Research Article

Clinical Evaluation of Pulp Regeneration of Non-Vital Mature Permanent Anterior Teeth.

Ahmed A. Youssef *, Magdy M. Ali ** and Amr H. El-Bolok***
* Department of Endodontic, Faculty of Dentistry, Minia University.
** Department of Endodontics, Faculty of Dentistry, Beni Suif University.
*** Department of Oral Pathology, Faculty of Dentistry, Minia.

Abstract
Aim: To asses clinically the effect of using platelet-rich fibrin (PRF) in revascularization of non – vital mature teeth. **Materials and methods:** A total of twenty necrotic mature permanent anterior teeth were enrolled for pulp regeneration using two different revascularization techniques They were randomly divided into 2 groups (n=10): group A; was treated by blood clot (BC) revascularization technique (conventional) and Group B; Revascularization was done using platelet-rich fibrin (PRF). Clinical assessments were performed after 6 and 12 months. **Results:** After 12 months of follow up of regenerative endodontic procedure (REP), all cases in both groups free of pain, facial swelling and tenderness on palpation. **Conclusions**: Both PRF and BC revascularization techniques were successful clinically regarding the resolution of signs and symptoms associated with the mature necrotic anterior teeth.
Key words: Blood clot; mature necrotic teeth; platelet-rich fibrin; pulp revascularization; regenerative endodontic procedure.

Introduction
Mature non-vital permanent anterior teeth have long been treated with conventional root canal therapy (RCT) which involves chemomechanical preparation of the root canal and obturation with artificial materials which be unsuccessful to regain pulp tissue vitality, results in the loss of protective mechanism and increases the brittleness of the treated tooth[1,2]. Additionally, loss of normal translucency and discoloration of the endodontic treated teeth are usually detected due to the loss of moisture inside the root as a subsequent to complete removal of the tooth pulp[3].

In regenerative endodontic procedures (REPs), blood clot which is formed in a disinfected root canal space by intentionally inducing bleeding from periapical tissue creates a three-dimensional scaffold that entraps undifferentiated stem cells and supports the in-growth of new tissue[4].

REPs have been focused on the treatment of immature permanent teeth with necrotic pulps as an alternative to MTA apexification[5]. Recently, REPs were promoted as a viable treatment option for necrotic mature teeth to reconstruct the neurovascular system in root canal space with an immune system that protects the tooth from any microbial damage after treatment[6,7].

Studies regarding the use of REPs on mature necrotic teeth is limited to case reports/series and few randomized clinical trials. Arslan et al.,[8] compared the clinical outcome of REPs in mature necrotic teeth using blood clot technique with that of conventional root canal treatment (RCT) and concluded that REPs have a higher success rate 92.3% to RCT 80% with no statistically significant difference (P>0.05). Also, Nageh et al.,[9] conducted a randomized clinical trial compared BC and PRF as scaffolds in regenerative treatment of mature necrotic teeth in which 60% of treated teeth had regained tooth sensibility at the end of the 12-month follow-up period.

The present study aimed to assess clinically the regenerative potential of mature permanent teeth with necrotic pulps using BC revascularization technique and PRF protocol. The null hypotheses were that no statistically significant difference could be found between the tested groups in terms of .

Materials and methods

Trial Design

Adouble-blinded randomized controlled trial RCT with two-arm parallel groups with an allocation ratio of 1:1 was conducted after it was approved by the ethical committee of the Faculty of Dentistry, Minia University (no. 58/306).

Sample size calculation

Sample size calculation was performed based on the results of Shivashankar et al.,[10] Twenty patients were randomly selected from patients seeking treatment from the outpatient clinic of the Endodontic department of the faculty.

Eligibility Criteria

Patients included in this study were in the age range of 18 - 40 years having necrotic single-rooted maxillary anterior tooth with mature root. The selected patients were without known allergic reactions to any of the antibiotics used or systemic disease. Pregnant females, patients with generalized chronic periodontitis, patients having non-restorable teeth were excluded from the study. Also, teeth were pulp space is needed for post or teeth with previous root canal treatment, teeth with developmental anomalies, and teeth with external and internal resorption[9,11,12]. The trial was introduced to patients, after the explanation of detailed treatment procedures, the possible outcomes, complications, and follow-up period needed. The patients were asked to sign a printed informed consent in either Arabic or English that explains the aim of the study.

Randomization and blinding

Randomization was done by using computer sequence generation (www.random.org) which provided a table for two equal groups with randomized participant numbers (n=10) in each group. Twenty papers were numbered from 1 to 20 and individually packed in opaque envelopes. Each patient picked an envelope before the beginning of the second visit. The number in the envelope determined the regene-rative protocol which was to be performed for the participant. The patient and assessor were not informed of what procedure he/she was subjected to.

Pre-operative assessment

Demographic data of all participants were recorded, followed by assessment and recording of the presence of swelling or tenderness related to the examined tooth. All patients had received a questionnaire based on a visual analog scale (VAS) to record their assessment of pain. The VAS scale has already been validated in other studies[13].

Interventions

At the first visit, the tooth was anesthetized by 1 carpule of mepivacaine 3% using infiltration technique followed by rubber dam isolation and tooth surface disinfection. Straight-line access cavity was prepared and the working length was determined clinically using electr-onic apex locator and confirmed radiogra-phically with an intraoral periapical radiograph. Root canals were prepared up to size #60–80 K file and irrigated by 1.5% NaOCl between each successive and after completion of the prepa-ration. Root canals was dried then, dentin bonding agent was applied to the access cavity walls and the coronal third of the canal to avoid coronal discoloration. Triple antibiotic paste (TAP) was injected an the access cavity was sealed by dry sterile cotton pellet and glass ionomer cement. Patients were recalled 3 weeks later.

At the Second visit, local infiltration anesthesia without vasoconstrictor was administered follo-wed by rubber dam isolation. The TAP was washed out by irrigation with 5ml sterile saline followed by the same amount of 17% EDTA for 1 minute and the final irrigation was done using 5 ml distilled water, root canal was dried and the regenerative procedure was done according to the group to which each patient belonged.
Group A: Intra canal bleeding was evoked by over-instrumentation using sterile #25–#40 K-file 2-3 mm beyond the apex, bleeding was controlled at a level just below the cementoe-namel junction (CEJ) until blood clot formation.
Group B: After the bleeding was evoked in the canal; 5 ml blood was drawn from the patient's right median cubital vein into a test tube without the addition of an anticoagulant and centrifuged immediately at 3000 rpm for 10

minutes[14]. The product attained contained three layers: the middle layer was the platelet-rich fibrin. The PRF was segregated and squeezed to form a membrane which was fragmented and placed incrementally in the canal to the level of the CEJ.

White MTA was placed 3 mm below the CEJ followed by a moist cotton pellet and a temporary filling restoration to allow for complete setting of the MTA. Temporary filling was removed and replaced by a glass ionomer cement base and resin composite restoration after 2 days to seal the access cavity.

All Patients were recalled at 6 and 12 months to evaluate treated teeth clinically by recording pain according to VAS, swelling and tenderness.

Statistical Analyses

Categorical data were presented as frequencies and percentages and were analyzed using Fisher's exact test. Numerical data were presented as mean, standard deviation (SD) values. Data were explored for normality by checking the data distribution, calculating the mean and median values and using Kolmogorov-Smirnov and Shapiro-Wilk tests.

Parametric data were analyzed using independent t-test. Ordinal data were analyzed using Mann Whitney U test. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with IBM® SPSS® Statistics Version 26 for Windows.

Results

This study was conducted on 20 cases that were randomly and equally allocated to the studied groups (i.e. 20 cases each), with each group having an equal number of both genders. The mean age of the cases in blood clot group was (24.20±4.64) years, and in the RPF group it was (25.40±5.58) years with no significant difference between both groups (p=0.607). Pre-operatively, the mean value of VAS was (3.20±1.87) for the blood clot group, while in RPF group it was (3.60±2.17) and the difference between both groups was not significant (p=0.739). During all subsequent follow-up intervals, all cases in both groups had VAS of zero. In both groups, facial swelling was found in 3(30%) cases pre-operatively, after 6 months only one case in the blood clot group had swelling, and after 12 months all cases were free of facial swelling. Pre-operatively, 2(20%) cases in both groups reported tenderness on palpation, after 6 months and until the end of the study, all cases were free (Table 1) (Figure1).

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Table (1): Summary statistics of demographic and clinical data

Features				Blood clot (I)	PRF (II)	p-value
Gender	Male		n	5	5	1ns
			%	50.0%	50.0%	
	Female		n	5	5	
			%	50.0%	50.0%	
Age			Mean±SD	24.20±4.64	25.40±5.58	0.607ns
VAS	Pre-operative		Mean±SD	3.20±1.87	3.60±2.17	0.739ns
	Other intervals			0.00±0.00	0.00±0.00	1ns
Swelling	Pre-operative	No	n	7	7	1ns
			%	70.0%	70.0%	
		Yes	n	3	3	
			%	30.0%	30.0%	
	After 6 months	No	n	9	10	1ns
			%	90.0%	100.0%	
		Yes	n	1	0	
			%	10.0%	0.0%	
	After 12 months	No	n	10	10	———
			%	100.0%	100.0%	
		Yes	n	0	0	
			%	0.0%	0.0%	
Tenderness	Pre-operative	No	n	8	8	1ns
			%	80.0%	80.0%	
		Yes	n	2	2	
			%	20.0%	20.0%	
	After 6 months	No	n	10	10	———
			%	100.0%	100.0%	
		Yes	n	0	0	
			%	0.0%	0.0%	
	After 12 months	No	n	10	10	———
			%	100.0%	100.0%	
		Yes	n	0	0	
			%	0.0%	0.0%	

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

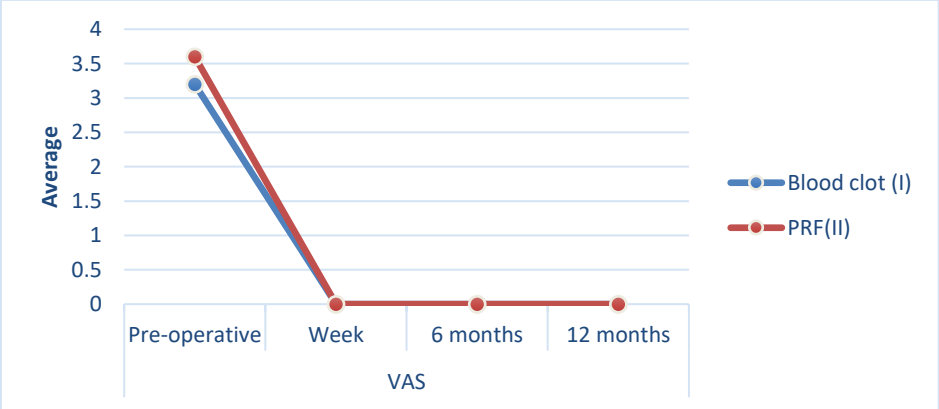

Figure (1): Line chart showing average VAS

Discussion

Root canal therapy has been used for the treatment of necrotic mature permanent, this protocol involves chemo-mechanical preparation of the root canal and sealing of the root canal with a biocompatible material[15]. REPs mostly proposed for the treatment of immature permanent necrotic teeth, includes disinfection with minimum to no mechanical preparation followed by bleeding induction inside the root canal and covering the blood clot with a biocompatible material[16]. For mature teeth, REPs has been suggested to overcome the drawbacks of RCT such as lack of sensation and immune mechanisms, increased susceptibility to root fracture of treated teeth and possibility of re-infections due to coronal leakage or micro-leakage[9,16]. This study evaluated the clinical efficacy of 2 different regenerative protocols in treatment of necrotic mature permanent teeth, namely BC and PRF.

Formation of a BC by inducing bleeding in the disinfected root canal creates a scaffold of fibrin that entraps undifferentiated mesenchymal stem cells capable of initiating new tissue development[17].

PRF, a second-generation platelet concentrate which form a scaffold allows the release of various growth factors which direct stem cell migration, proliferation, and differentiation, supplements angiogenesis and play an important role in the self-regulation of inflammatory and infectious phenomenon[18-20].

In the current study, adequate irrigation and debris removal from the root canal was achieved through mechanical preparation to K-file size #60–80 followed by a through disinfection procedure adopted from the AAE guidelines using 1.5% NaOCl to avoid cytotoxic effect of higher concentrations of NaOCL[21,22]. 17% EDTA was used after NaOCl to moderate the side effects of NaOCl and allowing the release of growth factors embedded in dentin[23].

TAP formula in which minocycline was replaced by Doxycycline[24] was applied as an intra-canal medication in for 21 days for the chemical disinfection of the canals. Doxycycline was found to cause less coronal discoloration than minocycline in TAP[25]. To avoid discoloration caused by either triple antibiotic paste or MTA which one of the drawbacks of REPs is teeth, dentin bonding agent was applied to the access cavity walls and coronal third of the canal before TAP injection[26].

3% Mepivacaine was used in the second visit without vasoconstrictor to facilitate bleeding into the canal system from periapical tissues. Violation of the apex by over-instrumentation was done to allow the migration stem cells into root canal space from the periapical area[22]. MTA was placed after clot formation, MTA is a hydrophilic and needs moisture to set leads to the upregulation of various cytokines and biologic markers[27,28].

In this study, the outcomes were assessed by assessor not participating in the study. The outcome was resolution of pain, swelling, and tenderness clinically. We used Visual Analogue Scale (VAS) to evaluate pain intensity as it sensitive to small changes in pain, its results are sensitive to treatment effects, reproducible, and can be applied in a variety of practice settings[29,30]. Holden et al.,[30], also recommended that the swelling was assessed visually as a binary outcome whether there is swelling or not.

The outcome of this study showed that there was no statistically significant difference between the two groups in pain preoperative VAS scores regardless of the time intervals in first and second visits, also no statistically significant difference was found between the two groups in 1 month, 3 months, 6 months, 9 months and 12 months. This can be due to the resolution of infection and swelling[31].

Conclusions

Regenerative endodontic procedures using either blood clot or platelet-rich fibrin revascularization protocol have the potential to be used as a treatment option for mature teeth with necrotic pulp. Studies with longer follow up period are required to assess different outcomes of this treatment.

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