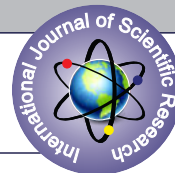


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COMPARATIVE CLINICAL, RADIOGRAPHICAL AND HISTOLOGICAL EVALUATION OF CORONAL PULPOTOMY TECHNIQUE WITH PRF AND A NOVEL CROSSLINKED PRF AS AN ALTERNATIVE TO PULPECTOMY FOR PRESERVING THE TOOTH VITALITY IN CONTEXT OF TISSUE ENGINEERING.



Dental Science

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ABSTRACT

Background: Removal of infected pulp tissue from the coronal portion leads to preservation of a remaining vital and functioning pulp. Preservation of pulp vitality by pulpotomy is one of the universally accepted treatment procedure with the aid of latest bioactive materials leading to a more conservative approach in the management of teeth involving the pulp based on clinical conditions. **Aim:** To comparatively evaluate the clinical and radiological and histological outcome of platelet-rich fibrin (PRF) and the novel crosslinked PRF (C-PRF) developed by us as pulpotomy agent in context of tissue engineering in permanent teeth. **Materials and Methods:** 20 permanent molar teeth from 20 healthy patient aged 18-40 years with permanent molar teeth cariously exposed pulps and diagnosed clinically as irreversible pulpitis were selected for the study. Written consent from the patients was taken. The patients were then randomly divided into 2 groups (n=10). One group received PRF pulpotomy another group received our novel crosslinked PRF (C-PRF) pulpotomy. In all patients coronal pulp was removed after preparation of the access cavity with rubber dam under Lignocaine 2 % local anaesthesia. After hemostasis, exposed pulps were covered using PRF membrane with Biodentine or C-PRF with Biodentine, and then all teeth were restored with glass-ionomer cement. Clinical and radiographic evaluation was undertaken at 1 day, 3month and 6 months intervals. The results were analyzed using appropriate statistical tests. After around six months one case of each group third molar teeth was extracted due to positional problem and causation of cheek ulceration. Histological examination of the extracted teeth was done. **Results:** Statistical Analysis was performed with help of Epi Info (TM) 7.2.2.2 EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC). Test of proportion was used to find the Standard Normal Deviate (Z) to compare the difference proportions. $p < 0.05$ was taken to be statistically significant. **Conclusion:** C-PRF could improve the prognosis of pulpotomy procedures of mature permanent

KEYWORDS

dental pulp, pulpotomy, pulp regeneration, tissue engineering, platelet-rich fibrin PRF, crosslinked PRF(C-PRF)

INTRODUCTION

Most cases of pain associated with pulpal exposure either by caries, trauma or the mechanical procedures are managed with two immediate treatment modalities; root canal therapy (RCT) and extraction.[1] However there are varying opinions based on the clinical scenario on how to manage when pulp that has been exposed by caries or through mechanical procedures. [2] Preservation of pulpal vitality is of paramount importance as the vital functioning pulp is capable to initiate several important functions like formation of dentin, providing nutritive support to the tooth, enabling a defensive function and a unique reparative capacity on dentin. [3]

Vital pulp therapy (VPT) is defined as a treatment procedure which aims to preserve and maintain pulp tissue that has been compromised but not destroyed by caries, trauma or restorative procedures in a healthy state or to be vital. [4] Vital pulp therapy includes direct and indirect pulp capping and pulpotomy procedures [5] Pulpotomy is a vital pulp therapy in which the coronal portion of the pulp is removed surgically and the remaining radicular pulp is preserved intact and a suitable material is placed over the radicular pulp to protect it from further insult and initiate healing and repair. [6]

Pulpotomy is based on the hypothesis that the inflammation and reduced vascularization, caused by bacterial invasion are confined to the coronal pulp, while the radicular pulp which when protected with hard tissue stimulating material, the pulp would heal and remain vital. [1,2]

Various materials have been introduced and advocated for use in pulpotomy procedures based on their important properties such as biocompatibility, sealing ability and antimicrobial efficacy when placed in direct contact with the radicular pulp. [3] Formocresol introduced by Buckley, is considered as the gold standard pulpotomy agent owing to its bacteriostatic and fixative properties. It was composed of formaldehyde and cresol which are carcinogenic and mutagenic which led to the search for more biocompatible alternatives in the form of Mineral Trioxide Aggregate (MTA), laser irradiation and

Biodentine. [7] Mineral Trioxide Aggregate (MTA) introduced in the year 1993, is one of the most commonly used and researched material in endodontics and for such pulpotomy procedures with successful clinical results. However, due to certain inherent drawbacks of MTA such as presence of toxic elements in the material composition, higher cytotoxicity, high pH, difficult handling characteristics and longer setting time lead to the search for the material that can potentiate the natural healing process and which is biocompatible and can overcome the limitations of MTA [3,8, 9] leading to the introduction of PRF and Biodentine.

Biologically based therapeutic agent PRF 10, is a second generation platelet concentrate introduced in the year 2001 by Dr Joseph Choucr, which is strictly autologous and helps to release the growth factor for a prolonged period, necessary for the regeneration of dentin pulp complex there by accelerating the healing process [3].

Studies comparing different preparations of PRF used in coronal pulpotomy techniques in context of pulp tissue regeneration have proved some promising results [11,12] But most of studies pointed out its difficult handling property and rapid disintegration. Crosslinking of PRF should improve its handling property and physical nature and better suit for pulp tissue engineering purposes.

MATERIALS AND METHODS

The present study was conducted in the Department of Conservative Dentistry and Endodontics of Gurunank Institute of Dental Sciences with ethical clearance of JIS University. Twenty patients in the age group of 18 to 40 years with good general health and no history of systemic illness were chosen for the study. Twenty molars were selected with history of mild pain on intake of hot and cold fluids, with no history of previous pain and swelling, no tenderness on percussion and no associated sinus opening adjacent to the involved teeth. Diagnostic tests were performed on all the patients which revealed lingering pain to hot and cold tests. Electric pulp tests (EPT) results showed a more intense response in the involved tooth as compared to the contralateral healthy mature permanent tooth. Radiographic

examination revealed presence of radiolucency of carious lesion or radiopacity of fractured restoration approaching the pulp with normal periodontal ligament space and lamina dura. There was no periapical lesion visible on the radiograph. (Figure) After complete clinical and radiographic examination, a diagnosis of vital teeth with signs and symptoms of irreversible pulpitis was made in the involved tooth in respect to all the cases.

Procedure was explained to the patient and the informed consent was obtained. Teeth were anesthetized, isolated with a rubber dam. Treatment included removal of caries and fractured restoration if any. Pulpotomy was performed using a high speed airtor hand piece and the coronal pulp tissue was removed to orifice level. Associated bleeding indicated pulp vitality; hemostasis was achieved with sterile normal saline with gentle application of small pieces of sterile cotton pellets within five minutes. (Figure-) Procedure was explained to the patient as an alternative to the conventional RCT to preserve the vitality of the teeth. Patients were randomly divided into two groups: Group I: The treatment modality of coronal pulpotomy using only PRF for ten patients. Group II: The treatment modality of coronal pulpotomy using crosslinked PRF(C-PRF) for ten patients.

PRF was prepared by drawing blood from median cubital vein into a 10 ml test tube without the addition of an anticoagulant. Hence to prevent the blood from coagulating after coming in contact with the glass tube, it was centrifuged immediately using a table top centrifuge. The product thus obtained consisted of the three layers: the top most layer of acellular platelet poor plasma, the middle layer of platelet rich fibrin and the bottom most layer of red blood corpuscles. (Figure) The PRF was segregated and was squeezed with a sterile gauze piece. (Figure) The PRF membrane obtained after centrifugation of the patients own blood was placed over the exposed pulp stumps. (Figure) Biodentine was manipulated and an approximately 2 mm thick layer of Biodentine was placed over the PRF with a plastic instrument. (Figure) It was packed using dry cotton pellets for five minutes and then the cotton pellets were removed. A layer of restorative glass ionomer cement was placed upon the Biodentine and permanent restoration was done in the same appointment using composite restorative material in all the ten patients. (Figure)

In Group 2 the PRF samples were crosslinked in freshly prepared TA solution in dependence for 10 minutes at room temperature. TA was used at a Concentrations of 1 wt% for preparing samples. After crosslinking, the gels were washed with normal saline for 5 min. to ensure that all excess TA was removed.

The patients were recalled after one day, three months and six months for clinical and radiographical evaluation. (Figure) Clinically status of pain, sinus tract, swelling, vestibular tenderness on palpation and tenderness on percussion and radiographically, periodontal ligament widening, internal/ external resorption and periapical/furcal radiolucency and calcifications were evaluated in both the groups. (Table 1, 2,)

RESULTS

Table 1 Radiographical finding after single visit pulpotomy procedure

Groups	Periodontal ligament widening		Periradicular lesions		Furcation bone loss		Internal/external resorption		Calcification	
	3 months	6 months	3 months	6 months	3 months	6 months	3 months	6 months	3 months	6 months
PRF Pulpotomy 10 cases	A	2	A	2	A	A	A	A	A	A
C-PRF Pulpotomy 10 cases	A	A	A	A	A	A	A	A	A	A

A: Absent

Table no. 2 Clinical findings after single visit pulpotomy procedure

Group	Pain		Swelling		Sinus tract		Vestibular tenderness		Tenderness on percussion	
	One day follow up	6 months follow up	One day follow up	6 months follow up	One day follow up	6 months follow up	One day follow up	6 months follow up	One day follow up	6 months follow up
PRF Pulpotomy 10 Cases	7	A	A	A	A	A	A	A	A	A
C-PRF Pulpotomy 10 cases	1	A	A	A	A	A	A	A	A	A

A: Absent

Table No. 3 Statistical analysis of radiographical finding

	Periodontal ligament widening at 6 months	Periradicular lesions at 6 months	P- Value
PRF Pulpotomy 10 Cases	2 (20%)	2 (20%)	
C-PRF Pulpotomy 10 Cases	0 (0%)	1 (10%)	0.99

Fisher exact test

*p<0.05 statistically significant; p>0.005 Non significant, NS

Table No.4 Statistical analysis of clinical finding

	Pain At One Day Follow Up	Tenderness On Percussion At One Day Follow UP	P- Value
PRF Pulpotomy 10 Cases	7 (70%)	6 (60%)	
C-PRF Pulpotomy 10 Cases	1 (10%)	1 (10%)	(0.51)
Chi Square Value	(0.43)		

*p<0.05 statistically significant; p>0.005 Non significant, NS

DISCUSSION

Several factors influence the treatment plans of a clinician when dealing a tooth with a pulpal exposure. The treatment of teeth involving irreversible pulpitis is conventionally done with root canal therapy. An infected pulp usually undergoes a degenerative process in a coronal to apical direction. When pulpal infection and inflammation are restricted to the coronal portion of the pulp chamber, there is every possibility that unaffected healthy pulp tissue remains in the radicular portion of the root canal system. Removal of the infected tissue should lead to preservation of a remaining vital and functioning pulp.[13, 14] Preservation of the vitality of the pulpal tissue, prevention of pain, swelling and finally preserve the tooth through the extirpation of the coronal pulp and prevention of radicular pulp inflammation/infection is the goal of pulpotomy treatment procedure. [15]

Pulpotomy can be carried out with various materials based on their biocompatibility, sealing ability and antimicrobial efficacy. Latest bioactive materials such as MTA and Biodentine have excellent biocompatibility and sealing abilities. In recent studies, limitations of MTA as difficulty in manipulation, increased cost, two visit treatments and longer setting time have been mentioned. [8] In a comparative study that evaluated the PRF and MTA as pulpotomy agent in immature permanent teeth concluded that success with PRF was more than MTA in root end closure with no statistically significant difference between the two groups. [9] Recently a study conducted to determine the physicochemical properties and dentin bond strength of MTA and Biodentine as a retrograde filling material revealed that Biodentine exhibited faster setting time, long term higher compressive strength and bonding to dentin than MTA. [16] So, in our study Biodentine was used above the PRF/C-PRF in pulpotomy treated permanent molars. Over it posterior restorative G.I.C was used as final restoration.

PRF often named as Choukroun's PRF, is a second-generation platelet concentrate. The PRF constitutes components of blood that are beneficial to improve wound healing. PRF can be considered as an immune concentrate with specific composition and a three dimensional architecture. It contains numerous growth factors like platelet derived growth factor, transforming growth factor β 1, insulin like growth factor which exhibits varied potent local properties such as cell migration, attachment, proliferation and differentiation. It has been shown as a good biomaterial for pulp regeneration in context of tissue engineering. PRF is both a healing and interpositional biomaterial [17]

Recent study proved that the TA-based hydrogel network could fulfil demands over a prolonged clinical period as well as physiological functions with antioxidant, antibacterial and anti-inflammatory properties. [18] Moreover, as reported in the work of Du et al., a hybrid hydrogel containing PEG, quaternized chitosan (QCS) and TA (PEGDA/QCS/TA) was developed based on mussel-inspired chemistry.[19] The incorporation of TA via multiple hydrogen bonding with PEG enabled properties such as anti-swelling, high mechanical strength, antimicrobial and excellent adhesive properties to versatile surfaces. In our own study, which is under publication, we have also found improvement of PRF physical characteristics after crosslinking with tannic acid. So, our novel C-PRF is compared with the conventional PRF in pulpotomy cases intended for use as a tissue engineering material.

In the present study few patients showed post-operative pain and discomfort in the 24 hrs follow up in the PRF group only and in the C-PRF group only one complained, which is not in accordance with the study conducted by Matsuo et al., which concluded that PRF pulpotomy was a sure success and three months was an adequate time to evaluate the success or failure of vital pulp therapy treatment procedures.[20]

The success rate of the VPT like pulpotomy is governed by adequate blood supply, obtaining hemostasis, biocompatibility of the restorative materials and the provision for the adequate seal. Control of hemorrhage/hemostasis is an important key to enhance the success. Hemorrhage of exposed dental pulp tissue is in part due to inflammatory response of the pulp to bacteria and their byproducts from carious dentin. Two other important factors in predicting pulpal response to VPT are the sealing ability and the non-toxicity of material. [17, 21]

The success in this study may also be attributed to the immediate placement of permanent coronal restoration, as demonstrated by Massler who concluded that the most important cause of failure in VPT is the presence of leakage during the healing process.[22]

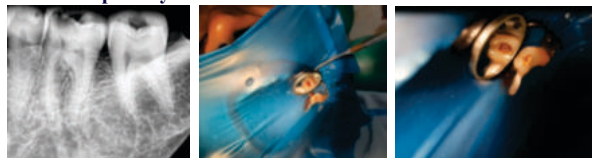
More success rate with the C-PRF may be due to its added antibacterial and haemostatic action of the tannic acid crosslinker that is present in excess in the placed C-PRF over the radicular pulp of the pulpotomy treated permanent molars

CONCLUSION-

Based on the present study results we can conclude that pulpotomy as a treatment option has an excellent clinical success rate using PRF whatever the cause of pulp exposure in permanent teeth with promising horizons in clinical application that may lead to paradigm shift in vital pulp therapy.

Using cross linked PRF (C-PRF) can further improve the handling property and prognosis of the pulpotomy. C-PRF can safely be used as tissue engineering material for pulp tissue regeneration and other soft connective tissue regeneration.

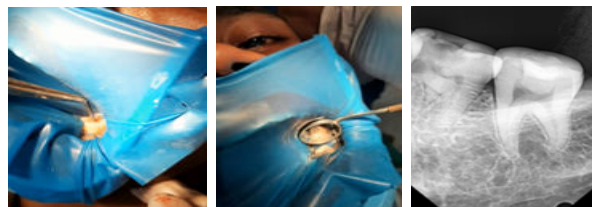
Case-1 Pulpotomy of Permanent Tooth with PRF



A.Pre- operative radiograph

B.Access opening showing vital

C.Haemostasis achieved with cotton pack

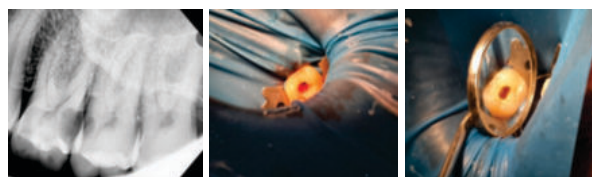


D. PRF placement in pulp chamber

E. Biodentine followed by G.I.C. restoration

F. 1-Yr Follow up radiograph

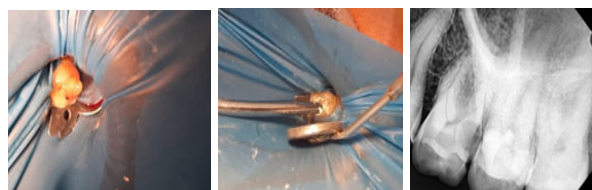
Case-2 Pulpotomy of Permanent Tooth with C-PRF (Crosslinked PRF)



A.Pre- operative radiograph

B. Access opening showing vital

C. Haemostasis achieved with cotton pack



D. PRF placement in pulp chamber

E. Biodentine followed by G.I.C. restoration

F. 1-Yr Follow up radiograph

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