Original Article

Assessment Of Different Disinfection Protocols On Regenerative Potential Of Mature Permanent Teeth With Necrotic Pulps: A Randomized Double Blinded Clinical Trial

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Abstract

Aim: This preliminary randomized, prospective, controlled trial aimed to compare the radiographic outcomes of three different disinfection protocols in the treatment of mature permanent teeth with necrotic pulps. **Methodology:** The trial has been reported according to the Preferred Reporting Items for Randomized Trials in Endodontics 2021 guidelines. The study protocol was registered at the clinical trial registry (ClinicalTrials.gov) with identifier number NCT05557994. Thirty-six patients with mature necrotic anterior teeth with large periapical lesions were randomly allocated into three groups (n = 12): group A, disinfected with triple antibiotic paste, group B, disinfected with activation of Xp-endo finisher, and group C, disinfected using diode laser. The follow-up was for 12 months. Peri-radicular healing was assessed using standardized radiographs taken at baseline, and at 6 and 12 months after treatment. Statistical analysis was conducted using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction for intergroup comparisons and Freidman's test followed by Nemenyi post hoc test for intragroup comparisons. The significance level was set at $p \leq .05$ within all tests. **Results:** There was a significant increase in peri-radicular healing in all groups at 6 and 12 months, compared to that at baseline ($p < .001$). **Conclusions:** The findings of this preliminary trial indicate the potential for using single visit REPs using diode laser disinfection as a treatment option for mature teeth with necrotic pulps. A higher level of evidence obtained through adequately powered clinical trials and longer follow-up periods are required to conclusively validate the different outcomes of REPs.

Keywords
mature necrotic teeth, diode laser, periapical healing, regenerative endodontic procedures.

Introduction

RCT has been used for many years as a traditional protocol for the treatment of mature permanent teeth with necrotic pulps, this protocol involves chemo-mechanical preparation of the root canal and sealing of the radicular space with a biocompatible material. More recently, regaining pulp vitality in necrotic permeant fully mature teeth using REP has been the subject of controversy among endodontic scientists in an attempt to overcome the drawbacks of RCT such as lack of sensation and immune mechanisms, and susceptibility to root fracture of treated teeth.

Regenerative endodontic procedures have recently been suggested for treating mature permanent teeth with closed and fully formed apices **(Digka et al., 2020; Glynis et al., 2021).** Published case reports of mature teeth treated with REPs have revealed favorable outcomes in the resolution of signs and symptoms and in the healing of apical periodontitis **(Abou Samra et al., 2018; Paryani & Kim, 2013; Saoud, Martin, et al., 2016; Saoud et al., 2014;**

Shah & Logani, 2012). Moreover, in a recent histologic study, Arslan, **Şahin, et al. (2019)** revealed that the ingrowth of vital tissue within the root canal system is achievable after REPs in mature teeth. The identified tissue was a combination of a fibrous connective and bone-like substance combined with vascular-like structures. This finding was innovative because it revealed for the first time the potential of REPs in mature teeth to produce tissue components and structures that resemble those identified within the root canals of immature teeth **(Martin et al., 2013).** Research findings have also suggested that this tissue may contribute to the re-establishment of the innate immune system, which could control root canal system reinfection **(He et al., 2017).** Several studies have subsequently been conducted that indicate that REPs may gradually evolve as a viable treatment option in mature teeth with pulp necrosis and apical periodontitis **(Arslan, Ahmed, et al., 2019; Brizuela et al., 2020; El-Kateb et al., 2020; Jha et al., 2019).**

To promote regeneration, the pulp space and dentinal walls must be sufficiently disinfected before performing pulp regeneration procedures, and the required degree is possibly higher than that in traditional endodontic therapy **(Yang et al.,2016).** Antibiotics appear to be suitable intra-canal medication. Triple antibiotic paste (TAP) consisting of metronidazole, ciprofloxacin and minocycline is considered to be a successful regimen in managing the root canal pathogen of mature permanent teeth with necrotic pulp. Recently, new methods of disinfection have been described to control the limitations of conventional disinfecting methods by either diminishing the bacterial number to an adequate level or eliminating the toxicity to periapical stem/ progenitors. Other concerns regarding the use of TAP include tooth discoloration and bacterial resistance.

Laser is an acronym for light amplification by stimulated emission of radiation. It is classified according to power into low power lasers (LPL) which is also called low intensity lasers (LIL) and low-level laser therapy (LLLT) which is used in bio-modulation, stimulation of healing, decreasing inflammation, decontamination and pain relief. The beneficial effect of (LLLT) on bone was proved by many authors, who observed that the collagen production, osteoblastic activity and increased bone mineral density was promoted by tissue modulation characteristics of (LLLT) **(El Mekkawi et al.,2020).** XP Endo Finisher file (*FKG, La Chaux-de-Fonds, Switzerland)* is a recently introduced irrigant activation system. The instrument has the small core size of #25, no taper, non-cutting design and the alloy properties that enable the file to easily adapt the root canal anatomy by transformation to austenite phase, allowing the file to expand to 6 mm in diameter, clean difficult to reach areas and improve root canal disinfection **(Eren et al.,2018).**

The aim of the present study was to assess the radiographic healing in mature permanent teeth with necrotic pulps that were treated using different disinfection protocols. The null hypothesis was that no statistically significant difference would be found between the tested groups in terms of periapical healing.

MATERIALS AND METHODS

Trial design

The study was conducted after obtaining the approval of the ethical committee of the Faculty of Dentistry at Minia University, Minia, Egypt; approval **(no. 77/488).** The study was also registered in the Clinical Trials Registry (ClinicalTrials.gov) with identifier number **(NCT05557994)**. This trial design was Prospective, parallel, randomized, double blinded clinical trial as the participants and outcome assessors will be blinded. The Allocation ratio was 1:1.

Eligibility criteria

The Inclusion criteria were18- 40 years old patients free from any systemic disease that may affect normal healing and predictable outcome and having non-vital, single-rooted, restorable anterior teeth including a single root canal with mature roots and closed apices with periapical lesion score ≥3 according to the classification of **Ørstavik et al. (1986).** The exclusion criteria were any known sensitivity or adverse reactions to pharmaceuticals necessary to complete the trial, non-restorable coronal portion of teeth and radiographic evidence of external or internal root resorption.

Preoperative assessment

A total of 36 Samples from outpatient clinic of Minia university were recruited and referred for preoperative baseline radiograph for accurate assessment and standardization of follow up records. A preoperative radiograph was

taken using the standardized paralleling technique with number 2 periapical film (*Kodak International*), which was mounted on a custom-made anterior bite block (i.e., radiographic stent) connected to the X-ray tube via an adapter ring to evaluate the size of the periapical lesion. Viewing conditions were standardized using a slide viewer. The periapical status was assessed by using the periapical index [PAI] according to **Ørstavik et al. (1986).** Each tooth was assigned a number of PAI scores by using visual references for the 5 categories within the scale.

Treatment procedures (interventions)

Patients were asked to rinse their mouth using chlorhexidine mouthwash (*Hexitol; Arab Drug Company*). They were then anaesthetized with 1 carpule of mepivacaine 3% (*Scandonest; Novocol Pharmaceutical of Canada, Inc*.) by using the infiltration technique. After rubber dam isolation, the tooth surface was disinfected, and a straight-line access cavity was prepared. The working length was determined clinically by using an electronic apex locator (*Root ZX II; J Morita Corporation)* and then confirmed radiographically with an intraoral periapical radiograph. Root canals were prepared up to a size #60 K-file (*Dentsply/ Maillefer*) **(Nageh et al., 2018).** Between each successive file, irrigation was performed with 1.5% sodium hypochlorite (NaOCl) (*Clorox, Pmoctoz and Gamble, Egypt*) using side-vented irrigation needles (Ultradent) 2 mm away from the working length. Root canals were copiously and slowly irrigated with 20 ml of 1.5% sodium hypochlorite (NaOCl) solution for 5 minutes alternatively with 20 ml of 17% ethylene diamine tetra acetic acid (EDTA) solution for 1 minute with intermediated rinse of distilled water and dryness with paper points in between irrigations. Master cone was selected which corresponding to the same size of the master apical file and confirmed by periapical radiograph using the standardized paralleling technique. The root canal was dried with suitable size paper points which corresponding to the same size of the master apical file. The method of disinfection was selected according to the alphabetical letter that was written on the paper in the envelope that the patient was picked.

Grouping

Sequence generation was done for the patient's number (from 1 to 36) by using computer sequence generation (www.random.org) which provided a table for group A, group B and group C with randomized participant numbers (12 number in each group)

- **Group A: (n=12)** disinfection using triple antibiotic paste (conventional).
- **Group B: (n=12)** disinfection using dynamic agitation of Xp-endo finisher.
- **Group C: (n=12)** disinfection using Diode laser.

Group A: TAP disinfection (n = 12)

A homogenous creamy mix of 1:1:1 of Ciprofloxacin, Metronidazole and Doxycycline were injected into the canal to a level just below the cemento-enamel (Figure 1) junction then the access cavity was sealed with a temporary glass ionomer cement.

Figure 1: Access cavity after injection of TAP (Metronidazole, Ciprofloxacin and Doxycycline)

The patient was recalled 3 weeks later, TAP was removed by copious irrigation with 20ml of saline followed by 17% EDTA for 1 minute using side vented needle placed 1 m shorter than working length and final irrigation was done using 5 ml distilled water.

Group B: XP-FINISHER disinfection (n = 12)

The X-Smart Plus motor was used at 800 rpm and 1 Ncm of torque with the final cleaning file XP-Endo Finisher. XP-finisher was placed in the root canal heating irrigation solution to 37 ° C so to allow the instrument to reach the austenitic phase. The kinematics consisted of movements from 7 to 8 mm input and output, and this procedure lasted for 60 seconds.

Group C: Diode laser disinfection (n = 12)

Diode laser decontamination and disinfection were applied with 810-nm laser by means of a 200- μ m optical fiber at a power output of 2.5 W in pulsed mode (10 msec on and 10 msec off) for 5 sec.

Bleeding induction procedures (for all groups)

The canal was dried and intentionally over-instrumentation 2-3 mm beyond the apex into the periapical region was done to create a biological scaffold for the regenerative process. Intra-canal bleeding was controlled at a level just below the cementoenamel junction (CEJ) using a dry cotton pellet. Then, the canals were kept for 7 minutes to allow for blood clot formation. **(Figure 2)**

Figure (2): Induced bleeding with a blood clot formation.

White MTA powder was mixed with supplied sterile water in a 3:1 powder/liquid ratio on paper pad using metal spatula to obtain a putty-like consistency. A 3-mm-thick layer was placed directly over the blood clot and finally the tooth was restored using glass ionomer restoration.

Radiographic Assessment

In the 6th and 12th month's visits, standardized reproducible radiographs were taken in the same manner that was used at baseline. The periapical status assessment was done using the PAI score. The periapical radiographs that were taken at baseline, 6th and 12th were given a PAI score by a single examiner who was blinded to the treatment groups.

(Table 1:): Periapical Index **(Orstavik D et al. 1986)**

Score	Criteria
1	Normal periapical structures
2	Small changes in bone structure
3	Changes in bone structure with some mineral loss
4	Periodontitis with well-defined radiolucent area
5	Severe periodontitis with exacerbating features

Statistical analysis:

Numerical data were presented as mean and standard deviation values. They were analyzed for normality using the Shapiro-Wilk test and they were found to be non-parametric. They were analyzed using Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction for intergroup comparisons and Freidman's test followed by Nemenyi post hoc test for intragroup comparisons. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows.

RESUITS

A-Intergroup comparisons:

- **Baseline:**
  There was no significant difference between different groups (p=0.053). The highest value was found in XP-finisher group (4.42±0.51), followed by TAP group (3.92±0.51), while the lowest value was found in Laser group (3.83±0.72).
- **6 months:**
  There was a significant difference between different groups (p=0.001). The highest value was found in XP-finisher group (3.50±0.67), followed by TAP group (2.42±1.00), while the lowest value was found in Laser group (2.33±0.49). Post hoc pairwise comparisons showed XP-finisher to have a significantly higher value than TAP (p=0.009) and Laser (p=0.003).
- **12 months:**

There was a significant difference between different groups (p<0.001). The highest value was found in XP-finisher group (3.17±0.83), followed by TAP group (1.83±0.39), while the lowest value was found in Laser group (1.58±0.51). Post hoc pairwise comparisons showed XP-finisher to have a significantly higher value than TAP (p=0.002) and Laser (p<0.001).

B-Intragroup comparisons:

- **TAP:**

  There was a significant difference between values measured at different intervals (p<0.001). The highest value was measured at baseline (3.92±0.51), followed by 6 months (2.42±1.00), while the lowest value was found at 12 months (1.83±0.39). Post hoc pairwise comparisons showed value measured at baseline to be significantly higher than values measured at 6 (p=0.010) and 12 months (p=0.006).
- **XP-finisher:**

  There was a significant difference between values measured at different intervals (p<0.001). The highest value was measured at baseline (4.42±0.51), followed by 6 months (3.50±0.67), while the lowest value was found at 12 months (3.17±0.83). Post hoc pairwise comparisons showed value measured at baseline to be significantly higher than values measured at 6 (p=0.003) and 12 months (p=0.005).
- **Laser:**

  There was a significant difference between values measured at different intervals (p<0.001). The highest value was measured at baseline (3.83±0.72), followed by 6 months (2.33±0.49), while the lowest value was found at 12 months (1.58±0.51). Post hoc pairwise comparisons showed value measured at baseline to be significantly higher than values measured at 6 (p=0.006) and 12 months (p=0.005).

Table (2): Mean and standard deviation (SD) values for (PAI) score for different groups

Time	(PAI) score (Mean±SD)			p-value
	TAP	XP-finisher	Laser	
Baseline	3.92 ± 0.51^{Aa}	4.42 ± 0.51^{Aa}	3.83 ± 0.72^{Aa}	**0.053ns**
6 months	2.42 ± 1.00^{Bb}	3.50 ± 0.67^{Ab}	2.33 ± 0.49^{Bb}	**0.001***
12 months	1.83 ± 0.39^{Bb}	3.17 ± 0.83^{Ab}	1.58 ± 0.51^{Bc}	**<0.001***
p-value	**<0.001***	**<0.001***	**<0.001***	

Values with different upper and lowercase superscript letters within the same horizontal row and vertical column respectively are significantly different *; significant (p ≤ 0.05) ns; non-significant (p>0.05)

DISCUSSION

Regaining pulp vitality in necrotic permeant fully mature teeth using REP has been the subject of controversy among endodontic scientists in an attempt to overcome the drawbacks of RCT such as lack of sensation and immune mechanisms, and susceptibility to root fracture of treated teeth. The importance of promoting sterile intracanal environment resulting in genetic induction and continuity of root formation was often quoted in literature. This study evaluated the regenerative potential of mature permanent teeth with necrotic pulps after disinfecting the root canal using diode laser and compare it with Xp-endo finisher activation and conventional triple antibiotic paste. In the current study, adequate irrigation and debris removal from the root canal especially the apical third by reaching apical canal preparation to K-file size #60–80 [(4)]. Violation of the apex by over-instrumentation using K-file #20–40 allows the migration of thin blood vessels and mesenchymal stem cells into root canal space from the periapical area [(4)]. Irrigation with 1.5% NaOCl which is a tissue solvent and has strong antimicrobial properties was used with side vented needle to avoid apical extrusion of NaOCl into the periapical tissue [(5)]. Also, a 5.25% concentration was not used due to it has been found that NaOCl has a cytotoxic effect on stem cells in the apical tissues, denatures growth factors in

dentin as transforming growth factor type beta (TGF-b) and also decreases odontoblastic differentiation [6]. 17% EDTA was used after NaOCl to moderate the side effects of NaOCl, it can affect the release of growth factors embedded in dentin thereby increasing their bioavailability [7]. These growth factors are potent stimulators of stem cell proliferation and differentiation [8].

For group A, triple antibiotic paste was used for root canal disinfection. TAP consists of 3 antibiotics: metronidazole, ciprofloxacin and doxycycline. The combination of the 3 antibiotics was proved to sufficiently eradicate bacteria from infected root canals [9]. According to **Kim et al** [10] it should be limited to the root canal because of the potential risk of tooth discoloration, despite the biologic success. one of the suitable techniques for preventing discoloration would be preventing its contact with the coronal dentin through dentin bonding agent. Furthermore, two recent studies suggested that intracanal medicament concentration currently used in regenerative endodontics had detrimental effect on the survival of human stem cell of apical papilla and human dental pulp cells which may adversely affect the successful outcome of endodontic regeneration [11,12]. For group B, activated XP finisher, a new instrument for final finishing and cleaning of dentinal walls, was used for root canal disinfection. The instrument has the small core size of #25, no taper and the alloy properties that enable the file to easily adapt the root canal anatomy by transformation to austenite phase, allowing the file to expand to 6 mm in diameter and to clean difficult to reach areas [13].

According to results of recent studies, XP-finisher showed better results in elimination of biofilm than positive pressure activation and also eradication of bacteria in dentinal tubules up to 50 μm deeper than conventional irrigation [14,15,16], which is why we decided to use this instrument for our research. For group C, Diode laser (810nm) was used for root canal disinfection depending on results of recent studies performed on anti-microbial effects of diode laser that suggest the diode laser could be more effective in reducing intra canal bacterial count and penetration in the depth of 500 microns in dentin [17,18]. Photo-biomodulation properties of Low-Level-Laser-Therapy (LLLT) claimed to affect positively physiological properties of cells and molecules through non-thermal means [19]. A bacteria-free canal is a prerequisite for tissue regeneration, but tissue will not grow into an empty space. Rather, a scaffold is essential to aid the ingrowth of new tissue into the canal space. Induction of a blood clot, with its constituent growth and differentiation factors from the periapical tissues, may act as a scaffold for the ingrowth of new tissue in the disinfected root canal.

The blood clot consists of cross-linked fibrin. It serves as a pathway for the migration of cells from the periapical area [20]. Given the importance of a bacteria-free environment, a coronal seal with Mineral trioxide aggregate (MTA) was used in this study, which possesses an excellent sealing ability. After clot formation, MTA was placed directly into the root canal over the blood clot to obtain a tight seal in the coronal portion of the tooth because it is hydrophilic and needs moisture to set. Also, it has been found that placement of MTA after REP leads to the upregulation of various cytokines and biologic markers such as interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-8, alkaline phosphatase, bone sialoprotein, osteopontin, and BMP-2 [21]. PAI score was used to quantify the apical lesions precisely as it is an accurate measurement method that possesses repeatability and is frequently used to assess the results of root canal treatment in permanent teeth. All teeth included in this study showed clear lesions of the periapical tissue (PAI score ≥ 3) before beginning the regenerative treatment. The results of our study showed a statistically significant reduction in PAI score in all groups after 6^{th} and 12^{th} months of treatment which can be attributed to the maintenance of a sterilized canal system with the help of disinfection protocols used in our study. These results were in agreement with **Arslan et al.** who stated that REP has the potential to be used as a treatment option for mature teeth with large periapical radiolucencies. Placement of triple antibiotic paste in the canals reduces the bacterial load greatly by penetrating the dentinal tubules [22]. XP-Endo finisher file can remove dentin from the root canal surface, guide chemical solutions to the hard-to-reach areas and enhances the dissolution of biofilm and microorganisms [23]. Also, laser disinfection which used in group C result in production of the laser energy which able to remove the smear layer and tissue debris from the canal wall [24]. In the three experimental groups of this study, widening of the apical foramen and apical clearing lead to the removal of infected cementum which further help to facilitate healing and lead to deposition of new cementum and connective tissue that may have sealed off the apical foramen or accessory canals inside the apical root

canal [25]. Induction of periapical bleeding into the disinfected canals during REP may be another possible explanation for healing by bringing components of the innate and adaptive immune system, such as cytokines, complement components, immunoglobulins phagocytes, and antimicrobial peptides which allows localization and opsonization of bacteria to facilitate phagocytosis. Regarding Effect of time periods, our study showed a statistically significant reduction in PAI score after 6 months while the lowest value was found in Laser group (1.58±0.51) after one year.

The possible mechanisms for increased reduction in size of lesion in group C could have been the initiation of the pro-inflammatory cells and osteoblast by LASER irradiation, which was in accordance with the study by **Shah et al [26]**. Accumulation of evidence regarding the successful outcome of REPs in treating mature teeth with necrotic pulp using different protocols could contribute to the development of a specific position statement and recommendations similar to the available ESE position statement **(Galler et al., 2016)** for immature teeth revascularization procedures.

CONCLUSION

Within the limitations of this study the following can be concluded; Single visit regenerative endodontics can be applied to cases with necrotic pulps and periapical lesions where thorough disinfection is recommended. The study concluded that Diode laser group was most effective root canal disinfectant and aided in the healing of periapical lesions.

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