

## PLATELET-RICH PLASMA PULPOTOMY IN PRIMARY MOLARS

Talat Mohamed Beltagy\*

### **ABSTRACT**

**Background:** In spite of the technological advances in dental restoratives, there is a constant need for the development of a novel biologically-based autologous capping substitute that overcomes the side effects of various synthetic-based biomaterials used for pulpotomies.

**Purpose:** To evaluate clinically and radiographically the success of platelet-rich plasma (PRP) medicament in primary molars pulpotomy.

**Materials and methods:** A randomized controlled clinical trial in which 20 children aged 5-8 years were selected. Each child had at least two lower deeply carious primary molars (1st and/or 2nd) indicated for pulpotomy and randomly divided into two groups; The PRP (study group) in one quadrant and formocresol (control group) in the opposite one. All teeth were treated with the same conventional and standard pulpotomy technique. All patients were followed up clinically and radiographically at 3, 6, 9 and 12 months post-operative. Data were statistically analyzed using Fisher exact test.

**Results:** The clinical and radiographic success showed no statistically significant difference between both groups ( $p > 0.05$ ). The overall clinical success rate of pulpotomized teeth at the end of recall time was 100 % for PRP and 90% for FC group, whereas the overall radiographic success was 90% for PRP and 85% for FC group.

**Conclusion:** From the present study, it was concluded that PRP had a successful outcome, a potent therapeutic medicament and had a promising alternative to the presently used pulpotomy medicament.

**KEYWORDS:** Platelet-rich plasma, Formocresol, Pulpotomy.

### **INTRODUCTION**

The objective of vital pulp therapy in primary teeth is to preserve the pulp vital instead of replacing it with an inert filling and to maintain the integrity and function of the teeth till their time of normal exfoliation<sup>(1)</sup>.

Formocresol (FC) was considered the gold standard for primary teeth pulpotomy<sup>(2)</sup>, but due to some significant side effects as cytotoxicity, potential mutagenicity, immune sensitization, and carcinogenicity, various alternative pharmacotherapeutic medicaments/techniques have

\* Assistant Professor of Pedodontics, Faculty of Dentistry, Kafrelsheikh University, Egypt.

been proposed to have efficacies equivalent to or better than FC with a wider margin of safety<sup>(3)</sup>. Also, non-pharmacological pulpotomy techniques have been well-documented<sup>(4)</sup>.

In spite of great scientific and technological advances in synthetic dental materials, researches still reported their mild to moderate cytotoxic effects on pulpotomies. Therefore, it is important to develop new biocompatible and biologically-based therapeutics directed at preserving pulp vitality, increasing tooth integrity by stimulation of body's natural healing<sup>(5)</sup>, forming biological tissue<sup>(6)</sup>, and neutralizing the side effects of previously used synthetic based biomaterials<sup>(7)</sup>.

In dental medicine, a recent innovation has been prepared using blood components; platelet-rich plasma (PRP). This concentrate of the first generation is a novel biologically active component. It collects the power of recent physical, chemical and biological science for solving the actual problems in clinical pedodontics<sup>(2,5)</sup>. Marx et al.<sup>(8)</sup> in 1998 were the first who applied the PRP in mandibular grafts.

The PRP is an autologous source of highly concentrated growth factors, platelets, the native concentration of fibrinogen, WBCs, phagocytes, vasoactive and chemotactic agents<sup>(9)</sup>. A PRP blood clot contains 95% platelets (338% higher than untreated blood), 4% RBCs, and 1% WBCs<sup>(10)</sup>. Tsay et al.<sup>(11)</sup> found that platelet count can exceed 2 million/ $\mu$ l in PRP, thus it increases by 160%-740%. Therefore, it can consider jump starts the cascade of regenerative events via three mechanisms: increasing of local cell multiplication, decreasing the early macrophage proliferation, and degranulating the  $\alpha$ -granules in platelets via inhibition of excess inflammation, which contains both the synthesized and pre-packaged growth factors<sup>(12)</sup>.

The PRP prevents the extensive fibrosis, tissue necrosis and promotes the tissue healing as it has a high inductive potential on stem cells<sup>(13)</sup>. The treatment can be seen very safe because PRP is made of patient's self-blood<sup>(14)</sup>. Kalaskar and Damle<sup>(15)</sup>

showed 100% success rate of lyophilized freeze-dried platelet as pulpotomy agent compared to 60% of calcium hydroxide. Another study with Solomon et al.<sup>(16)</sup> who found that the use of autologous platelet concentrate as a pulpotomy agent recorded better successful outcome clinically and radiographically than Biodentine.

The clinical published data about the efficacy of biologic growth factors on vital pulp therapy were limited<sup>(2,17)</sup>, yet, this study aimed to evaluate, clinically and radiographically, the success of platelet-rich plasma as a pulpotomy medicament in primary molars.

## MATERIALS AND METHODS

### Clinical and radiographic study

Ethical approval of this research was documented from Ethics Committee, Faculty of Dentistry, Tanta University. All children's parents participating in this study were thoroughly informed of the objective and procedures of the research and informed consents were obtained from them. The selected twenty patients aged 5-8 years old, with their mean age  $6.45 \pm 0.6$  years. Each patient had at least two lower deeply carious (1<sup>st</sup> and/or 2<sup>nd</sup>) primary molars indicated for pulpotomy.

### Clinical inclusion criteria<sup>(4)</sup>

- All patients were healthy, cooperative and had no systemic illness.
- The absence of any clinical signs and symptoms of pulpal necrosis.
- The treated primary molars would be restorable.
- Any hemorrhage should be stopped from the amputated pulp stumps within 5 minutes if using a pledget of sterile moist cotton pellets.

### Radiographic inclusion criteria<sup>(4)</sup>

- The caries radiolucency approaching pulp.
- At least 3/4 of the root length should remain.

- No evidence of external and/or internal root resorption, widening of periodontal ligament (PDL) space, furcal or periapical radiolucency and canal calcification or pulp stone.

The forty deeply carious primary molars were randomly assigned into two equal groups (20 molars each) according to the pulpotomy medicament used. The PRP group in one quadrant and FC group (control) in the opposite (a split mouth design).

### Pulpotomy procedure

After administrating profound local anesthesia, complete teeth isolation occurred using a rubber dam. A #4 round bur in a high-speed under cooling system was used to remove the roof of the pulp chamber. Large sharp sterile spoon excavator was used for the amputation of the coronal pulp and then the cavity was irrigated with normal saline and dried with sterile cotton pellets. Hemostasis was accomplished using a compressed pledget of sterile moist cotton pellets for 5 minutes.

### PRP gel preparation

One ml of peripheral venous blood was drawn from the patient's median cubital vein and transferred into a sterile evacuated polystyrene tube contained Acetic acid Citrate Dextrose (ACD TUBE, C.D. RICH) for anticoagulation and preservation of high concentration of platelets.

The first spin was performed at 2400 r.p.m for 10 minutes using Centurion Scientific Benchtop centrifuge (UK) (Fig 1A). This step separates the blood into three layers; top-most platelet poor plasma (PPP) layer, middle PRP layer, and bottom-most red blood cell layer (Figs 1B-1D).

Using a sterile microbibe, the PRP and PPP were collected in a 5ml sterile glass tube without anticoagulant that undergoes the second spin at 3600 r.p.m for 15 minutes, the PPP was concentrated at the upper two-thirds of the centrifuged sample, whereas the platelet pellet was concentrated at the bottom.

The PPP was removed and discarded soon after centrifugation leaving PRP at the lower third alone<sup>(18)</sup>. PRP was activated with 10% calcium chloride (CaCl<sub>2</sub>, Biomed, Egypt) (50µl per ml of PRP), in Eppendorf 1.5 ml tube 15 min before PRP clinical application<sup>(19-22)</sup> (Fig 2).

In PRP group, after achieving hemostasis, PRP gel was placed in contact with the pulp stumps by the previously trimmed highly absorbable sterile dental collagen membrane<sup>(2)</sup> (CollaGuide/Korea) that was adjusted to the entire pulp chamber floor (Fig 3).

In FC group (Fig 4), a sterile cotton pellet was dipped in Buckley's formula, dampened twice between gauzes to remove the excess and was placed on the amputated coronal root orifice for 5 min<sup>(23)</sup>.

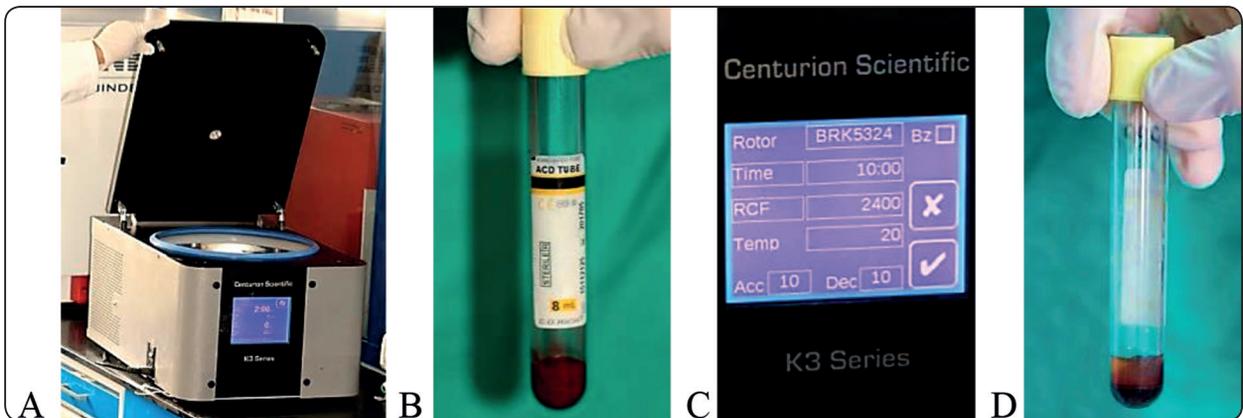


Fig. (1) Centurion Scientific centrifuge (A). A sterile ACD tube contained 1ml venous blood for PRP preparation (B). The first spin program (C). The three separated layers of the centrifuged blood (D).

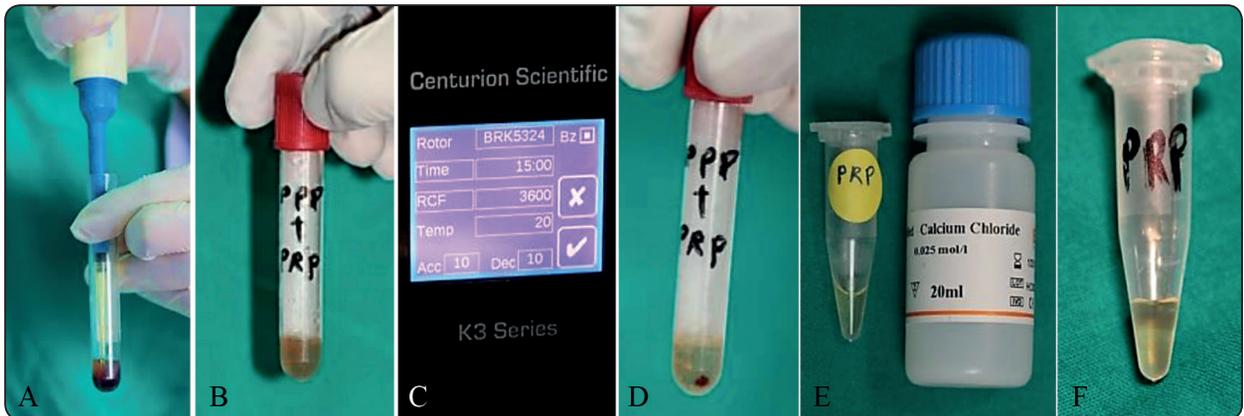


Fig (2) The PPP and PRP were transferred to a sterile glass tube using microbitter (A, B). The second spin program (C). The PPP and PRP after centrifugation (D). The separated PRP with 10% CaCl<sub>2</sub> for clot activation (E). The PRP gel (F).

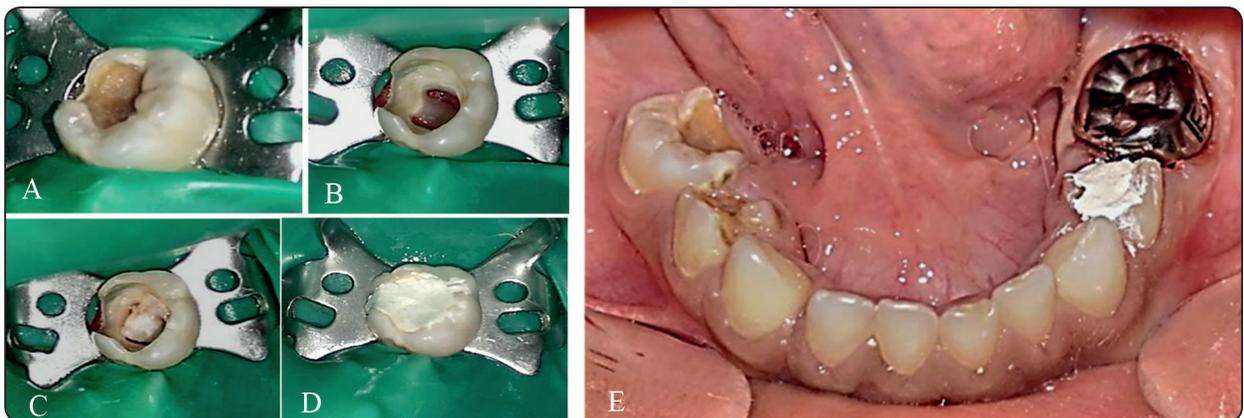


Fig. (3) Preoperative photograph of carious lower left 2nd primary molar indicated for PRP pulpotomy (A). Hemostasis of the pulp stumps (B). The PRP was placed over the pulp stumps with the collagen membrane (C). The cavity was sealed with IRM (D). Final restoration with stainless steel crown (E).

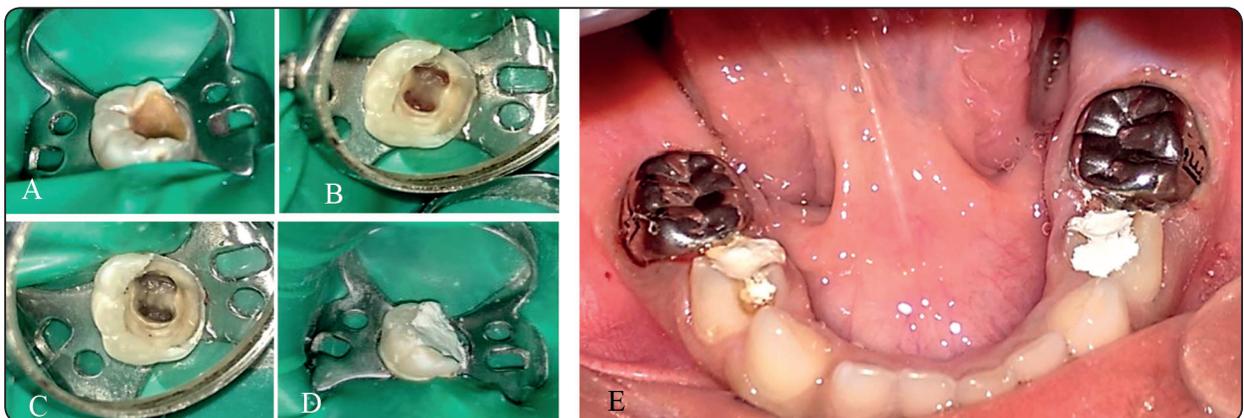


Fig. (4) Preoperative photograph of carious lower right 2nd primary molar indicated for FC pulpotomy (A). Hemostasis of coronal pulp tissue (B). The pulp stump post-formocresol application (C). The cavity was filled with IRM (D) and covered with stainless steel crown (E).

In both groups, the coronal cavities of treated teeth were sealed with reinforced zinc oxide eugenol (IRM, Caulk Dentsply, USA), restored with stainless steel crowns (3M) and cemented with glass-ionomer luting cement (Ketac Cem, 3M ESPE, USA). The patients were directed to maintain good oral hygiene and were recalled for clinical and radiographic evaluation at 3, 6, 9 and 12 months.

The criteria for clinical success were the absence of the following: symptoms of pain, tenderness to percussion, abscess, soft-tissue swelling or sinus tract, and pathologic tooth mobility at recall visits. Whereas, the radiographic criteria for success were: normal PDL space, the absence of internal and/or external root resorption, no signs of any radiolucency in the furcation and/or the periapical area, and no canal calcification.

The overall success rate, both clinically and radiographically, was assessed for each patient. If a pulpotomized treated tooth deviated from one of the success criteria, it was regarded as a treatment failure. All data were statistically analyzed using Fisher exact test.

**RESULTS**

Twenty children (13 girls and 7 boys) were included in this study, 40 lower primary molars were

selected for pulpotomy and divided equally into two groups, the first group was treated with PRP and the second one was treated with FC. All pulpotomized teeth were evaluated clinically and radiologically at 3, 6, 9 and 12 months postoperative.

**Clinical evaluation**

Clinical results are shown in table (1) revealed that all molars treated with PRP were clinically symptoms-free during recall visits, while the FC group had two molars (10%) with clinical failure; one molar presented with chronic abscess at 9 months and another elicited pain upon percussion at 12 months (Fig 5). The overall clinical success rate at the end of the study was 100% and 90% for PRP and FC group, respectively. The failure rate for both groups during recall time was not statistically significant ( $p > 0.05$ ). Soft tissue swelling, abnormal tooth mobility, fistula/sinus were not reported in both groups during the entire follow-up period.

**Radiographic evaluation**

Radiographic evaluation illustrated in table 2 and figures (6&7). The overall radiographic success was 90% for PRP and 85% for FC at the end of the study. The failure rate for both groups during recall time was not statistically significant ( $p > 0.05$ ).

TABLE (1) Clinical evaluation of PRP and FC pulpotomies during recall time.

Groups	Follow-up period							
	3 months		6 months		9 months		12 months	
	Success	Failure	Success	Failure	Success	Failure	Success	Failure
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
PRP	20 (100)	0 (---)	20 (100)	0 (---)	20 (100)	0 (---)	20 (100)	0 (---)
FC	20 (100)	0 (---)	20 (100)	0 (---)	18 (90)	2 (10)	18 (90)	2 (10)
p*	1.000		1.000		1.000		1.000	

N (%) = Number of teeth and percentage for each group. PRP= Plasma-rich platelet. FC= Formocresol.

\*Significant at  $p < 0.0$



Fig. (5) FC group at 12 months; photograph showing chronic abscess of lower 2nd primary molar.

At 3 and 6 months of recall time, there was no radiographic failure in both groups. In PRP group, radiographic failures were 10%; one molar showed furcal radiolucency at 9 months, and another showed root canal calcification of apical third of one root at 12 months (Figs 8, 9), whereas, in FC group, the radiographic failures were 15%; one molar (5%) showed periapical radiolucency, and another (5%) showed internal and external root resorption associated with periapical radiolucency at 9 months (Fig 10). Widened PDL space was detected in the last case at 12 months (Fig 11). The radiographic failure rate of both groups at 3, 6, 9 and 12 months was not statistically significant ( $p>0.05$ ).

TABLE (2) Radiographic evaluation of PRP and FC pulpotomies during recall time.

Groups	Follow-up period							
	3 months		6 months		9 months		12 months	
	Success	Failure	Success	Failure	Success	Failure	Success	Failure
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
PRP	20 (100)	0 (---)	20 (100)	0 (---)	19 (95)	1 (5)	18 (90)	2 (10)
FC	20 (100)	0 (---)	20 (100)	2 (---)	18 (90)	2 (10)	17 (85)	3 (15)
p*	1.000		1.000		1.000		1.000	

N (%) = Number of teeth and percentage for each group. PRP= Plasma-rich platelet. FC= Formocresol. \*Significant at  $p < 0.05$ .

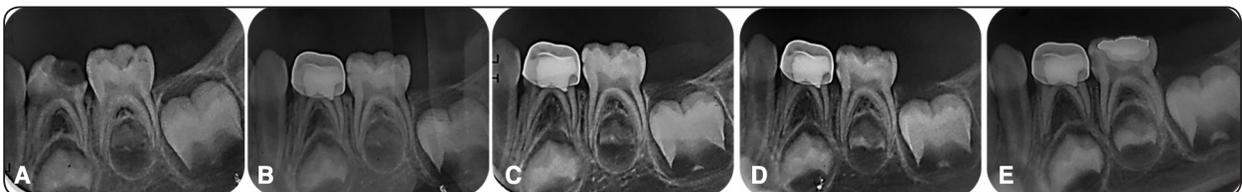


Fig. (6) Preoperative intraoral peri-apical (IOPA) radiograph of a PRP case showing deep carious lower 1st primary molar (A). Follow-up radiographs at 3, 6, 9, and 12 months, respectively (B, C, D, E).



Fig. (7) Preoperative IOPA radiograph showing a deep caries of lower 2nd primary molar indicated for FC pulpotomy (A). Radiographs at 3, 6, 9 and 12 months postoperative, respectively (B, C, D, E).



Fig. (8) Preoperative IOPA for a case in the PRP group (A). 3-month post-treatment of lower 2nd primary molar (B). Radiograph showing furcal radiolucency at 9 months (arrow) (C).

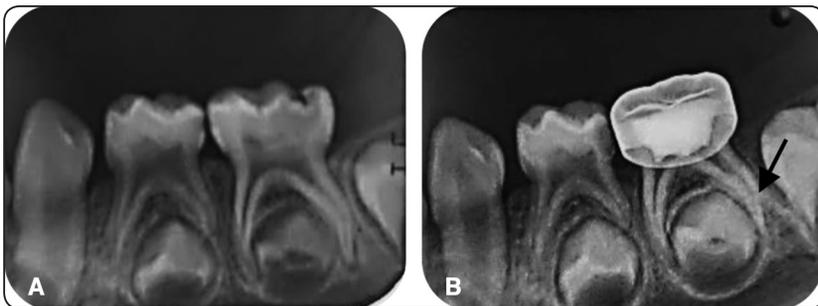


Fig. (9) Preoperative IOPA radiograph of a case in the PRP group (A). 12-month post-treatment showing calcification at the apical third of distal root canal of lower 2nd primary molar (arrow) (B).



Fig. (10) Preoperative IOPA radiograph of carious lower 2nd primary molar that indicated for FC pulpotomy (A). 9-month post-treatment showing periapical radiolucency associated with internal/external root resorption (B).



Fig. (11) Preoperative IOPA radiograph showing deep caries of lower 2nd primary molar indicated for FC pulpotomy (A). 3-month postoperative (B). Radiographic failure due to widening of periodontal ligament space of the mesial root at 12 months (arrow) (C).

## DISCUSSION

The success of pulpotomy procedure depends on various vital factors; the important one is the right choice of material used. The ideal material is not yet been identified. Therefore, it is important to develop new inherently biocompatible and biologically based therapeutics to maintain the pulp vital<sup>(3,6)</sup>.

The most suitable and successful approach to therapeutic pulp therapy seems to be the regenerative medicine since its main principle is to mimic the physiological events of growth and development<sup>(24)</sup>. Recently, PRP represents the attractive agent in regenerative medicine and a novel approach to tissue regeneration, gained by sequestering and concentrating the platelets<sup>(25,26)</sup>. Moreover, autologous preparation avoids the risks of diseases transmission and immunogenic reactions, non-expensive, and it can be simply achieved by commercially available systems that can be easily utilized and prepared in the dental operating room<sup>(27)</sup>. All these advantages encourage the use and evaluation of PRP clinically and radiographically in this study as pulpotomy medicament in primary molars.

The ages of the patients in this study ranged from 5-8 years to avoid the lack of cooperation that may be very common in children below 5 years, while in children above 8 years, more than 3/4 of their primary roots may show physiologic root resorption<sup>(28,29)</sup>.

In the current study, PRP preparation obtained by two-step centrifugation of plasma, usually 1/10 of the initial blood volume<sup>(30)</sup>, and treated with Acetic acid Citrate Dextrose as an anticoagulant, and 10% calcium chloride as an activator<sup>(31)</sup>.

To avoid the platelet activation and degranulation, ACD is the preferred anticoagulant, as it is considered one of the best supports in preserving the platelet viability. Soon after centrifugation, The PRP must be separated from the PPP to avoid the

slow diffusion of the platelets concentrate into the PPP over time that would decrease the PRP platelets count<sup>(30)</sup>.

Adding  $\text{CaCl}_2$  to PRP, as a clot activator<sup>(31)</sup>, neutralizes the anticoagulant effect, and converts fibrinogen into fibrin, leads to platelet gel concentrate, and automatically activates  $\alpha$ -granules results in the release of significant high concentrations of growth factors, which have a significant role in the regulation of the growth and development of numerous tissues<sup>(10,32)</sup>. Also, it avoids the risk of disease transmission or life-threatening coagulopathies due to antibody formation associated with the use of exogenous activators, such as bovine thrombin<sup>(11)</sup>.

Because of PRP should be only activated when they are ready to use<sup>(30)</sup>, and they also need to survive as much as possible, the collagen membrane used in this study seems to be useful to survive PRP in the traumatized pulp tissue and to accelerate and promote regeneration that may significantly improve the clinical outcomes<sup>(2,10,33)</sup>. Also, it was found that the platelet concentrate secretes about 70% of their stored growth factors within 10 min that approximate to 100% within the first hour. Furthermore, additional amounts of growth factors are synthesized for approximately 8 days till they are depleted and dead<sup>(30)</sup>. Amongst the other advantages, the collagen membrane acts as a barrier<sup>(2,34)</sup> preventing the unwanted IRM that comes into direct contact with vital pulp tissue and protecting it from the undesirable forces of the restorative procedures.

In the present study, formocresol was used as a control group since it is still the gold standard medicament for pulpotomy in primary dentition because of its ease of use with excellent clinical results.

The clinical success rate in this study was 100% and 90% for PRP and FC, respectively. This agrees with Kalaskar and Damle<sup>(15)</sup> who reported 100% PRP success rate. This can be explained by the pre-

packaged growth factors contained in  $\alpha$ -granules of PRP<sup>(35)</sup> leading to local stem cells stimulation added to its synergistic effect on the prevention of infection and necrosis<sup>(2)</sup> due to its anti-microbial efficacy, healing-promoting properties<sup>(36)</sup>, and biocompatibility with the pulp tissue<sup>(37)</sup>. Also, this is confirmed by the explanation of Nagar and Viswanath 2012<sup>(38)</sup> who stated that PRP inhibits inflammation by decreasing the early macrophage proliferation, increasing angiogenesis via increasing the tissues vascularity and granulation tissue, and epithelial cell production, and it has also an antimicrobial effect.

This study also showed 90% clinical success rate for formocresol in comparison with the findings of Havale et al.<sup>(39)</sup>, Agamy et al.<sup>(28)</sup>, Markovic et al.<sup>(40)</sup>, Huth et al.<sup>(41)</sup>, and Ruby et al.<sup>(42)</sup> who reported 86.7%, 90%, 91%, 96% and 100%, respectively. They explained that their clinical failures as post-operative chronic abscesses and pain with percussion may be attributed to chronic inflammation of inter-radicular areas and periodontal inflammation, respectively.

On the other hand, failure of pulpotomy is normally detected radiographically, as the tooth may be clinically asymptomatic.

Radiographically, the success rate was 90% for both groups. The success rate of PRP compared to the results of Kalaskar and Damle<sup>(15)</sup> who reported 100% success. While, the success rate of FC group compared to Havale et al.<sup>(39)</sup>, Thaliyath & Joseph<sup>(43)</sup> and Ansari & Ranjpour<sup>(44)</sup> who recorded radiographic success of 56.7%, 67.7%, and 85%, respectively.

The signs of the radiographic failure seen in PRP group were minor root canal calcification and pathological furcal radiolucency. The exact reason is difficult to explain. Hence, further investigation with histological corroboration in this area is needed. However, PRP has been shown to stimulate cell proliferation of fibroblasts and osteoblasts increasing osteogenesis and to upregulate osteocalcin in these cells, and this may be a contributing factor to the

minor canal obliteration in this study<sup>(45,46)</sup>. Furcal radiolucency may be due to misdiagnosis of existing radicular pulpal inflammation before pulpotomy procedure<sup>(47)</sup>.

While in FC group, Widening of PDL space, furcal radiolucency, and periapical radiolucency associated with internal and external root resorption were reported as radiographic failure. These findings agree with Eidelman et al.<sup>(48)</sup> and Olatosi et al.<sup>(47)</sup> who reported a case with internal root resorption that recorded 7% and 4.2%, respectively. Holan et al.<sup>(49)</sup> also found 4 teeth (13.8%) with inter-radicular and/or periapical radiolucent defects which were associated with internal and external root resorption.

The radiographic failure in this study may be due to seepage of the smaller size of the formocresol molecules into the periapical region via the pulpal canals or into the furcation area through the accessory canals or via the thin and permeable pulpal floor, which is a nature in primary molars<sup>(49)</sup>. Also, the misdiagnosed chronic radicular pulpal inflammation prior to pulpotomy is more likely to be a contributing factor<sup>(50)</sup>.

However, the main factors almost making it almost difficult to compare two of published studies are the lack of a standardized protocol of PRP preparation, lack of commonly modern quality accepted evaluation criteria and the duration of recall time which may affect the final outcome. In addition, few little PRP pulpotomies are present in published studies.

## RECOMMENDATION

Further researches on this topic are required with regard to histological studies that are also needed to determine the pulpal response to this material. Moreover, a larger sample size and longer follow-up periods clinically, radiographically and histologically are also highly recommended. Likewise, it is important to study and determine the possible effects of PRP on succedaneous teeth.

## CONCLUSION

The successful outcome of vital pulp therapy in this study confirms the efficacy of PRP as a potent therapeutic medicament in pulpotomy of primary teeth. Also, the findings in the present study suggest that PRP had a promising effect, and it could be an alternative to the currently used pulpotomy medicament.

## REFERENSES

- 1- Fuks A. Current concepts in vital primary pulp therapy. *Eur J Paediatr Dent* 2002; 3:115-120.
- 2- Maden M, Orhan O, Ertuğrul F, Sengüven B. The inflammatory response of the pulp after direct capping with platelet-rich plasma and enamel matrix derivative: A controlled animal study. *OJST* 2014; 4:14-21.
- 3- Kumar Praveen NH, Nayak Rashmi, Bhaskar Vipin K, Mopkar Pujan P. Pulpotomy Medicaments: Continued Search for New Alternatives- A Review. *OHDM* 2014; 13:883-890.
- 4- Gupta G, Rana V, Srivastava N, Chandna P. Laser Pulpotomy—An Effective Alternative to Conventional Techniques: A 12 Months Clinicoradiographic Study. *Int J Clin Pediatr Dent* 2015; 8: 18-21.
- 5- Somani R, Zaidi I, Jaidka S. Platelet rich plasma-A Healing aid and perfect Enhancement Factor: Review and Case Report. *Int J Clin Pediatr Dent* 2011; 4:69-75.
- 6- Keswani D, Pandey R, Ansari A, Gupta S. Comparative evaluation of platelet-rich fibrin and mineral trioxide aggregate as pulpotomy agents in permanent teeth with incomplete root development: a randomized controlled trial. *J Endod* 2014; 40:599-605.
- 7- Hiremath H, Saikalyan S, Kulkarni S, Hiremath V. Second-generation platelet concentrate (PRF) as a pulpotomy medicament in a permanent molar with pulpitis: a case report. *Int Endod J* 2012; 45:105-112.
- 8- Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich Plasma: growth factor enhancement for bone graft. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; 85:638-640.
- 9- Anila S, Nandakumar K. Applications of Platelet Rich Plasma for Regenerative Therapy in Periodontics. *Trends Biomater Artif Organs* 2006; 20: 78-83.
- 10- Khoshzaban A, Heidari-keshel S, Aghazadeh S, Bashtar M. Radiographic & histopathological analysis in calvarias bone regeneration process by platelet-rich plasma, platelet-rich plasma–gel And auto bone chips in rat. *JPS* 2010; 1:40-45.
- 11- Tsay RC, Vo J, Burke A, Eisig SB, Lu HH, Landesberg R. Differential growth factor retention by platelet-rich plasma composites. *J Oral Maxillofac Surg* 2005; 63:521-528.
- 12- Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. *J Extra Corpor Technol* 2004; 36:28-35.
- 13- Kotosovilis S, Markou N, Pepelassi E, Nikolidakis D. The adjunctive use of platelet- rich plasma in the therapy of periodontal intraosseous defects: a systematic review. *J Periodontal Res* 2010; 45:428-443.
- 14- Fukaya M, Ito A. A New Economic Method for Preparing Platelet-rich Plasma. *Plast Reconstr Surg Glob Open* 2014; 2: e162.
- 15- Kalaskar RR, Damle SG. Comparative evaluation of lyophilized freeze dried platelet derived preparation with calcium hydroxide as pulpotomy agents in primary molars. *J Indian Soc Pedod Prev Dent* 2004; 22:24-29.
- 16- Solomon RV, Faizuddin U, Karunakar P, Sarvani GD, Soumya SS. Coronal Pulpotomy Technique Analysis as an Alternative to Pulpectomy for Preserving the Tooth Vitality, in the Context of Tissue Regeneration: A Correlated Clinical Study across 4 Adult Permanent Molars. *Case Rep Dent* 2015; 2015: ID 916060, 12 pages.
- 17- Danilović V, Petrović V, Marković D, Aleksić Z. Histological evaluation of platelet rich plasma and hydroxyapatite in apexogenesis: study on experimental animals. *Vojnosanit Pregl* 2008; 65:128-134.
- 18- Döri F, Huszár T, Nikolidakis D, Arweiler N, Gera I, Sculean A. Effect of platelet-rich plasma on the healing of intra-bony defects treated with a natural bone mineral and a collagen membrane. *J Clin Periodontol* 2007; 34:254-261.
- 19- Gaßling VW, Açil Y, Ingo N, Springer IN, Hubert N, Wiltfang J. Platelet-rich Plasma and Platelet-rich fibrin in human cell culture. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 108:48-55.
- 20- Nofal E, Elkot R, Nofal A, Eldesoky F, Shehata S, Sami M. Evaluation of carboxytherapy and platelet-rich plasma in treatment of periorbital hyperpigmentation: A comparative clinical trial. *JCD Version of Record online* 3 January 2018; DOI: 10.1111/jocd.12478.

- 21- Aggour RL, Gamil L. Antimicrobial Effects of Platelet-rich Plasma against Selected Oral and Periodontal Pathogens. *Pol J Microbiol* 2017; 66: 31-37.
- 22- Amable PR, Carias RB, Teixeira MV, da Cruz Pacheco I, Corrêa do Amaral RJ, Granjeiro JM, et al. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. *Stem Cell Res Ther* 2013; 4:67.
- 23- 23-Al-Mutairi MA, Bawazir OA. Sodium hypochlorite versus Formocresol in primary molars pulpotomies: a randomized clinical trial. *Eur J Paediatr Dent* 2013; 14:33-36.
- 24- Petrović V, Pejčić N, Čakić S. The Influence of Different Therapeutic Modalities and Platelet Rich Plasma on Apexogenesis – a Preliminary Study in Monkeys. *Adv Clin Exp Med* 2013; 22: 469-479.
- 25- Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Aleksic Z, Kenney EB. Effectiveness of a combination of platelet-rich plasma, bovine porous bone mineral and guided tissue regeneration in the treatment of mandibular grade II molar furcations in humans. *J Clin Periodontol* 2003; 30:746-751.
- 26- Tabatabayi MH, Tavakoli A, Ameghani BA. Regenerative property of PRF used as capping material in pulpotomy in dogs. *Biomed Res* 2017; 28: 4634-4639.
- 27- Meury T KLSTAM. Effect of platelet-rich-plasma on bone marrow stromal cell differentiation. [Abstract]. *ASBMR 26th annual meeting* 2007.
- 28- Agamy HA, Bakry NS, Mounir MM, Avery DR. Comparison of mineral trioxide aggregate and formocresol as pulp-capping agents in pulpotomized primary teeth. *Pediatr Dent* 2004; 26:302-309.
- 29- Godhi B, Sood PB, Sharma A. Effects of mineral trioxide aggregate and formocresol on vital pulp after pulpotomy of primary molars: An in vivo study. *Contemp Clin Dent* 2011; 2:296-301
- 30- Marx RE. Platelet-Rich Plasma (PRP): What Is PRP and What Is Not PRP? *Implant Dent* 2001; 10: 225-228.
- 31- Anitua E, Sánchez M, Orive G, Andía I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Biomaterials* 2007; 28: 4551-4560.
- 32- Anitua E, Andía I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost* 2004; 91:4-15.
- 33- Butterfield KJ, Bennett J, Gronowicz G, Adams D. Effect of platelet-rich plasma with autogenous bone graft for maxillary sinus augmentation in a rabbit model. *J Oral Maxillofac Surg* 2005; 63:370-376.
- 34- Soheilifar S, Soheilifar S, Bidgoli M, Torkzaban P. Barrier Membrane, a Device for Regeneration: Properties and Applications. *Avicenna J Dent Res* 2014; 6: e21343.
- 35- Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB. Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. *J Periodont Res* 2002; 37:300-306.
- 36- Yang LC, Hu SW, Yan M, Yang JJ, Tsou SH, Lin YY. Antimicrobial activity of platelet-rich plasma and other plasma preparations against periodontal pathogens. *J Periodontol* 2015; 86:310-318.
- 37- Arora N, Ramanayake T, Ren Y, Romanos G. Platelet-rich plasma: a literature review. *Implant Dent* 2009; 18: 303-310.
- 38- Nagar P, Viswanath D. Platelet Rich Plasma/Platelet Rich Fibrin- Uses in Pediatric Dentistry: A Review. *J Dent Oral Biosc* 2012; 3:43-46.
- 39- Havale R, Anegundi R, Indushekar KR, Sudha P. Clinical and Radiographic Evaluation of Pulpotomies In Primary Molars With Formocresol, Glutaraldehyde and Ferric Sulphate. *OHDM* 2013; 12:24-31.
- 40- Markovic D, Zibojinovic V, Bucetic M. Evaluation of three pulpotomy medicaments in primary teeth. *Eur J Paediatr Dent* 2005; 6:133-138.
- 41- Huth KC, Paschos E, Hajek-Al-Khatar N, Hollweck R, Crispin A, Hickel R, et al. Effectiveness of 4 pulpotomy techniques: Randomized controlled trial. *J Dent Res* 2005; 84:1144-1148.
- 42- Ruby JD, Cox CF, Mitchell SC, Makhija S, Chompu-Inwai P, Jackson J. A randomized study of sodium hypochlorite versus formocresol pulpotomy in primary molar teeth. *Int J Paediatr Dent* 2012; 13:1-8.
- 43- Thaliyath A, Joseph T. Glutaraldehyde: an alternative agent in pulpotomy for deciduous teeth. *J Indian Soc Pedod Prev Dent* 1996; 14: 76-79.
- 44- Ansari G, Ranjpour M. Mineral trioxide aggregate and formocresol of primary teeth: a 2-year follow-up. *Int Endod J* 2010; 43: 410-418.

- 45- Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin Oral Implants Res* 2006; 17:212-219.
- 46- van den Dolder J, Mooren R, Vloon AP, Stoelinga PJ, Jansen JA. Platelet-Rich Plasma: Quantification of Growth Factor Levels and the Effect on Growth and Differentiation of Rat Bone Marrow Cells. *Tissue Eng* 2006; 12:3067-3073.
- 47- Olatosi O, Sote E, Orenuga O. Effect of mineral trioxide aggregate and formocresol pulpotomy on vital primary teeth. *Niger J Clin Pract* 2015; 18:292-296.
- 48- Eidelman E, Holan G, Fuks AB. Mineral trioxide aggregate vs. formocresol in pulpotomized primary molars: A preliminary report. *Pediatr Dent* 2001; 23:15-18.
- 49- Holan G, Eidelman E, Fuks AB. Long term evaluation of pulpotomy in primary molars using mineral trioxide aggregate or formocresol. *Pediatr Dent* 2005; 27:129-136.
- 50- Curzon MJ, Roberts JF, Kennedy DB: Editors. *Kennedy's Paediatric Operative Dentistry*. 4th ed. Oxford: Wright; 2002. pp 15-18, 159-161.