Regenerative Endodontic Procedures for the treatment of necrotic mature teeth with apical periodontitis. A systematic review and meta-analysis of randomized controlled trials.

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Abstract

Introduction: Regenerative endodontic procedures (REPs) are intended to repair and regenerate part of the pulp-dentin complex. The aim of this study was to systematically appraise existing evidence on the effectiveness of REPs on mature teeth with pulp necrosis and apical periodontitis.

Methods: Electronic database and hand searches were implemented on 8 databases of published and unpublished literature from inception to January 3, 2021, for the identification of randomized controlled trials (RCTs) or prospective clinical trials. Related keywords included: "regenerative", "pulp revascularization", "revitalization procedure", "necrotic mature teeth". Random effects meta-analysis was conducted assessing as main outcome treatment success. Risk of bias was assessed through the Cochrane RoB 2.0 tool and the quality of the evidence was assessed with the Grading of Recommendations Assessment, Development and Evaluation (GRADE).

Results: Of 337 initial hits, four RCTs were eligible for inclusion, while 3 were included in the quantitative synthesis. Overall, there was no difference in the relative risk (RR) for successful/ unsuccessful treatment outcome between either REPs or conventional treatment (3 studies, RR= 1.03; 95% Confidence Interval: 0.92, 1.15; p=0.61; heterogeneity I-squared: 0.0%, p=0.53; Prediction Interval: 0.51, 2.09). Risk of bias ranged from low to raising some concerns, while the quality of the evidence was graded as moderate.

Conclusions: Based on moderate quality evidence, REPs appear as a viable treatment alternative for mature necrotic teeth with periapical lesions, at present. Further, well-designed RCTs might also provide confirmatory evidence in this respect, while also frame a backbone for standardization of the therapeutic protocol of REPs.

Keywords: Regenerative endodontic procedures, necrotic mature teeth, apical periodontitis, conventional endodontic treatment, meta-analysis

Introduction

Regenerative endodontic procedures (REPs) are defined as biologically based procedures designed and performed to repair or replace damaged tooth structures and to regenerate part of the pulp-dentin complex[1]. Different terms have been used across the years to describe this procedure, such as regeneration, pulp revascularization and revitalization. Several studies have also shown that REPs may achieve favorable outcomes leading to resolution of signs and symptoms and/or demonstrating complete healing of periapical tissues, root canal walls thickening and continuation of root maturation, coupled with apical closure. This holds true frequently in combination with regaining pulp connective tissue vitality[2-4].

During the last 20 years, REPs have been carried out more frequently, mainly as therapeutic procedures for immature teeth with pulp necrosis and apical periodontitis. In 2011, researchers revealed that the induction of bleeding from the apical area within a disinfected root canal system results in the stimulation of a considerable amount of stem cells[5]. In this respect, tissue engineering strategies are currently being investigated and seem promising as future adjunct tools for clinical practice. These involve the assessment of scaffolds, growth factors and/or collected stem cells for the regeneration of the pulp-dentin complex[6]. Specifically, scaffolds such as PRP (platelet rich plasma)[7,8], gelatin hydrogel[9] and platelet fibrin[10] have been examined and proposed for use during REPs.

The advent of these therapeutic perspectives has further elucidated the potential of REPs to be used as alternative perspectives in the treatment of mature teeth with pulp necrosis and apical periodontitis. In essence, the initial idea about the precursors of REPs belongs to Nygaard-Østby back in 1961, who had found that blood clotting has a good potential to act as root canal filling in necrotic mature teeth, where the size of the apical foramen was intentionally increased[11]. In recent years, a number of clinical reports have been published related to this therapeutic initiative, suggesting that REPs might be a useful alternative approach compared to conventional root canal treatment; also providing advanced biological properties. So far, there are indications that REPs might have a favorable outcome compared to traditional methods, when applied to mature permanent teeth with closed and fully formed apex. Individual treated cases of mature teeth using a regenerative approach have shown that a favorable outcome may be feasible in terms of resolution of signs and symptoms and healing of apical periodontitis[12-15]. In addition, in a recent histologic study, Arslan et al. (2019) showed that ingrowth of a vital tissue within the root canal system is feasible following REPs in mature teeth[16]. The identified tissue was a combination of fibrous connective and bone-like substance coupled with some vascular-like structures. This finding was innovative as it revealed for the first time the potential of REPs in mature teeth, to produce tissue components and structures that resemble the ones

identified within the root canals of immature teeth[17,18]. It has also been supported that this tissue may contribute to the reestablishment of innate immune system which could control root canal system reinfection[19]. Subsequently, a number of clinical trials have been performed indicating that REPs may gradually evolve as a viable treatment option in mature teeth with pulp necrosis and apical periodontitis [20-23].

To our knowledge and until now, there has been no systematic approach to collectively appraise the existing evidence from clinical trials on the effectiveness of REPs in mature teeth with pre-existing pulp necrosis and inflammation of periapical tissues. In addition, given the level of evidence identified, the rationale and transparent indications for future research shall be more adequately framed.

Therefore, the aim of the present study was to systematically review and appraise contemporary literature in this respect, both qualitatively and quantitatively, and also identify the dynamics of REPs to be established as a therapeutic procedure alternative to conventional treatment in clinical practice, framed under the aforementioned settings. The null hypothesis is that there is no difference in the success rate of REPs compared to conventional treatment approaches in mature permanent teeth with pulp necrosis and apical periodontitis.

Methodology

Protocol and Reporting

The protocol of the present systematic review was developed *a priori* and after implementation of the search strategy was registered in the Open Science Framework (https://osf.io/5xp7c/). Reporting for the review follows the PRISMA guidelines[24].

Search strategy

Electronic search was conducted in April 24, 2020 and updated in January 3, 2021, within published and unpublished literature, separately and by two examiners, without date restrictions. The main formal databases were the MEDLINE via PubMed, the Embase, the Web of Science Core Collection, the Cochrane Central, and the Cochrane Database for Systematic Reviews. Unpublished reports were sought through clinicaltrials.gov (U.S National Library of Medicine), Open Grey, and ISRCTN registry. Hand searching was conducted in the retrieved for full-text evaluation articles in order to identify any additional potentially eligible for inclusion publication. Full search strategy for MEDLINE via PubMed is presented in **Appendix 1.**

Eligibility criteria

Study design: randomized controlled trials (RCTs), prospective clinical trials.

Participants: all patients undergoing endodontic treatment in mature teeth. No age or gender restriction.

Intervention: REPs (any), as defined by the authors of the primary studies.

Comparators: conventional non- surgical root canal treatment (NSRCT), or any other type of REPs.

Outcome: treatment success based on clinical and/or radiographic criteria [no clinical symptoms/ decrease or elimination of lesions], response/sensitivity to thermal/electric pulp test (EPT).

Exclusion criteria: case reports/series. Studies without at least one control and one test group, studies including previously treated patients/teeth, as well as studies with ineligible results for this review were also excluded.

Study selection

Titles and abstracts of initially retrieved articles were screened independently by 2 reviewers. The full text of potentially eligible articles was examined in a second stage, by both reviewers and ultimate inclusion of articles was based on consensus, after consultation with a third author if discrepancies were identified throughout the process.

Data collection process

Data were extracted and recorded in standardized piloted forms. Specific characteristics of each study and information on study design, title, authors, date, population, interventions, comparators and outcomes were listed. Data were extracted by two reviewers and re-examined by a third. Inconsistencies were identified and settled after discussion and until a consensus was reached.

Risk of bias in individual studies

Risk of bias assessment for each study was aligned on study design. For RCTs, the Cochrane RoB tool 2.0 was used[25]. This tool includes a five- domain evaluation of the risk of bias. In essence, it is based on bias arising from: 1. Randomization process, 2. Deviations of patients from the intended interventions, 3. Missing outcome data, 4. Measurement of the outcome, and 5. Selection of the reported result. Each domain comprises of a number of detailed items, which guide the investigators/ reviewers through the evaluation process and a final rating of "low", "some concerns", and "high risk" of bias may be reached. Specifically, a study is rated as "low" risk of bias when all domains are low risk of bias, as "some concerns", when at least one domain is rated as such, and "high" risk of bias when it is judged as "high" risk of bias in at least one domain, or as raising "some concerns" for

multiple domains that would contribute to loss of confidence in the results. Further information on the specific items is provided in the **Appendix Table 1**.

Summary measures and data synthesis (primary analyses)

Quantitative synthesis of the studies' findings was performed, after exploration of heterogeneity levels, both clinically and statistically, across individual reports[26]. Random effects meta-analysis was conducted in view of the heterogeneity anticipated. Pooled estimates were presented if 2 or more studies were deemed eligible for a single comparison. Estimates were presented as risk ratios with corresponding confidence bounds (95% Confidence Intervals) in view of the nature of the anticipated outcomes. More specifically, treatment success and response to (electrical) stimuli were considered. Prediction Intervals (95% PIs) were also calculated. Authors were contacted for additional data request, in case not fully reported within the published document. All analyses were performed with Stata version 15.1 software (Stata Corporation, College Station, Tex, USA).

Risk of Bias across studies

Publication bias was explored through standard funnel plots and Egger's regression test, if 10 or more studies were included in the quantitative synthesis[27].

Additional analyses

Sensitivity analyses were considered, if applicable, to explore and isolate the effect of studies with high risk of bias on the overall effect. In addition, sensitivity analyses were considered if variability in population characteristics was identified across studies (*i.e.*, multiple age groups).

Assessment of the Quality of the Evidence

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) was performed to assess the overall quality of the evidence as formulated by the research question, interventions, comparators and outcomes for evaluation[28]. According to GRADE, the overall body of evidence is rated as high, moderate, low and very low. Assessment is made on the following the domains: risk of bias, inconsistency, indirectness, imprecision and publication bias. For RCTs, the first 4 domains of the quality of evidence may be downgraded on the basis of either 'serious' or 'very serious' risks (1 or 2 levels respectively); publication bias may either be suspected or undetected (1 level-downgrade).

Results

Search details

Eligibility and selection of studies for qualitative and quantitative synthesis is presented in Figure 1. From an initial number of 337 results, 13 articles passed through full-text assessment process and ultimately 4 were deemed eligible for inclusion in the qualitative synthesis[20-23]. Three of these qualified for the meta-analysis[20,21,23]. The list of excluded studies after full text assessment is presented in **Appendix 2**. Reasons for exclusion are outlined in **Figure 1**.

Study design and characteristics

A detailed presentation of the characteristics of the included studies appears in **Table 1**. All four studies were randomized controlled trials, of 2-arm parallel design. All were conducted in University settings, while spanning in different locations, namely, Turkey, Chile, Egypt and India. Total number of patients reported per study was within the range of 18 to 46, with the same number of contributing teeth. All assessed teeth were permanent necrotic mature teeth of any type and periapical lesions, while El-Kateb *et al.*[22] included solely maxillary anterior central and lateral incisors. All but one study included adult patients[17]. Jha *et al.*[23], included patients within the age range of 9 to 15 years old, however, all contributing teeth presented with completed root development.

Interventions included REPs with induction of bleeding for blood clot formation and stimulation of stem cells of the apical region[20,22,,23], or use of encapsulated human umbilical cord mesenchymal stem cells in a plasma-derived biomaterial[21]. All but one study[22], reported control groups being treated with conventional non-surgical root canal treatment (NSRCTs) procedures[20,21,23]. The study of El-Kateb *et al.*[22], involved comparison of two REP intervention groups with the use of different maximum rotary instrumentation sizes. All studies involved assessment of successful treatment based on clinical and radiographic procedures as the primary outcome of interest, within 12 to 18 months follow-up examination. In addition, two studies evaluated tooth response to thermal or EPT[20,21] **(Table 1)**.

Ethylene-diamine-tetra-acetic acid (EDTA) was reported as final irrigation solution in all four studies in variable concentrations ranging from 5 to 17%[20-23]. Calcium hydroxide was used as intra-canal medicament either in isolation or in combination with other, in two studies[21,22] whereas a triple antibiotic paste was used for interappointment medication, comprising doxycycline, metronidazole and ciprofloxacin in the other two studies[20,23].

Risk of bias within studies

Overall, risk of bias was rated as low in 3 of the included studies[20-22], while as raising some concerns in the remaining one[23]. Domains pertaining to randomization procedure including allocation concealment, blinding, missing data and pre-registration of a protocol were adequately reported in the three studies. Reporting of randomization was inadequate, while also no *a priori* registered protocol was identified for the study of Jha *et al.*[23] **(Table 2, Appendix Table 1)**.

Effects of Interventions, Meta- Analyses and Additional Analyses

As previously noted, 3 studies contributed to meta-analysis or additional analyses[20,21,23]. Studies reporting on successful treatment outcomes, as well as tooth response to electrical testing were considered eligible for mathematical synthesis.

According to the overall estimate, there was no difference in the relative risk (RR) for successful/ unsuccessful treatment outcome between either REPs or NSRCTs (3 studies, RR= 1.03; 95% CI: 0.92, 1.15; p=0.61; heterogeneity I- squared: 0.0%, p=0.53). Prediction Interval also ranged between a RR of 0.51 and 2.09 illustrating the variability of the true effect in different conditions, studies or settings **(Table 3, Figure 2)**.

As for the response outcome to EPT, there was an increased RR for positive response in the REP group compared to the NSRCT, by 4.31 times (2 studies, RR=4.31; 95%CI: 1.36, 13.62; p=0.01; I-squared: 19.5%, p=0.27) **(Table 3, Figure 3)**.

Sensitivity analysis considered age rage and the results of this analysis are outlined in Table 3. Evidently, when only studies including adult patients were considered the associated RR did not effectively change (2 studies, RR= 1.05; 95% CI: 0.90, 1.22; p=0.54; I- squared: 14.9%, p=0.28) **(Table 3)**.

Publication bias or effect of high risk of bias in individual studies could not be investigated, due to the scarcity of the existing published reports (*i.e.*, <10).

Quality of the evidence

The quality of the existing evidence for successful treatment of teeth, comparing REPs and NSRCTs was moderate overall, mainly because of concerns in one study about the potential for inclusion of risk of bias **(Table 4)**. In terms of the outcome pertaining to the response to EPT, after either REPs or NSRCTs, again the quality of the evidence was identified as moderate, however, the reason for downgrading was imprecision, due to the fact that only two studies contributed to the

syntheses, apparently yielding a relatively wide confidence bound **(Table 4)**. Overall, and for both outcomes, based on GRADE assessment, further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Discussion

Findings in context

The present systematic review and meta-analysis is the first to evaluate whether REPs provide an alternative treatment for mature teeth with necrotic pulp and apical periodontitis. Research in the field has revealed that most of the evaluated teeth indicated for treatment with REPs presented a history and etiologic factor for treatment, of a traumatic dental injury. A relevant review with previously published data, reported that across all the REP cases studied, the aetiology of treatment was due to trauma in 34% of the cases, while dens evaginatus ranked second in terms of prevalence, comprising 23% of the cases[2]. Based on the results of this review we failed to reject the null hypothesis of no difference in success rate of treatment with REPs, compared to traditional endodontic procedures, and our findings depicted considerably high favourable events for regenerative treatment outcomes. However, the findings should be viewed with caution, due to the paucity of well-designed controlled clinical trials in the field, substantiated also by the small number of teeth included across the studies. Nevertheless, the level of consistency of the design, methodology and settings of the eligible trials is considered adequate, and this is confirmed by the low overall levels of heterogeneity identified across the studies. More specifically in the present review, a total of 68 necrotic mature teeth were evaluated regarding the outcome of REPs. There is moderate quality evidence that REPs may offer a viable treatment option in the management of mature teeth with pulp necrosis and apical periodontitis. An additional important finding was the increased possibility of positive response to electric pulp testing with REPs. Despite the fact that no untreated control teeth were used by the included studies to compare the positive reactions, it may be inferred that this factor may constitute a strong predictor for the positive outcome of REPs.

In all included studies, teeth were diagnosed as non-vital with established periapical pathology. The presence of apical periodontitis creates a challenge for the disinfection of the root canal system and the outcome may be less predictable. A review back in 2012, based on outcomes from immature teeth, revealed a link between the duration of time from the establishment of pulp necrosis and the prognosis of REPs[29]. The need to maintain the level of disinfection in high quality standards during and after the regenerative treatment, has evidently been considered a key factor, and if adequately handled, most likely improving the outcome and prognosis of the procedure[30-33]. The use of high

concentration irrigants and the application of intra-canal and inter-appointment medicament has been identified as the most successful approach to achieve complete disinfection, however biocompatibility considerations should be taken into account. In all studies included in the present review, a two-step disinfection protocol was performed which did not significantly differ between each other. Sodium hypochlorite (NaOCl) was the main irrigation solution with a range of concentrations used between 1-2.5%. The use of a reduced concentration solution shows that all studies tried to follow a low toxicity disinfection protocol in order to protect any vital remnants of stem cells of the apical papilla. In addition to reduced concentrations of NaOCl, EDTA was also used as final irrigation solution, in all four studies[20-23]. Its importance lays in the advantage EDTA offers when used as a final rinse solution, by increasing the survival ability and expression of the stem cells of the apical papilla. It further neutralises the negative properties of sodium hypochlorite and releases growth factors trapped within the dentin matrix, which are considered to play a significant role in the differentiation and metabolism of the cells[34]. Coupled with cytokines, growth factors are also known to control the haemostasis, inflammation, and maturation during wound healing[34-36]. Galler *et al.* has also showed that EDTA effect on dentine promotes the migration, differentiation and adhesion of the dental pulp stem cells (DPSC)[37]. Thus, EDTA has been widely adopted by a number of researchers and clinicians as final rinse before the induction of bleeding and blood clot formation.

In all assessed studies, no differences were observed in terms of treatment outcomes, based on inter-appointment medicament used. This observation is in agreement with the results of relevant studies and international recommendations about the use of calcium hydroxide and triple antibiotic paste during REPs[38]. Based also on the results of the present review, two of the included studies used a calcium silicate material (Biodentine) above the blood clot[21,22], whereas the third one used a commercial brand of MTA[20]. Several studies have shown that the use of these materials may induce favourable outcomes during REPs [14,15,22,39,]. All four studies reported induction of bleeding from the periapical tissues to form an adequate blood clot. This procedure seems to perform well as it has been verified by several published studies. The results of a recent review about platelet concentrations in REPs in immature teeth, showed no statistically significant differences between blood clot and platelet-rich plasma (PRP) or platelet-rich fibrin (PRF) used as potential scaffolds during REPs, for the promotion of canal wall thickening and the sustained development of the root[40]. Accordingly, Zhou *et al.* compared the results of a combination technique with both PRF and blood clot, versus blood clot alone, and did not report any differences in the outcome [41].

The use of mostly single rooted teeth except the study by Jha *et al.*[23] might potentially constitute a significant drawback for the establishment of the REPs as an adequate alternative treatment option in cases of mature necrotic teeth with apical periodontitis. However, this is not uncommon, as it has

been reported also in cases of immature necrotic teeth with apical periodontitis. According to the results of a recent review, only 46 immature posterior teeth (molars) have been reported to be treated using REPs, based on up- to- date published data[42]. Evidently, there is still a long way for the complete establishment and acceptance of REPs as an adequate treatment option in all categories of teeth (anterior-posterior), presented with pulp necrosis and apical periodontitis.

In the same line, the width of the apical foramen has been considered as a potentially significant predictor for the successful outcome of the REPs, although this could not be investigated by the present meta-analysis, as it did not constitute an independent risk factor investigated by the primary studies. A number of studies have shown that sizes of apical foramen even smaller than 1mm could re-vascularize successfully[43], or a positive outcome might be established, when the size of apical foramen ranged between 0.5 and 1 mm[44]. The latter might also help avoid over-instrumentation of root canals, thus jeopardizing the structural integrity of teeth[20]. The number of visits needed for treatment completion is not unanimously agreed and accepted among the clinicians and researchers. However, reports have emerged in the literature, comprising single-visit REPs, with indeed positive outcomes[45-47].

In conclusion, REPs appear as a viable treatment option for clinicians, when teeth with pulp necrosis and apical foramen with diameter size up to 1 mm are to be endodontically managed. In case these techniques are considered non-eligible or fail, conventional endodontic procedures can be always carried out as an alternative. The re-access and treatment using MTA apical plug may be subsequently considered the therapeutic approach of choice, especially when a persistent infection has been established.

Strengths and Limitations

The present study was the first systematic review including a quantitative synthesis, which comprehensively assessed the body of evidence regarding regenerative treatment approaches to mature teeth. We implemented a rigorous methodology in terms of design, conduct and reporting, following respective guidelines. Eight databases were searched for relevant up to date primary clinical trials, an *a priori* protocol[48] was drafted after initial search implementation, while pooled estimates were presented to achieve the desired precision in our findings. An assessment of the risk of bias as well as the quality of the evidence stemming from the investigated interventions was applied. In essence, risk of bias of the included studies is expected to raise some concerns regarding the internal validity of the included available studies and evidence, albeit only one study was suboptimal with regard to risk of bias domains; thus, some impact on the confidence of the conclusions of this review cannot be precluded. The identified and eligible reports showed evidence of clear and transparent methodological

output, following currently available reporting guidelines, overall[49]. In this respect, and following considerations about risk of bias and quality of the evidence for the outcomes under study, it is expected that further studies are developed, framed under the same or improved standards of conduct and reporting. Concerns were raised for a sole study with regard to randomization scheme, where no allocation concealment was described and potential baseline differences or effects due to lack of concealment might have posed a problem. Lack of protocol registration in this study did not allow for any comparisons with the reported results to be conducted. In addition, future attempts should be directed at serving as confirmatory research based on similar core outcomes, interventions and settings with the identified studies. Future studies should be designed, accounting for more prolonged evaluation periods and follow- up timing for the teeth under investigation. Further insights within the investigation of the use of encapsulated cells, and PRP or PRF scaffolds for REPs might also prove beneficial biological vehicles. The generalizability of the study results spans on teeth with complete root development, mostly single- rooted and in adult patients, while treatment procedures were monitored under university settings.

The present review is not free of limitations. Not all pre-defined methodological outputs were finally implemented, due to the paucity of the existing studies in the field. In essence, publication bias could not be examined as less than 10 studies contributed to the meta-analysis, while likewise, not all pre-defined sensitivity analyses were performed. Nevertheless, our findings demonstrate the current state of the art with regard to regenerative endodontic procedures for mature teeth.

Conclusions

REPs present a favourable outcome comparable to conventional endodontic procedures in necrotic mature teeth. Long standing debates in endodontic community on the limitations of apical preparation, apical size and its control, obturation materials, effect of heating and more, might have no place in this relatively new treatment procedure. Endodontists and researchers are urged to focus their research and clinical observation practices on the exploration of the biological base of this novel approach, in order to determine a standardised therapeutic protocol leading to a more predictable treatment outcome. The need for further clinical studies in the field remains imperative.

References

1. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod 2007;33:377–90.
2. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Endod Topics 2013;28:2–23.
3. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative Endodontic Therapy: A Data Analysis of Clinical Protocols. J Endod 2015;41:146–54.
4. Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: Biological basis of regenerative endodontic procedures. Pediatr Dent 2013;35:129–40.
5. Lovelace TW, Henry MA, Hargreaves KM, Diogenes A. Evaluation of the Delivery of Mesenchymal Stem Cells into the Root Canal Space of Necrotic Immature Teeth after Clinical Regenerative Endodontic Procedure. J Endod 2011;37:133–8.
6. Albuquerque MTP, Valera MC, Nakashima M, Nör JE, Bottino MC. Tissue-engineering-based strategies for regenerative endodontics. J Dent Res 2014;93:1222–31.
7. Sachdeva GS, Sachdeva LT, Goel M, Bala S. Regenerative endodontic treatment of an immature tooth with a necrotic pulp and apical periodontitis using platelet-rich plasma (PRP) and mineral trioxide aggregate (MTA): a case report. Int Endod J 2015;48:902–10.
8. Torabinejad M, Turman M. Revitalization of Tooth with Necrotic Pulp and Open Apex by Using Platelet-rich Plasma: A Case Report. J Endod 2011;37:265–8.
9. Nagy MM, Tawfik HE, Hashem AAR, Abu-Seida AM. Regenerative Potential of Immature Permanent Teeth with Necrotic Pulps after Different Regenerative Protocols. J Endod 2014;40:192–8.
10. Shivashankar VY, Johns DA, Vidyanath S, Ramesh Kumar M. Platelet Rich Fibrin in the revitalization of tooth with necrotic pulp and open apex. J Conserv Dent 2012;15:395–8.
11. Östby BN. The role of the blood clot in endodontic therapy an experimental histologic study. Acta Odontol Scand 1961;19:323–53.
12. Shah N, Logani A. SealBio: A novel, non-obturation endodontic treatment based on concept of regeneration. J Conserv Dent 2012;15:328-32.
13. Paryani K, Kim SG. Regenerative endodontic treatment of permanent teeth after completion of root development: a report of 2 cases. J Endod 2013;39:929-34.
14. Saoud TMA, Sigurdsson A, Rosenberg PA, Lin LM, Ricucci D. Treatment of a large cystlike inflammatory periapical lesion associated with mature necrotic teeth using regenerative endodontic therapy. J Endod 2014;40:2081–6.

15. Saoud TM, Martin G, Chen Y-HM, Chen K-L, Chen C-A, Songtrakul K, et al. Treatment of Mature Permanent Teeth with Necrotic Pulps and Apical Periodontitis Using Regenerative Endodontic Procedures: A Case Series. J Endod 2016;42:57–65.
16. Arslan H, Şahin Y, Topçuoğlu HS, Gündoğdu B. Histologic Evaluation of Regenerated Tissues in the Pulp Spaces of Teeth with Mature Roots at the Time of the Regenerative Endodontic Procedures. J Endod 2019;45:1384-89.
17. Martin G, Ricucci D, Gibbs JL, Lin LM. Histological findings of revascularized/revitalized immature permanent molar with apical periodontitis using platelet-rich plasma. J Endod 2013;39:138-44.
18. Shimizu E, Ricucci D, Albert J, Alobaid AS, Gibbs JL, Huang GT, Lin LM. Clinical, radiographic, and histological observation of a human immature permanent tooth with chronic apical abscess after revitalization treatment. J Endod 2013;39:1078-83.
19. He L, Kim SG, Gong Q, Zhong J, Wang S, Zhou X, Ye L, Ling J, Mao JJ. Regenerative Endodontics for Adult Patients. J Endod 2017;43:S57-S64.
20. Arslan H, Ahmed HMA, Şahin Y, Doğanay Yıldız E, Gündoğdu EC, Güven Y, et al. Regenerative Endodontic Procedures in Necrotic Mature Teeth with Periapical Radiolucencies: A Preliminary Randomized Clinical Study. J Endod 2019;45:863–72.
21. Brizuela C, Meza G, Urrejola D, Quezada MA, Concha G, Ramírez V, et al. Cell-Based Regenerative Endodontics for Treatment of Periapical Lesions: A Randomized, Controlled Phase I/II Clinical Trial. J Dent Res. 2020;99:523–9.
22. El-Kateb NM, El-Backly RN, Amin WM, Abdalla AM. Quantitative Assessment of Intracanal Regenerated Tissues after Regenerative Endodontic Procedures in Mature Teeth Using Magnetic Resonance Imaging: A Randomized Controlled Clinical Trial. J Endod 2020;46:563-574.
23. Jha P, Virdi MS, Nain S. A Regenerative Approach for Root Canal Treatment of Mature Permanent Teeth: Comparative Evaluation with 18 Months Follow-up. Int J Clin Pediatr Dent 2019;12:182–8.
24. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. J Clin Epidemiol. 2009;62:1006-1012.
25. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ 2019;366:l4898.
26. Koletsi D, Fleming PS, Michelaki I, Pandis N. Heterogeneity in Cochrane and non-Cochrane meta-analyses in orthodontics. J Dent 2018;74:90–4.

27. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Increase in studies of publication bias coincided with increasing use of meta-analysis. Br Med J 1997;316:629–34.
28. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. An emerging consensus on rating quality of evidence and strength of recommendations. Bmj. 2008;336(7650):924–6.
29. Nosrat A, Homayounfar N, Oloomi K. Drawbacks and Unfavorable Outcomes of Regenerative Endodontic Treatments of Necrotic Immature Teeth: A Literature Review and Report of a Case. J Endod 2012;38:1428–34.
30. Lin A, Joe J. Regenerative Endodontics Versus Apexification In Immature Permanent Teeth With Apical Periodontitis : A Prospective Randomized Controlled Study. 2017;43:2017.
31. Chaniotis A. Treatment Options for Failing Regenerative Endodontic Procedures: Report of 3 Cases. J Endod 2017;43:1472–8.
32. Lin LM, Shimizu E, Gibbs JL, Loghin S, Ricucci D. Histologic and histobacteriologic observations of failed revascularization/revitalization therapy: a case report. J Endod 2014;40:291–5.
33. Zizka R, Buchta T, Voborná I, Harvan L, Šedý J. Root Maturation in Teeth Treated by Unsuccessful Revitalization: 2 Case Reports. J Endod 2016;42:724–9.
34. Steed DL. The role of growth factors in wound healing. Surg Clin North Am. 1997;77:575–86.
35. Werner S, Grose R. Regulation of Wound Healing by Growth Factors and Cytokines. Physiol Rev 2003;83:835–70.
36. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 2008;16:585–601.
37. Galler KM, Widbiller M, Buchalla W, Eidt A, Hiller K-A, Hoffer PC, et al. EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. Int Endod J 2016;49:581–90.
38. AAE org. Considerations for a Regenerative Endodontic Procedure, 2013. Accessed 07/01/2021
39. Nagas E, Uyanik MO, Cehreli ZC. Revitalization of necrotic mature permanent incisors with apical periodontitis: a case report. Restor Dent Endod 2018;43:e31.
40. Del Fabbro M, Lolato A, Bucchi C, Taschieri S, Weinstein RL. Autologous Platelet Concentrates for Pulp and Dentin Regeneration: A Literature Review of Animal Studies. J Endod 2016;42:250–7.
41. Zhou R, Wang Y, Chen Y, Chen S, Lyu H, Cai Z, et al. Radiographic, Histologic, and

Biomechanical Evaluation of Combined Application of Platelet-rich Fibrin with Blood Clot in Regenerative Endodontics. J Endod 2017;43:2034–40.

42. Tzanetakis GN, Giannakoulas DG, Papanakou S, Gizani S, Lygidakis N. Regenerative endodontic therapy of immature permanent molars with pulp necrosis: a cases series and a literature review. Eur Arch Paediatr Dent 2020 Epub ahead of print.
43. Laureys WGM, Cuvelier CA, Dermaut LR, De Pauw GAM. The critical apical diameter to obtain regeneration of the pulp tissue after tooth transplantation, replantation, or regenerative endodontic treatment. J Endod 2013;39:759–63.
44. Fang Y, Wang X, Zhu J, Su C, Yang Y, Meng L. Influence of apical diameter on the outcome of regenerative endodontic treatment in teeth with pulp necrosis: a review. J Endod 2018;44:414–31.
45. Topçuoğlu G, Topçuoğlu HS. Regenerative Endodontic Therapy in a Single Visit Using Platelet-rich Plasma and Biodentine in Necrotic and Asymptomatic Immature Molar Teeth: A Report of 3 Cases. J Endod 2016;42:1344–6.
46. Shin SY, Albert JS, Mortman RE. One step pulp revascularization treatment of an immature permanent tooth with chronic apical abscess: a case report. Int Endod 2009;42:1118–26.
47. McCabe P. Revascularization of an immature tooth with apical periodontitis using a single visit protocol: a case report. Int Endod J 2015;48:484–97.
48. Koufatzidou M, Koletsi D, Fleming PS, Polychronopoulou A, Pandis N. Outcome reporting discrepancies between trial entries and published final reports of orthodontic randomized controlled trials. Eur J Orthod 2019;41:225–30.
49. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, Elbourne D, Egger M, Altman DG. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c869.

Figure Legends

Figure 1. PRISMA flow diagram of study screening procedure and selection

Figure 2. Random effects meta-analysis for successful treatment outcome, comparing regenerative endodontic procedures (REPs), and conventional non- surgical root canal treatment (NSRCT).

Figure 3. Random effects meta-analysis for positive response to electrical stimuli, comparing regenerative endodontic procedures (REPs), and conventional non- surgical root canal treatment (NSRCT).

Table 1. Characteristics of included randomized controlled trials, based on the described populations, interventions, comparisons, and outcomes.

Study	Population	Intervention	Comparison	Outcome	Notes
Arslan et al., 2019[17] [Turkey]	46 patients (11 female, 35 male; REP age, 20.58, ±2.53; NSRCT age, 20.66±1.27), 46 teeth of any type (mature, necrotic with apical lesions)	REP [triple antibiotic paste and EDTA 5%/ bleeding induction for blood clot formation]	NSRCT [calcium hydroxide and EDTA 5%/gutta-percha cones and epoxy resin–based sealer-cold lateral condensation technique]	1. successful cases [based on clinical and radiographic healing] 2. number of teeth positive to EPT	Clinical & radiographic assessment up to 12 mo follow-up
Brizuela et al., 2020[18] [Chile]	36 patients (25 female, 11 male; age range overall, 16-58), 36 teeth of any type (mature with apical lesions)	REP [calcium hydroxide/ encapsulated human umbilical cord mesenchymal stem cells in a plasma-derived biomaterial]	NSRCT [calcium hydroxide/gutta-percha cones+Topseal sealer-continuous wave condensation technique]	1. successful cases [based on clinical and radiographic healing] 2. response to thermal and EPT	Clinical & radiographic assessment at 12 mo follow-up
El- Kateb et al, 2020[19] [Egypt]	18 patients (11 female, 7 male; age, 25.5, range: 20- 34), 18 teeth (necrotic mature maxillary anterior with apical lesions)	REP [calcium hydroxide and EDTA 17%/ bleeding induction for blood clot formation and mix with Biodentine] with rotary instrumentation till size X3	REP [calcium hydroxide and EDTA 17%/ bleeding induction for blood clot formation and mix with Biodentine] with rotary instrumentation till size X5	1. Signal intensity through MRI 2. clinical and radiographic healing	Clinical & radiographic assessment at 12 mo follow-up
Jha et al, 2019[20] [India]	30 patients (age range 9- 15); 30 teeth of any type (permanent mature with apical lesions)	REP [EDTA 17%/ SealBio technique, stimulating stem cells of the region and calcium sulfate-based cement]	NSRCT [gutta-percha cones-cold lateral condensation technique]	1.successful healing (combined radiographic and clinical criteria)	Clinical & radiographic assessment at 18 mo follow-up

EPT, electric pulp testing; EDTA, ethylenediaminetetraacetic acid; mo, months; MRI, magnetic resonance imaging; NSRCT, non- surgical root canal treatment; PA, periapical; REP, regenerative endodontic procedure

Table 2. Risk of bias (RoB) assessment of the included Randomized Controlled Trials (RCTs), according to the Cochrane RoB 2.0 tool.

Study	Randomization	Deviations from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall
Arslan et al, 2019	Low	Low	Low	Low	Low	Low
Brizuela et al., 2020	Low	Low	Low	Low	Low	Low
El- Kateb et al., 2020	Low	Low	Low	Low	Low	Low
Jha et al., 2019	Some Concerns	Low	Low	Low	Some Concerns	Some Concerns

Table 3. Results of meta-analyses [outcome: overall success; positive response to electrical stimuli] and sensitivity analyses [outcome: overall success in adult patients].

Synthesis	No. Studies	Risk Ratio	95% CIs	p- value	I^2 (%)	Tau- squared
Overall success	3	1.03	0.92, 1.15	0.61	0.0	0.0
Positive response to electrical stimuli	2	4.31	1.36, 13.62	0.01	19.5	0.16
Overall success (Age- subgroup[1])	2	1.05	0.90, 1.22	0.54	14.9	0.002

[1] one study including non- adult patients has been excluded

Table 4. Summary of Findings Table for the quality of the evidence according to Grading of Recommendations Assessment, Development and Evaluation (GRADE).

REPs vs NSRCTs for successful treatment and positive stimuli response

Patient or population: patients with necrotic mature teeth with periapical lesions
Settings: university
Intervention: REPs
Comparison: NSRCTs

Outcomes	Illustrative comparative risks* (95% CI) Assumed risk	Corresponding risk	Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
Success of treatment	**Study population**		**RR 1.03** (0.92 to 1.15)	112 (3 studies)	⊕⊕⊕⊖ **moderate**[1]	none
	906 per 1000	**933 per 1000** (833 to 1000)				
Positive response to electrical stimuli	**Study population**		**RR 4.31** (1.36 to 13.62)	82 (2 studies)	⊕⊕⊕⊖ **moderate**[2]	none
	105 per 1000	**454 per 1000** (143 to 1000)				

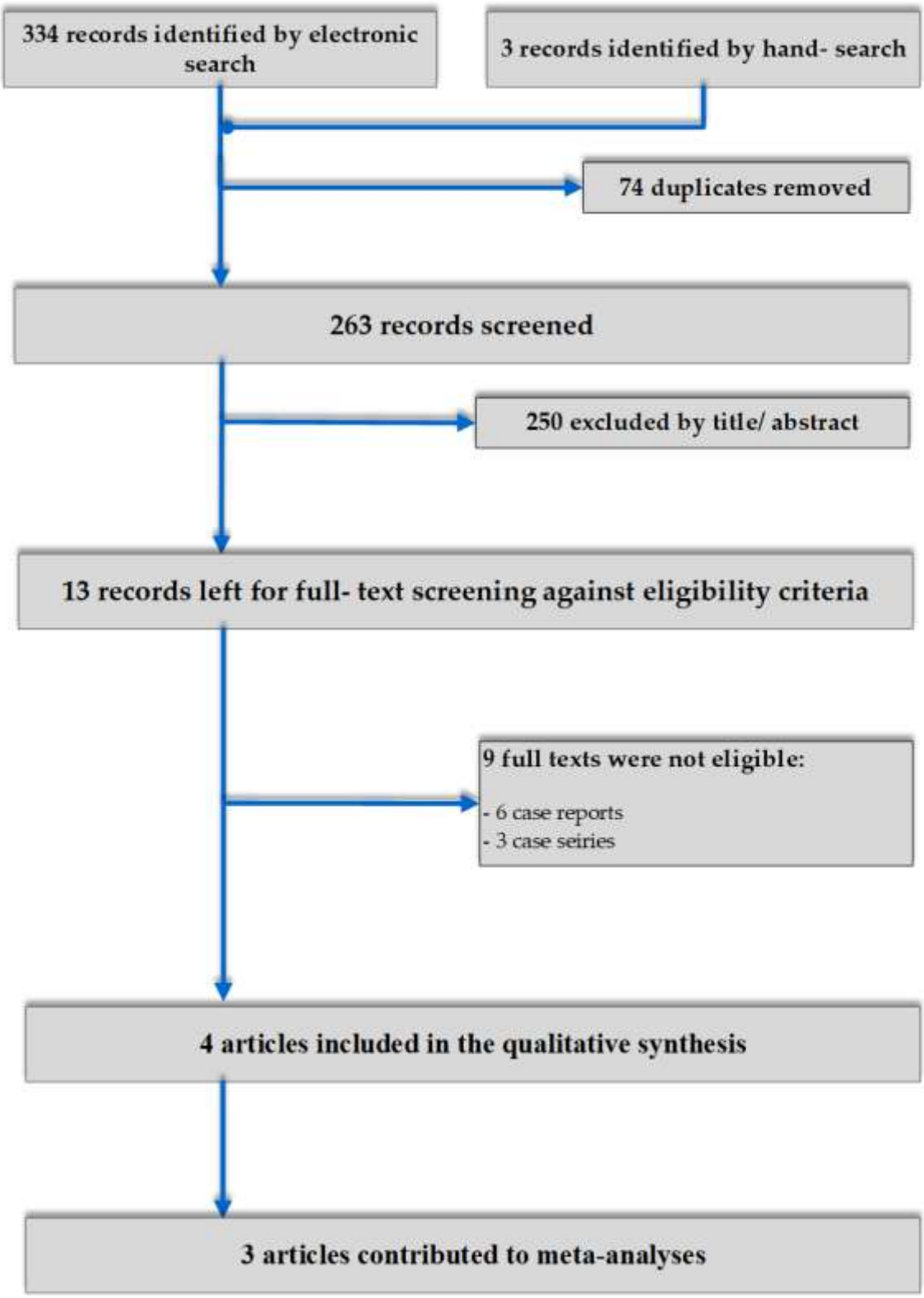
*The basis for the illustrative comparative risks pertains to risk in the control and the intervention group. .**Assumed risk** corresponds to the risk for reaching the outcome in the control group (ie success of treatment and positive response to electrical stimuli). The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the control group plus the **relative effect** of the intervention (and its 95% CI). This is equivalent to the overall risk in the intervention group.

CI: Confidence interval; **NSRCT:** conventional root canal treatment; **REP:** regenerative endodontic procedure; **RR:** Risk ratio

GRADE Working Group grades of evidence [explanation of GRADE ratings]
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] downgraded due to risk of bias (quality of included studies)
[2] downgraded due to small sample size and wide confidence bounds (imprecision)

Figure 1


334 records identified by electronic search
3 records identified by hand- search
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263 records screened
250 excluded by title/ abstract
13 records left for full- text screening against eligibility criteria
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- 6 case reports
- 3 case seiries
4 articles included in the qualitative synthesis
3 articles contributed to meta-analyses

Figure 2

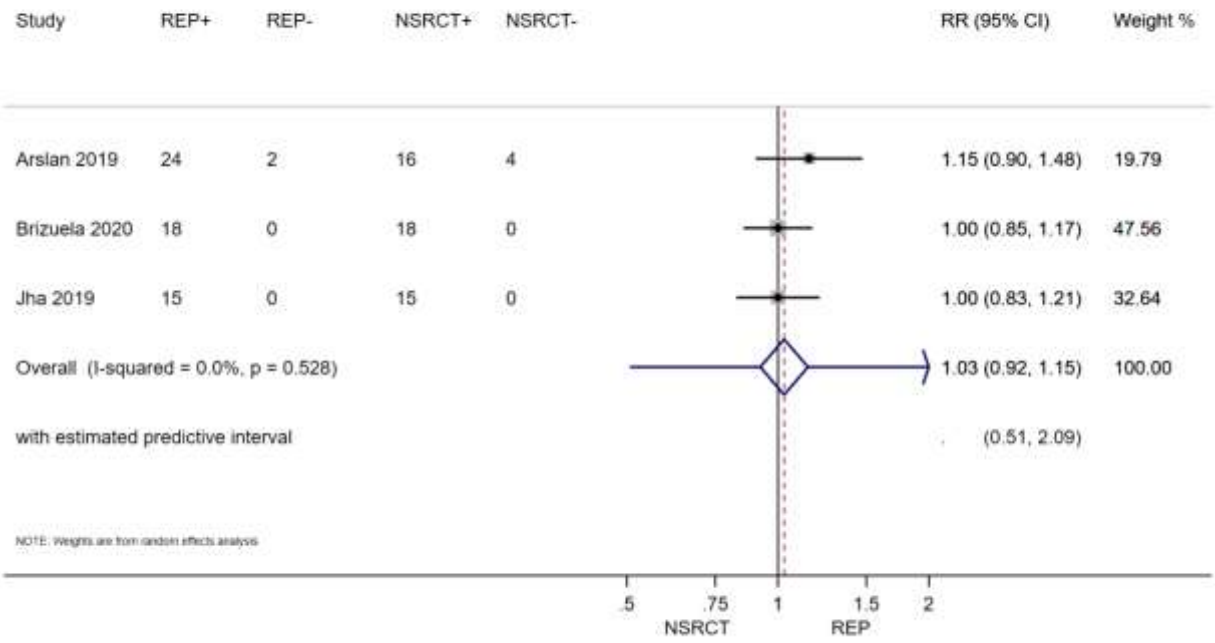
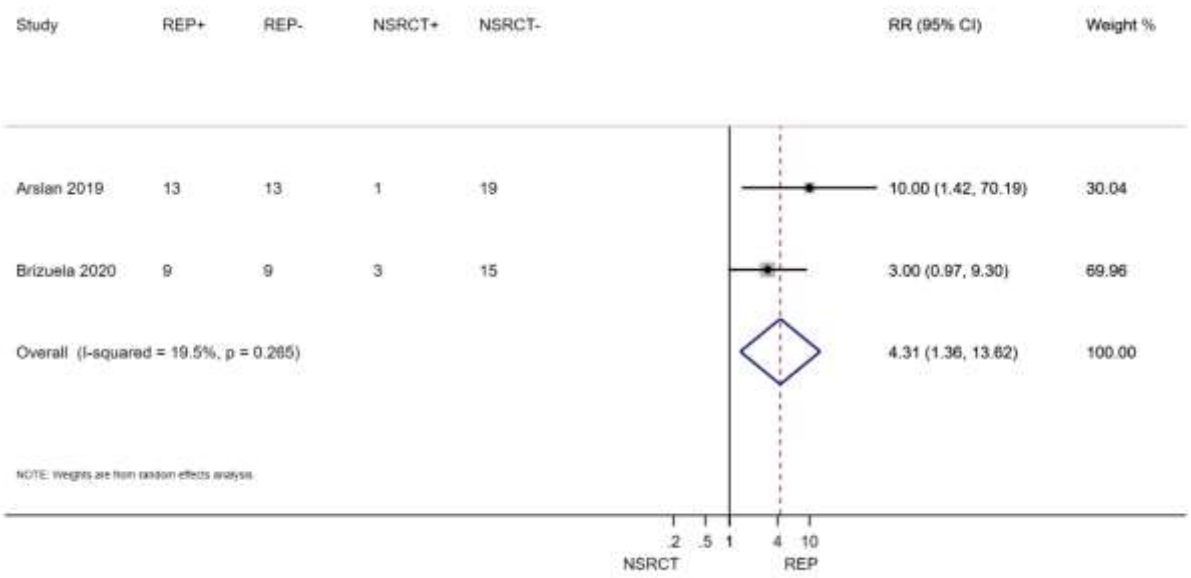

Study
REP+
REP-
NSRCT+
NSRCT-
RR (95% CI)
Weight %
Arslan 2019 24 2 16 4 1.15 (0.90, 1.48) 19.79
Brizuela 2020 18 0 18 0 1.00 (0.85, 1.17) 47.56
Jha 2019 15 0 15 0 1.00 (0.83, 1.21) 32.64
Overall (I-squared = 0.0%, p = 0.528) 1.03 (0.92, 1.15) 100.00
with estimated predictive interval (0.51, 2.09)
NOTE: Weights are from random effects analysis
.5
.75
1
1.5
2
NSRCT
REP

Figure 3


Study
REP+
REP-
NSRCT+
NSRCT-
RR (95% CI)
Weight %
Arslan 2019 13 13 1 19 10.00 (1.42, 70.19) 30.04
Brizuela 2020 9 9 3 15 3.00 (0.97, 9.30) 69.96
Overall (I-squared = 19.5%, p = 0.265) 4.31 (1.36, 13.62) 100.00
NOTE: Weights are from random effects analysis
.2 .5 1 4 10
NSRCT
REP