequently, no obturation material is used in RET and as an alternative, the root canal is filled with biological scaffolds such as blood that is induced from the apical area manually by extending the file to the periapical area, or autologous platelet-rich/poor plasma or collagen with/without hydroxyapatite or platelet-rich fibrin which may be combined with the stem cell.

RET is controversial in mature teeth due to the risk of recurrent infections through the non-obturated root canal and flare-up that might outweigh the benefits of regenerative treatment, counting complete root formation that is redundant in mature teeth where the root walls are thick and the apex is closed. Definite scientific evidence of the beneficial effects of this treatment in mature teeth should be provided before proposing RET as an alternative treatment. Therefore, this systematic review aimed to assess the level of evidence of clinical and radiographical outcomes of RET in mature teeth and run a meta-analysis to compare its success rate to conventional root canal treatment (CRCT).

Review

The protocol of this systematic review was registered in PROSPERO (CRD42020215802) (12) and followed the PRISMA statement (13). PubMed (National Library of Medicine), Science Direct (Elsevier), Web of Science core collection (Clarivate Analytics) were searched for relevant articles, published in the English language, from January, 2010 to December, 2021. This was supplemented by Manual searches in the reference lists of reviews and included studies to identify publications that might have been missed during the electronic database searches. The search terms (Appendix 1) used are mature permanent tooth/teeth, mature tooth/teeth, mature necrotic pulp, mature non-vital tooth/teeth, apical periodontitis, periapical lesion, regenerative endodontics, pulp regeneration, tooth/pulp revascularization, pulp revitalization, non-obturation endodontic, root canal therapy. The Boolean operators

'AND' and 'OR' were employed to combine the keywords and generate the search strategy.

Inclusion criteria and study selection
Clinical studies that assessed the efficacy of RET in mature necrotic permanent teeth with or without apical periodontitis were included. While studies on the animal, laboratory, reviews, and clinical studies of immature permanent teeth were excluded.
The primary outcome was the success rate of the RET assessed by the absence of clinical signs and symptoms (pain, swelling, inflammation, and probing), and radiographic finding (changes in periapical lesion and root canal walls). The secondary outcome was teeth response to the sensibility/vitality test which could be an indicator of vital tissue presence (14).
Title and abstract screening followed by full-text assessment were undertaken by two independent reviewers. Any disagreement was resolved by discussion and consensus. Data were extracted in standardized tables by both reviewers. A kappa score of >0.80 was observed between them on the various domains of data extraction.

Data extraction
Relevant data were extracted following the Cochrane Handbook for Systematic Reviews of Interventions guidelines (15) which consisted of study and participant characteristics (Table 2), types of intervention and comparator (Table 3), and primary outcome measures (table 4).

Risk of bias and quality assessment
The quality assessment was assessed according to the study design. The revised Cochrane Risk of Bias Tool for Clinical Randomized Trials (RoB 2.0) and risk of bias because of the randomization process, deviations from the intended interventions, missing outcome data, measurement of the reported result, and overall bias were appraised to classify the selected studies into a low risk of bias, some concerns, and a high risk of bias (16).
The Joanna Briggs Institute (JBI, University of Adelaide) tools were used to assess the quality of case reports (17) and case series (18). Evaluation parameters of the case reports were as follows; a clear description of the patient's demographic characteristics, case history, current clinical condition, assessment method, intervention, post-intervention condition, adverse effects, and lessons provided by the case report. The parameters of the case series were as follows; clear criteria for participants' inclusion, measuring the condition in reliable, standard and valid method, consecutive inclusion of participants, complete inclusion of participants, clear reporting of clinical information, outcomes, site clinic demographic and appropriate statistical analysis. For each parameter in both types of mentioned studies, the included articles could be awarded a "yes", "no", "unclear" or "not applicable". The overall quality of each case report and case series were allocated into three categories as follows: (i) low risk of bias (met at least 75% of the criteria), (ii) moderate risk of bias (met between 50% and 74% of the criteria), (iii) high risk of bias (met less than 49% of the criteria) (19).

Data analysis
Statistical analysis was performed using Review Manager (RevMan, Version 5.4., Cochrane Collaboration, 2020). The outcome of interventions with direct comparison was analysed using proportion (%) for the primary outcome and Yes/No for the secondary outcome. The risk ratio (RR) with a 95% confidence interval (CI) was used to evaluate the association between the incidence of success and treatment type (RET and CRCT). Heterogeneity was tested using I^2 statistic. Fixed-effects model was used for low/moderate heterogeneity while the random-effect model was applied for significant heterogeneity ($I^2 \geq 50\%$).

Review data: study selection
Figure 1 illustrates a flow diagram on the selection, inclusion, and exclusion of studies according to PRISMA. The search yielded 1172 hits; 1152 hits without duplicates were screened; 27 were relevant

and obtained in full text. Subsequent full article screening excluded an additional 8 references (20-27). The reasons for exclusion are presented in Table 1.
Eventually, the remaining 19 studies (6, 9, 10, 14, 28-42) were included and subjected to data extraction, methodologic quality assessment, and data synthesis. From these included studies, 2 were involved in Meta-analysis.

Characteristics of the included studies
11 case reports (9, 30-38, 40), two case series (6, 10), one single armed clinical study with no control (14) and five randomized clinical trials (RCTs) (28, 29, 39, 41, 42) were involved in the current review with a total of 222 patients, 76.1% of them had RET. The age of the patient varied from 9-76 years old. Female gender was prominent with 66 patients (51.6 %) compared to 62 Male patients (48.4%). The maxillary central incisor was the most involved tooth (82.1%) of all treated teeth (single-rooted teeth and mandibular first molar). The aetiology of pulp necrosis was mainly trauma followed by failed previous endodontic treatment, crown fracture and caries. The cases were diagnosed as asymptomatic apical periodontitis (39%), symptomatic apical periodontitis (22%), acute apical abscess (17.1%), chronic apical abscess (14.6%), and avulsed tooth, chronic pulpitis and symptomatic irreversible pulpitis (2.4% each). Internal root resorption was diagnosed in one case (38) and root perforation in another (35). Radiographical evidence of periapical lesions was detected in approximately 166 teeth (98.8%) that have Periapical Index ≥2. The avulsed tooth (32), mid-rooted fracture (35) and chronic pulpitis (37) cases were associated with no periapical lesions (Table 2).

Quality assessment and ROB
Three RCTs (28, 29, 39) were assessed as low risk whereas two had some concerns (41, 42). One clinical study and one case series were assessed as low risk (6, 14) whereas one case series (10) was presented a moderate risk of bias. Although two of 11 case reports (38, 40) have some concern regarding clear describe of the patient's history, overall bias was low risk (>75%) (9, 30-38, 40) (Figure 2).

Treatment protocol
1) Disinfection: the main irrigant in all cases was 1-6% sodium hypochlorite (NaOCl). Collectively, 139 cases (82.2 %) used 17% EDTA (6, 9, 14, 28-31, 34, 37-40, 42), whereas 18 cases (10.7%) used unspecified antimicrobial solution following the NaOCL (10). Additionally, triantibiotic solution was used before NaOCl in 3 cases (1.8%)(40). 36 cases (21.3%) used the Endoactivator system (39). The 3 cases (1.8%) used 10 ml of chlorhexidine gluconate irrigation (31) (Table 3).
2) Size of apical preparation was varied based on root canal diameter and operator judgment. Apical preparation of the maxillary central incisors was ranged from 0.30 mm up to 1mm using either hand, rotary or reciprocal files (6, 9, 14, 28-31, 33, 34, 37, 38, 41). For maxillary lateral incisor and premolars, the apical preparation was ranged from 0.30 to 0.60 mm (6, 30, 31, 33, 38, 41) whereas apical prepa-

Table 1
Excluded studies with reasons of exclusion

Study ID	Reason of exclusion
Chrepa, 2015 (21)	RET was not done completely it was initiated only to evaluate whether evoked bleeding from the periapical tissues elicits the influx of MSCs into the root canal system in mature teeth with apical lesions. After that, the root canal was filled through conventional Root canal therapy
Santiago, 2015 (24)	Studies were involved a young immature tooth
He, 2017 (23)	Review of previously published cases and no new case was presented
Gaviño Orduña, 2017 (22)	The trauma occurred when the tooth was immature with no history of tooth complete development earlier
Song, 2017 (25)	The studies involved immature teeth
Timmerman and Parashos, 2017 (26)	Teeth involved have open apices with no history of tooth complete development earlier
Al Khasawnah, 2018 (20)	Calcium hydroxide-iodoform-silicon oil paste (CHISP) as temporary canal filler and Pulpdent with Gutta-percha were used as permanent canals filler instead of regenerative induction
Zaky, 2020 (27)	In-vivo study involved animals

Table 2
Characteristics of the included studies

Study ID	Study design	Cases Number	Age	Gender	Tooth involved	Aetiology of pulp necrosis	Diagnosis	Preoperative periapical lesions
Shah and Logani, 2012 (10)	Case series	18	15-76	11 M & 7 F	Not specified	Not specified	Acute or chronic apical abscess	Yes or No
Paryani and Kim, 2013 (9)	Case report	2	14	F	Incisor # 8	Uncomplicated crown fracture	Symptomatic apical periodontitis	Yes
			11	F	Incisor #9	Uncomplicated crown fracture	Asymptomatic apical periodontitis	Yes
Saoud, 2014 (33)	Case report	2	23	F	Incisor #8	Trauma for 15 years ago	Acute apical abscess	Yes
			23	F	Incisor # 7	Trauma at since years ago	Symptomatic apical periodontitis	Yes
Nevins and Cymerman, 2015 (31)	Case report	3	48	F	Premolar 29	Previously treated pulp	Acute apical abscess	Yes
			40	F	Incisors #8, 9	Previously treated pulp	Acute apical abscess	Yes
			28	F	Incisor #8	Previously treated pulp	Symptomatic apical periodontitis	Yes
Saoud, 2015 (34)	Case report	2	26	M	Incisor #9	Trauma 10 years ago and previously treated tooth	Acute apical abscess	Yes
			12	M	Molar #19	Previously treated 17 months ago	Chronic apical abscess	Yes
Wang, 2015 (36)	Case report	1	39	F	Premolars #20, 29	Fractured dens evaginatus	Symptomatic apical periodontitis	Yes
Priya, 2016 (32)	Case report	1	11	M	Incisor # 9	Trauma	Avulsed tooth	NA
Saoud, 2016 (6)	Case series	4	11-21	2F & 2M	Incisors # 8, 9, 8, 25 & Molar #30	Trauma and caries	Chronic and acute abscess	Yes
Saoud, 2016 (35)	Case report	2	15	M	Incisors #8	Trauma	Symptomatic irreversible pulpitis	Yes
			16	M	Incisors# 8	Trauma	Acute apical abscess and perforating root resorption	Yes
Kaval, 2017 (38)	Case report	1	14	M	Incisors #10	Not stated	Symptomatic apical periodontitis and internal resorption root	Yes
Xu and Zhou, 2018 (37)	Case report	1	15	F	Premolar #13	Caries	Chronic pulpitis	No
Nagas, 2018 (30)	Case report	1	21	F	Incisors #9, 10	Trauma 7 years ago	Symptomatic apical periodontitis	Yes
Nageh, 2018 (14)	Clinical Study	15	18-40	No gender preference (F>M)	Central incisors	Caries	Symptomatic or asymptomatic apical periodontitis	Yes or No
Jha, 2019 (42)	Randomized Clinical Trial	30 (15RET & 15 CRCT)	9-15	No gender preference	Not specified	Not stated	Periapical periodontitis	Yes
Arslan, 2019 (28)	A Preliminary Randomized Clinical Study	46 (26 RET & 20 CRCT)	18-30	CRCT (13M, 7F). RET (22M, 4F)	Anterior & premolar (single root) #7, 8, 9, 10, 11, 24, 25, 26, 27, 28	Not stated	Symptomatic or asymptomatic apical periodontitis. Acute and chronic abscess	Yes
El-Kateb, 2020 (29)	Randomized Clinical Trial	18 (Control: test is 1:1)	20-40	Control (3M & 6F) Test (4M & 5F)	Incisors #7, 8, 9	Trauma (n = 13) and Defective restoration (n =5)	Asymptomatic apical periodontitis and 4 teeth with chronic apical abscess	Yes

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Table 2
Characteristics of the included studies

Brizuela, 2020 (39)	Randomized Clinical Trial	36 (CRCT; control: RET; test is 1:1)	16-58	CRCT (13F & 5M) RET (12F & 6M)	Maxillary or mandibular incisors/canines & mandibular premolars	Not stated	Symptomatic or asymptomatic apical periodontitis. Acute and chronic abscess	Yes
Feitosa, 2021 (40)	Case report	3	18-40	No gender predilection	Premolar (single root) #35,15,25	Not stated	Irreversible pulpitis or pulp necrosis	Yes
Mittal, 2021 (41)	Randomized Clinical Trial	36	16-34	No gender predilection	Maxillary anterior, mandibular anterior and posterior teeth	Not stated	Pulp necrosis	Yes or No

CRCT=Conventional root canal treatment; RET=Regeneration endodontic treatment

ration of molars mesial and distal canals was reached the maximum of 0.30 mm and 0.40 mm respectively (6, 34). Moreover, the preparation was confined to the coronal pulp canal on top of the fracture line with no apical preparation in the case of horizontal root fracture (35). Controversy, massive apical preparation was done to avulsed tooth up to 2 mm (32) (Table 3).
3) Number of visits. Treatment of 137 (81.1%) cases were completed in two visits (9, 28-31, 34, 36, 37, 39, 41, 42). Whereas 20 (11.8%) cases were accomplished in three visit (6, 33, 38), other four cases (2.4%) in one visit (32, 40) and one case (0.6%) in 4 visits (34) and 7 cases (4.1%) in 2-3 visit (10, 35) (Table 3).
4) Medicament material. $Ca(OH)_2$ was only used in 43 (25.4%) cases (6, 28, 29, 34, 38, 39) or combined with antibiotic in 4 cases (2.4%) (9, 35). Triple antibiotic (metronidazole, ciprofloxacin and minocycline or clindamycin) was used in 30 (17.8%) cases (30, 33, 35, 36), mixture of metrogyl, ciprofloxacin and tetracycline paste in 33 (19.5%) cases (10, 42), metronidazole with ciprofloxacin in 55 (32.5%) cases (14, 31, 33, 41), ciprofloxacin powder in 1 (0.6%) cases (9) and doxycycline solution (before replanted) in 1(0.6%) case (32) (Table 3).
Scaffold used and coronal barrier materials. The scaffold used was mainly a blood clot (58.6%) (6, 10, 28-30, 33-35, 37, 38, 41, 42) followed by 2% calcium chloride with Platelet-Poor Plasma plus umbilical cord Mesenchymal stem cell (10.7%) (39). Platelet-rich fibrin (14.2%) (14, 41), collagen with or without hydroxyapatite (10.7%) (9, 31, 41), platelet-rich plasma (1.2%) (36) and auto-transplantation of the pulp (1.8 %) were also utilized. MTA or Biodentin were the main coronal barrier material to be used for the majority of the cases (Table 3).
5) The follow-up period ranged from 1 to 60 months. In four RCTs studies (28, 29, 39, 41), which represents 66.9% of the regenerated cases, the follow-up periods were accomplished within 12 months. Additionally, one RCT was pursued till 18 months (42), while in case series (6, 10) and case report (9, 30-38) it reached up to 2.5-3 years roughly. The longest follow-up period was approximately 5 years (60 months) (14) (Table 3).

Clinical and radiographical outcomes of RET

Failure was reported in 4 cases (2.4%) with clinical signs and symptoms persistent though one case showed healing radiographically by reducing the size of the lesion (28). 165 (97.6%) cases were assessed as success clinically and radiographically with no signs and symptoms associated with the periapical lesion healing or completely healed at the end of follow-up time. 3.6% revealed deposition of hard

Table 3

Regenerative Endodontic treatment (RET) protocol of the included studies

Study ID	Visits	Irrigants	Apical preparation	Medicaments material	Scaffold used	Barrier	Follow-up
Shah and Logani (10)	2-3	2.5% NaOCl and Antimicrobial solution	2-4 file sizes larger than the master apical file at working length	TAP (metrogyl, ciprofloxacin and tetracycline)	Blood	A calcium sulfate-based cement	6 months recall till 3 years for 5 cases, 2 ½ years for 5 cases, 2 years for 5 cases and 6-months for 3 cases
Paryani and Kim (9)	2 visits with 1 week interval for tooth # 8- and 22-days intervals for tooth #9 respectively	5.25% NaOCl followed by 17% EDTA	The apical foramen was enlarged up to 0.6 mm with a # 60 K-file	Calcium hydroxide for tooth #8 Ciprofloxacin powder for tooth #9	Blood + Collacote (Absorbable Collagen)	MTA	1 month, 2 months, 1 year and 3 months, 22 months for tooth#8 1 month, 5 months and 18 months for tooth #9
Saoud (33)	3 visits with 1 week and 2 weeks intervals respectively	2.5% NaOCl followed by sterile saline solution	Instrumented to a #100 and #35 hand K- file to the WL for cases #1 and #2 respectively	TAP (metronidazole 500 mg + ciprofloxacin 200 mg + minocycline100 mg mixed with sterile saline solution)	Blood	MTA	6 months and 1 year
*Nevins and Cymerman (31)	2 visits with 1 month interval	6% NaOCl followed by 17% EDTA 2% chlorhexidine gluconate 10 Ml (case 1) I & D was done on a tooth with buccal swelling	Working length was determined radiographically with #60 or #70 K-file	Ciprofloxacin and metronidazole mixed in equal amounts	Blood+ SynOss putty	MTA (case1) Bioceramic Putty (2 cases)	3-month intervals for 1 year for 2 cases and 6 months for 1 case
Saoud (34)	2 visits with 2 weeks' intervals (case #1) 4 visits and intervals of 1 week and 1 month and a half respectively (case 2)	2.5% NaOCl irrigation Saline solution and then irrigated with 17% EDTA	The canal was debrided to hand #60 K-files to the WL (case 1) Instrumentation of the canawasre done to sizes 30 in mesial and 40 in distal (case 2)	Metapaste	Blood	MTA	7 and 13 months forcases 1 and 8- and 14-months case 2
Wang (36)	2 visits with 2 weeks intervals	20 mL 2.5% NaOCl followed by 20 mL saline for each canal	Not stated	Ciprofloxacin, metronidazole, and minocycline (0.1 conc. mg/mL)	Autologous PRP	MTA	8 and 30 months
#Priya (32)	1 visit	Normal saline and 5.25% NaOCl	Root apex was enlarged to approximately 1.5-2 mm	Teeth were placed in doxycycline solution for about 15 to 20 minutes and replanted and stabilized	Autologous PRP	GIC	2 week, 2, 3, 6, 9 and 12 months
Saoud (6)	3 visits with 2 weeks interval	2.5% NaOCl irrigation	ProTaper Universal Rotary files to F5 (#40) for teeth #8 and #9, F3 (#30) for tooth #25, F2 (#25) for mesial canals and F4 (#35) for distal canals of tooth #30	Metapaste (calcium hydroxide)	Blood	MTA	ranged from 8-26 months
Saoud (35)	2 or 3 visits with 2 weeks interval	2.5% NaOCl solution followed by sterile saline solution and 17% EDTA solution	The coronal canal was debrided to #50 K-files. (case1) Gates-Glidden # 2 for the resorptive area of the canal in perforating case (case 2)	Calcium hydroxide Metapaste (case 1) TAP (case 2) (metronidazole, ciprofloxacin, and minocycline)	Blood	MTA	5,8,14 and 19 months for case 1 8, 15 and 19 months for case 2

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Table 3

Regenerative Endodontic treatment (RET) protocol of the included studies

Kaval (38)	3 visits with 4 weeks and 3 months' intervals respectively	1% NaCL followed by 17% EDTA and distilled water	K files #80 for the root canal coronal to the resorptive area and # 45 for apical canal	Calcium hydroxide	Blood	MTA	6 months and 2 years
Xu and Zhou (37)	2 visits with 14 days interval	5.25% NaOCl followed by 17% EDTA	The apical foramen was enlarged up to 0.6 mm with a # 60 K-file	A ciprofloxacin and metronidazole	Blood	MTA	3, 6 , 12 and 30 months
Nagas (30)	2 visits with 28 days interval	20 mL of 5.25% NaOCl, followed by 10 mL of saline and then with 17% of EDTA	Not stated	TAP (ciprofloxacin, metronidazole, and clindamycin)	Blood	MTA	1-month, then every 6 months for 60 months
Nageh (14)	2 visits with 21 days interval	1.5% NaOCl. 20 mL 17% EDTA followed by saline irrigation	Apical canal preparation to K-file #60–80	metronidazole and ciprofloxacin mixed with saline	Blood+ PRF	MTA	Every 3 months up to 1 year
Jha (42)	2 visits with 1 or 2 weeks intervals	2.5% NaOCl and final rinse with 17% EDTA	Rotary protaper universal files were used and apical widening was done with K-files #25-30	TAP	Blood	Calcium sulfate-Based cement (Cavit G)	6, 12, 18 months
Arslan (28)	2 visits with 21 days interval	5 mL of 1% NaOCl followed by 2 mL 5% EDTA and 5 mL distilled water	The root canal was enlarged using reciprocating nickel-titanium files ((#25 and #40) and stainless steel (#45-#80) hand files	CRCT group: calcium hydroxide REP Group: TAP	Blood	White MTA	12 months
El-Kateb (29)	2 visits	20 mL 1.5% NaOCl followed by a final rinse with 20 mL 17% EDTA for about 1 minute	Rotary instrumentation of the canals was performed with PTN files until sizes X3 (test group) and X5 (control group)	Calcium hydroxide	Blood	Biodentin	1, 3, 6, 9 and 12 months
Brizuela (39)	2 visits in 21 days interval	20 ml 2.5% NaOCl and Endoactivator system followed by 20 ml 17% EDTA	Selected Reciproc files	Calcium hydroxide	Blood+ PPP + UC MSCs +an absorbable gelatine sponge haemostat	Biodentin	6 and 12 months
Feitosa (40)	1 visit	TAP solution (ciprofloxacin, minocycline, and metronidazole followed by sterile saline and 17% EDTA for 5 minutes	Rotary files (WaveOne Gold)	None	pulp autotransplantation from extracted third molar	Biodentin	3, 6, 9, 12 months
Mittal (41)	2 visits with 2 weeks intervals	20 mL of 1.5% NaOCl) and 10 mL of saline	K-files #60-80 for maxillary anterior teeth, #30 for mandibular anterior and posterior teeth	Metronidazole and ciprofloxacin paste	Blood/PRF/ collagen/ hydroxyapatite-collagen (Four groups separately)	Biodentin	3, 6, 9, 12 months

*PPP (Platelet-Poor Plasma), UC MSCs (umbilical cord Mesenchymal stem cells), MTA (white mineral trioxide aggregate), SynOss (collagen hydroxyapatite scaffold), PRP (Platelet Rich Plasma), PRF (Platelet Rich Fibrin), GICs (Glass ionomer cements). *Amoxicillin 500 mg (4x1x10) was prescribed in the 1st visit, #Patient was given Doxycycline 100 mg (2x1x7) was prescribed in the 1st visit.*

tissue and narrowing the root canal space (31, 33, 38). Thickening of the root canal walls was evident in 3.6% (33, 34, 37, 38). Regaining the tooth sensibility using electrical pulp test (EPT) was demonstrated in 51 (30.2%) cases (9, 14, 28, 29, 32, 37, 39, 40). Interestingly, 36 (21.3%) cases responded positively to cold test with no response to heat or EPT (41) (Table 4).

Meta-analysis

The pooled data of the two RCTs compared RET to CRCT at 12 months follow-up (28, 39) showed no significant differences in

Table 4

Clinical and radiographical outcomes

Study ID	Signs & Symptoms	Sensibility and vitality	Periapical lesion	Root canal wall
Shah and Logani (10)	Tissue healing was excellent clinically	Not mentioned	Complete resolution or decrease in the size with increase in bone density	Increase cementum density radiographically
Paryani and Kim (9)	Asymptomatic with Probing depths ≤3 mm in one	Normal response to Endo-Ice and EPT (34 of 80) in first case and no response in the 2nd case	Complete resolution	Thinning of the apical one-third of the root canal in one of two cases
Saoud (33)	Asymptomatic	No response	The pulp cavity appeared to be obliterated by hard tissue formation in the apical portion	Thickening of the canal walls and closure of the apex
Nevins and Cymerman (31)	Asymptomatic	No response	Continuous healing	Radiopacity develops within the coronal and middle third of the root canal
Saoud (34)	Asymptomatic	No response	Complete healed	Thickening of the canal walls and the apex appeared to have closed
Wang (36)	Asymptomatic	No response	Continuous healing	No evidence of thickening in the root canal or root lengthening
Priya (32)	6 months: Symptomatic 12 months: Asymptomatic	Positive response to thermal and EPT	At 6 months: evidence of internal resorption with periapical radiolucency. At 9 and 12 months: resolution of periapical radiolucency	At 6 months: external root resorption and space were observed At 9 and 12 months: slight evidence of replacement resorption
Saoud (6)	Asymptomatic	No response	28.5% of teeth: complete healed. 71.5% of teeth: reduce in size	Not stated
Saoud (35)	Asymptomatic	No response	Not stated	Formation of hard tissue between fragments in horizontal root fracture
Kaval (38)	Asymptomatic	No response	Significant healing	Increase in root canal wall thickness with remineralization in the perforated resorptive area and between the coronal and root pulp tissue
Xu and Zhou (37)	Asymptomatic	Gradually regained pulp sensibility and responded positively to the electric pulp tester	No periapical lesion	Root wall thickening
Nagas (30)	Asymptomatic	No response to cold or EPT	Complete resolution	The dimensions of the root space had remained unchanged
Nageh (14)	Asymptomatic	60% of the patients regaining sensibility gradually to reach the highest level at 12 months	Complete healed	Not stated
Jha(42)	Asymptomatic	Not stated	13 Complete healed and 2 healing for RET	Not stated
Arslan (28)	Asymptomatic teeth are 80% in CRCT group and 92.3% in REP group	50% of REP-treated teeth responded positively	Absence and reduction of the radiolucency in 85% of CRCT and 92.4% of REP with	Not stated
El-Kateb (29)	Asymptomatic	66.7% in the X3 group and 88.9% in the X5 group had gradually regained the sensibility to reach the highest level at 12 months	Periapical healing was enhanced in all cases	The apical thirds of the canal increased from its baseline values to reach the highest values at the 3-months which became approximate to the normal contralateral tooth
Brizuela (39)	At 6 months: 5.6% of REP group had percussion pain. At 12 months: both groups had 100% efficacy	Positive response to cold (56%) and heat (28%) and EPT (50%)	No Significant changes in cortical involvement and dimensions of apical lesions	Significantly median anteroposterior reduction of 0.35 mm in CRCT group and 0.94 mm in the REP group
Feitosa (40)	At 3 months, slight twinges at the periapical region with no response to EPT At 6 months and 1 year, asymptomatic	Positive response to EPT at 6 months and revascularization evidence by Doppler imaging at 1 year	Complete regression of periapical lesions for patients 1 and 2 whereas the radiolucency in patient 3 was almost entirely diminished	Not stated
Mittal (41)	Asymptomatic and swelling and sinus tract had resolved completely	Positive response to cold test at 12 months with no response to heat or EPT	Periapical healing and resolution of apical periodontitis	Not stated

EPT (electrical pulp test

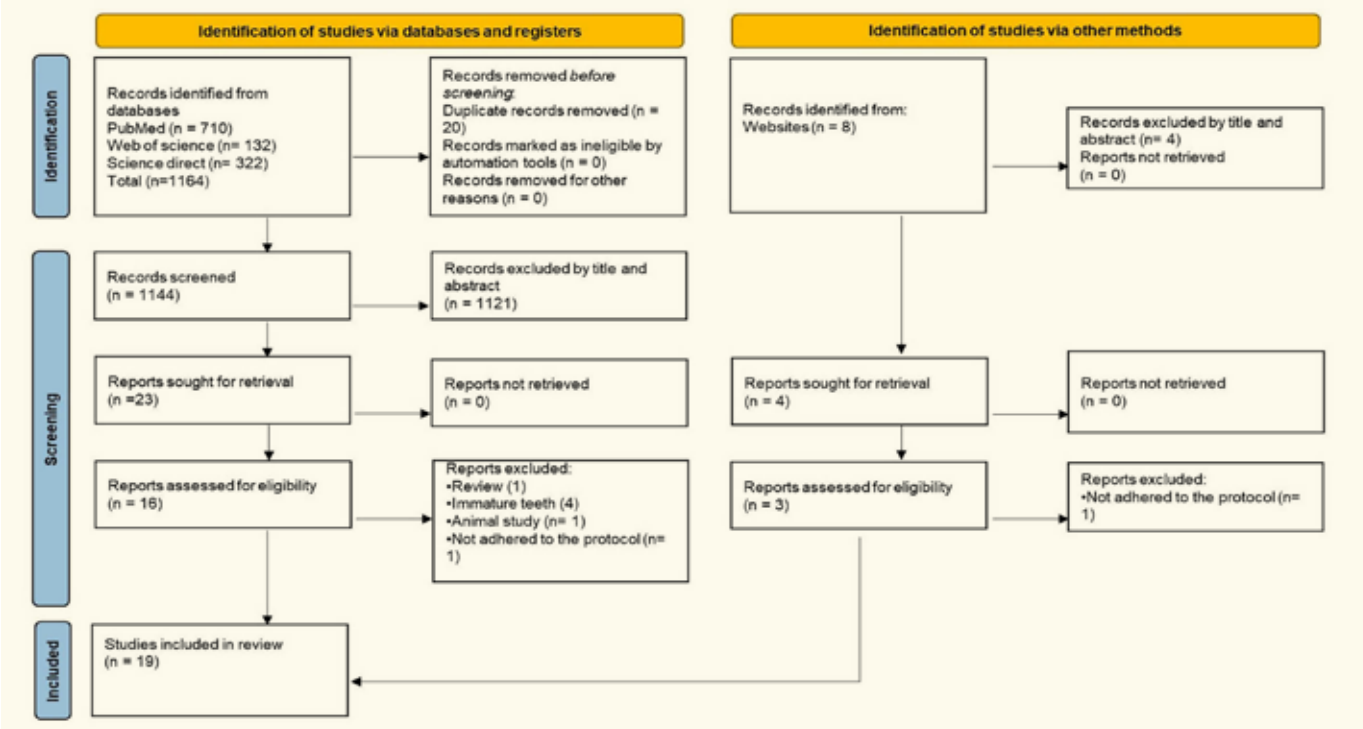

Figure 1
Literature search and screening according to PRISMA flow diagram on selection, inclusion, and exclusion of studies at each screening stage.

clinical and radiographical outcomes (P>0.05). While it showed a significant increase in positive response to electrical pulp test (EPT) in favouring of RET (I^2=9%; risk ratio; 3.97 95% CI: 1.39-11.30, P=0.010) (Figure 3).

Discussion

RET is built on the principles of regenerative medicine and tissue engineering and aimed to treat immature permanent teeth with pulpal necrosis by regenerating

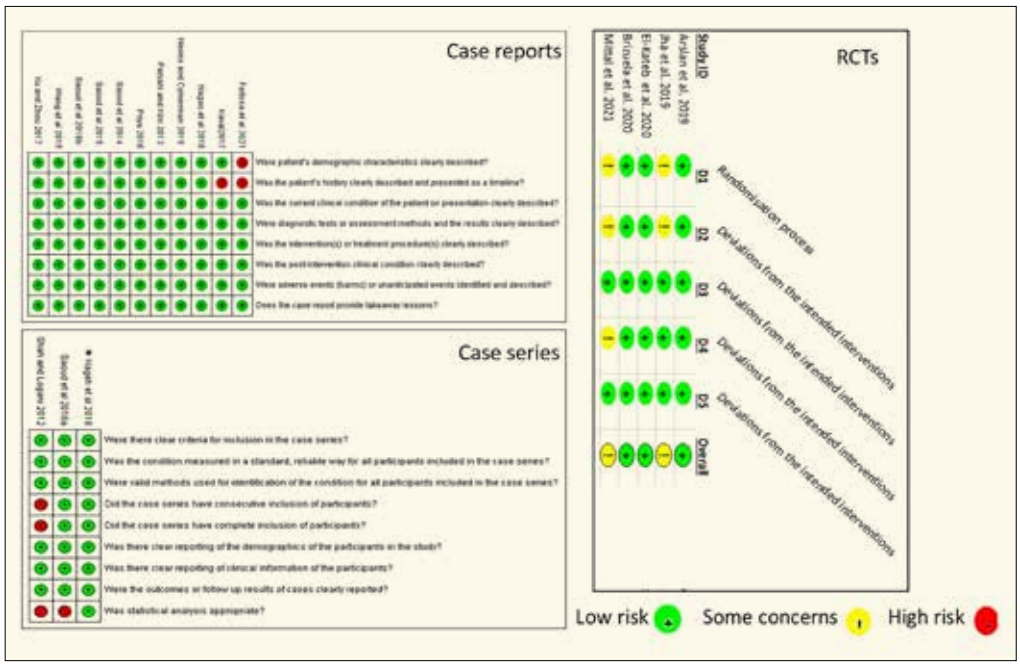

Figure 2
Quality assessment results of RCTs studies according to the revised Cochrane Risk of Bias Tool for Randomized Trials (RoB 2.0), case series according to the Joanna Briggs Institute tool and case reports according to the Joanna Briggs Institute too.

*The study design is single armed clinical study with no control, authors found IJB tool for case series was suitable to th quality assessment.

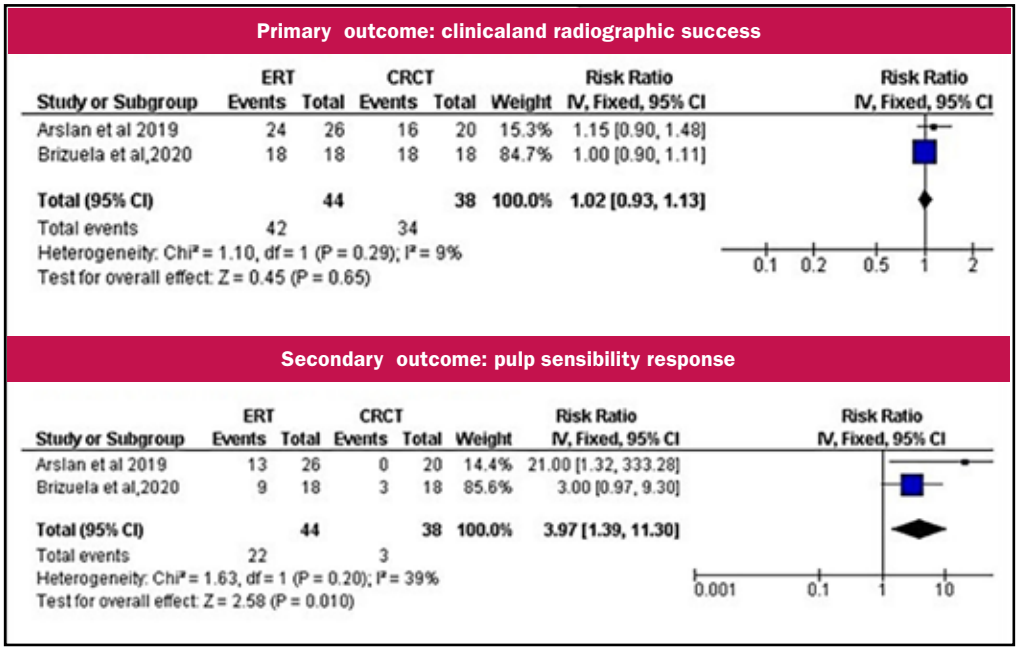

Figure 3
A forest plot of the clinical and radiographical success success of RET and CRCT at 12 months.

functional pulpal tissue applying protocols (43). Hence, researchers elected to find out more the efficacy of RET on permanent mature teeth (9, 10). To our knowledge, the first study that reported regenerative endodontic treatment for mature teeth was published in 2012 (10). This systematic review aimed to search with an earlier time frame to diminish the risk of missing any study to be included.

The majority of studies in this systematic review were case reports which represented the lowest levels of causation evidence due to the inherent bias (44), Therefore, the level of evidence of RET outcomes from these groups was considered low. However, these studies are the most commonly published articles in medical journals (45). Furthermore, the existing literature lacks RCTs that compare the RET to CRCT within standard treatment protocol, follow-up and reporting methods to reduce the heterogeneity. Thus out of five RCTs, only two studies were included in the meta-analysis. The other studies lack of comparator of CRCT (29, 41) or the findings at 12 months were not reported clearly (42).

Meta-analysis showed no significant difference in clinical and radiographical success rate between the RET and CRCT. This could be attributed to the disinfection protocol of the root canal area which is a crucial step in both treatments and the key to successful outcomes. It was reported that the main cause of CRCT failure was the persistence or occurrence of intraradicular or extraradicular infections (46) and failure of coronal barrier or seal (47, 48). Likewise, failure of RET was attributed to inadequate root canal disinfection (28) besides the loss of coronal restoration that instigates reinfection (32). Disinfection of the root canal is attained through a combination of mechanical debridement and irrigation along with intracanal medicaments (if required) to disrupt biofilms on the infected canal walls (49, 50). A low concentration of NaOCl (1.5%) followed by 17% EDTA was recommended during RET of immature tooth (7) to reduce the cytotoxic effect of NaOCl on the apical papilla stem cells which is essential for RET (51, 52). According to the findings, 1-6% NaOCl was the main irrigant used since mature teeth have closed apices confining the irrigant to the canal space so a high concentration of NaOCl might lack an adverse effect on stem cells survival (53). Mechanical root dentin debridement is not recommended in immature teeth as it increases the risk

of their thin root fracture (54), while it is required in fully mature teeth with a thick root to remove infected dentin especially at the apical third of the root.
Apical preparation was followed by apical foramen widening to different sizes, based on the tooth type and the operator judgment. The successful clinical and radiographic outcomes were demonstrated in the majority of the cases which might be attributed to blood-borne and apical papilla stem cells small size (10-100 µm) that allow them to enter the canal from the periapical area through small size orifice (54). In view of that, the size of the foramen could have no significant effect on the treatment outcome. On the other hand, apical foramen enlargement is contraindicated in CRCT due to the risk of pushing necrotic debris and microorganisms into the periapical tissues and triggering periapical inflammation (55).
The root canal was filled with obturation materials and sealers in the CRCT, whereas biological active host vital tissues were obtained by inducement in the RET. Interestingly, the periapical lesion can heal without root canal fillings if the intracanal bacterial load is effectively reduced (56). This concept could explain the success rate of the regenerated cases despite different protocols applied. Prominently, leaving empty root canals is not a professional standard of care since it could allow re-infections of the root canal. Particularly with the widening procedure of the apical foramen in RET which may facilitate the apical leakage if the proposed biological tissue sealing is failed.
Blood clot alone or in combination with growth factors and/or Mesenchymal stem cells were used as filling in RET to induce the regeneration process. The blood clot was successfully leading to pulp regeneration (10, 28, 29). However, executing the bleeding technique only in RET of mature teeth might have limitations compared to those in immature teeth due to the smaller quantity of stem cells in the former, thus the implementation of PRP/MSCs in RET of the mature tooth was recommended (57, 58). A marked difference in periapical healing and dentinal wall thickening of teeth and growth of pulp-like tissue were reported in some cases treated by revascularization with PRP and cell-based approach in different studies (32, 39). Only one study compared the success rate of RET based on the type of scaffold has been founded and it reported the efficacy of all scaffolds is comparable for clinical and radiographical outcomes however, positive response to cold was the highest with the PRF, followed by the collagen, hydroxyapatite and blood scaffolds (41). The findings of this review suggested that blood clot alone or in combination with growth factors were effective scaffolds. Furthermore, using blood scaffolds could be more practical and requires no chair-side time and effort in term of growth factors preparation.
Follow-up time is a fundamental factor in clinical studies as the degree of the success rate of any treatment may change over time (59). The follow-up time in this review varied according to patients' commitment with a minimum period of 6 months (10) and a maximum of 60 months (14). It was stated that most CRCT failures occurred within 3 years of treatment (60), however, RET failure occurred at least 1-2 years from initiation of treatment (61). This is in an agreement with the recommended follow-up period for RET in immature teeth by the American association of endodontic (7).
The secondary outcome of RET assessed in the current review is regaining the pulp sensibility/vitality. In the current review, approximately 50% of the cases have a positive response to the sensibility test. This is in accordance with the percentage of a positive response in immature teeth (62). Sensibility tests are not directly related to the pulp vitality but it depends on subjective response to an external stimulus to the nervous system (63). Some histological studies reported that the vital regenerated tissues in immature teeth with apical periodontitis treated by RET were cementum-like or bone-like tissues (64, 65). Alternatively, the researchers have confirmed the presence of vascularized pulp-like tissue in the mature tooth after RET by using doppler laser flowmetry (DLF) which is the best marker assessing pulp

Appendix 1

Search strategies for regenerative endodontic treatment for permanent mature teeth with pulp necrosis

((((((((((((((((((((((Mature permanent teeth) OR (Mature permanent tooth)) OR (mature teeth)) OR (mature tooth)) OR (mature necrotic teeth)) OR (mature necrotic tooth)) OR (mature non-vital teeth)) OR (mature non-vital tooth)) OR (apical periodontitis)) OR (periapical lesion)) OR (apical lesion)) OR (closed apex)) OR (closed apices)) AND (Endodontic regeneration)) OR (regenerative endodontics)) OR (pulp regeneration)) OR (pulp revascularization)) OR (pulp revitalization)) OR (regenerative endodontic therapies) OR (regenerative endodontics procedures) OR (tooth revascularization) OR (non-obturation endodontic treatment) AND (Endodontics) OR (Root Canal Therapy).

vitality through evaluating the vascular supply (39).

The findings of this review suggested that the positive response the to pulp sensibility test following RET could indicate the presence of a vital tissue (14) which is not necessary to be a pulp tissue (54). The negative response of pulp sensibility does not necessarily indicate a lack of vitality as it could be a sequence of false-negative and/or the deep extension of coronal barrier material into the root coronal portion (33). To the best of our knowledge, no histologic findings in mature teeth with necrotic pulp after RET have been reported yet and more evidence are needed to verify the type of tissue formed.

High heterogeneity between studies, the use of different treatment protocols, short follow-up periods, and lack of data in some included studies were among the limitations of this review. However, the findings of this review can be beneficial for guiding researchers and clinicians to explore a new approach for root canal treatment of permanent mature teeth and do more research on it. To sum up, more RCTs that have similar treatment protocol and case selection criteria with large sample size and long-term follow-ups comparing RET and CRCT had better to be established. This could increase the level of evidence that assesses both practitioners and patients to make treatment selection decisions.

Conclusions

With the limitation of this review, it appears that the adopted protocol of RET is comparable to CRCT and could be a potential approach to treat mature teeth with pulp necrosis and/or apical periodontitis. However, providing more evidence is essential to ascertain these findings.

Clinical Relevance

RET has a satisfactory clinical and radiographical outcome in necrotic pulp mature teeth with or without apical periodontitis however, the selection of the case to be treated should be based on solid evidence and agreement of the patient.

Conflict of Interest

The authors deny any conflicts of interest related to this study.

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