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Evaluation of regenerative endodontic procedure in human mature permanent teeth with necrotic pulp and periapical pathology using blood clot & prp: an invivo study

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Abstract:
Objective: *Aim of the present study was to evaluate the effectiveness of platelet rich plasma (PRP) and Blood Clot in Regenerative Endodontic Procedures (REP) of mature nonvital permanent teeth with or without periapical pathology.*
Material and Methods*: After gaining access, root canals were cleaned & irrigated with 1.5% NaOCL & saline, filled with Ca(OH)2 and sealed with cavit. On 2nd appointment irrigation was done with 1.5% NaOCl followed by 17% EDTA , finally with normal saline.*
Autologous PRP was injected into three teeth and fresh bleeding was induced within the root canals of rest under LA without vasoconstrictor. After placing 3 mm of MTA teeth were restored with composite . Follow up visits were done for clinical & radiological assessment using both IOPAR & CBCT.
Statistical analysis: *Z- test, p value ≤ 0.05 as statistically significant.*
Result: *Teeth were asymptomatic, functioning normally showing evidences of bone healing of apical radiolucencies. Positive pulp sensibility response could not be elicited.* ***Conclusion:*** *REP can be undertaken in mature nonvital permanent tooth with apical pathology using PRP/BC as scaffold.*
Key Words: *REPs, blood Clot, PRP, CBCT*

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I. Introduction:

Regenerative endodontic therapy has been defined as "biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of thepulp-dentin complex" (Murray et al 2007**[1]**).

The encouraging results of regenerative endodontics in immature permanent teeth had lead to an immense interest in try of regenerative endodontic procedures in mature permanent teeth with closed apices. With the success achieved with "revascularization" in healing of periapical lesions and hard tissue deposition at apical and lateral walls of the root canals (maturogenesis) in immatureteeth, the processes involved in the healing mechanism are now better understood. Role of variousstem cells, growth factors, Hertwig's epithelial root sheath (HERS) and their interactions in regeneration of tissueshave been documented.

Revascularization in Endodontics is the regenerative treatment that is **biologically based** andis achieved by the application of **tissue engineering** through **development of pulp dentin complex. Tissue engineering** is the employment of biologic therapeutic strategies and the use ofa combination of stem cells and three-dimensional scaffold materials, in the presence of suitable biochemical molecules, to improve or replace biologic function of a tissue. Advancements in tissue engineering are dramatically changing medicine and dentistry. Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences to restoring, maintaining, or replacing biologic function. It involves the interplay among stem cells, growth factors, and scaffolds (biologic matrices). It has become increasingly clear that the intentional manipulation of these three factors can lead to the regeneration of tissue function that would not otherwise occur if repair had taken place without intervention.

Pioneering work supporting the concept of regenerating dental tissues was reported in the 1960s when Dr. B.W. Hermann described the application of calcium hydroxide (Ca[OH]2) for vitalpulp therapy and Professor Nygaard Østby evaluated a revascularization method for reestablishing a pulp dentin complex in permanent teeth with pulpal necrosis[2].

II. Material and Methods:

Total number of 98 teeth in 68 patients aged between 15-50 years were included in the study after thorough clinical and radiological examination-both IOPAR & CBCT in axial, sagittal and coronal views **(Fig.1)** and pulp sensibility tests were done. The patients have been informed about the procedure and consent was taken. The research work was done for dissertation for post graduate study of the Institute under the guidance of same postgraduate teacher of the department. There after clearance from the institutional ethics committee was taken.

First appointment was conducted on 98 teeth in 68 patients. Under Local anaesthesia (2% lidocaine with 1:80,000 epinephrine) and rubber dam application (**Fig.2**), teeth were isolated. Access cavities were modified (**Fig.3**), working lengths were determined (**Fig.4)**. After cleaning and shaping, irrigation was done with copious amount of 1.5% NaOCl

(**Fig.5**). Canals were dried with paper points. Calcium hydroxide (Ultracal XS,Ultradent, USA) was used as intracanal medicament**(Fig.6)** and the access cavities were closed with cavit (3M ESPE)**.** Patient was advised to report after 4 weeks or whenever any problem arises.

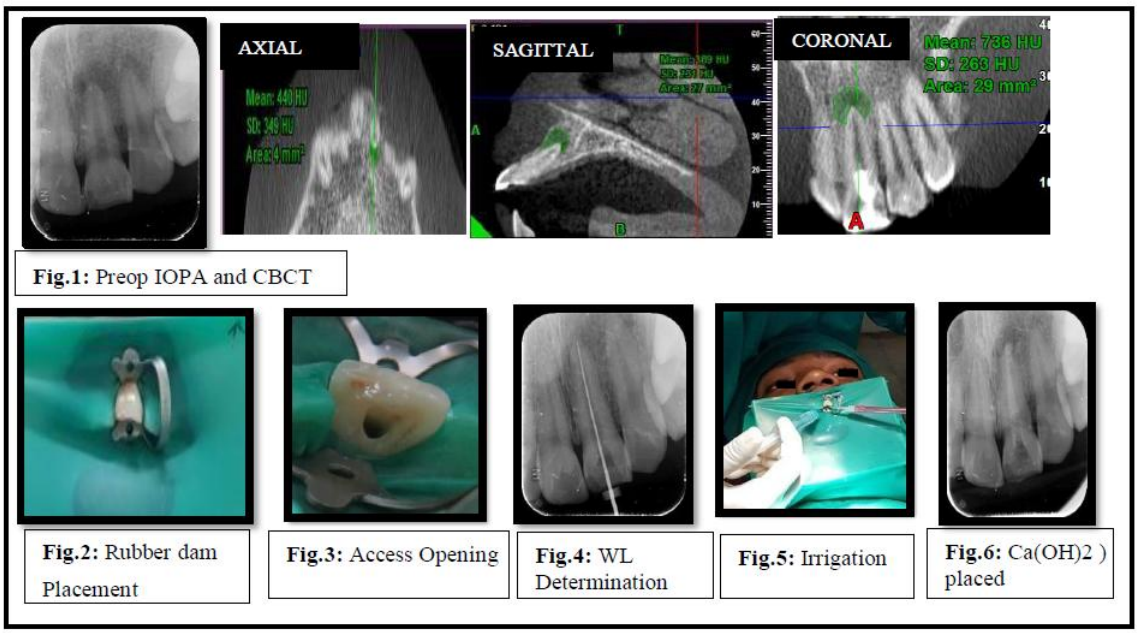


Fig.1: Preop IOPA and CBCT

Fig.2: Rubber dam Placement

Fig.3: Access Opening

Fig.4: WL Determination

Fig.5: Irrigation

Fig.6: Ca(OH)2) placed

66 patients [96 teeth(n)] responded to second appointment. Teeth were reassessed for absence of signs/symptoms of persistent infection , or if the canal was dry followed by temporary filling was removed; copious irrigation was done under isolation for washing the intracanal calcium hydroxide with 17% EDTA (Desmear,Ahmedabad,India) 20ml for 5minutes followed by final irrigation with normal saline [3]. Then the canal was dried with paper points which were then randomly allocated to three different groups [Control RCT group(n=35), PRP group(n=35) & Blood Clot group(n=26)]. In the **PRP group**, the protocol suggested by Dohan et al was followed to prepare the PRP. 10 ml of whole venous blood was taken from the patient's antecubital vein with the help of 10 ml syringe with 24G needle and collected in a glass test tube containing anticoagulant (3.8% sodium citrate) (**Fig.7, 8, 9**).It was first subjected to centrifuge at 1500 rpm (400 g, 8 cm radius of centrifugation) for 10 minutes in the centrifuge machine (REMI R- 8C) (**Fig. 10 & 11**).After the first centrifuge RBC is separated from the plasma (**Fig. 12**). Using a sterile syringe outermost layer containing Platelet poor plasma and platelet rich plasma including the buffy coat was transferred to other glass test tube (**Fig. 13**) and subjected to second centrifuge at 2700 rpm for 17 minutes (**Fig. 14**) in the centrifuge machine and thus RBC is separated from PRP & PPP (**Fig. 15**). Outermost layer (40%) PPP, which is removed with a syringe and discarded (**Fig.16**) and the remaining PRP is mixed with 10% calcium chloride and shaken well. The freshly prepared PRP was then taken in an insulin syringe and injected into the canal (**Fig.17**) below the level of cemento enamel junction and allowed to clot for 10-15 minutes (**Fig.18**). After 10-15 minutes for its clotting collaplug (SRI GOPAL KRISHNA LABSPVT. LTD. MUMBAI)
was placed over it for application of 3mm of MTA (Angelus) below CEJ(**Fig.19**). Waiting for another period of 15-20 minutes as its setting time, teeth were restored with composite resin (3M ESPE Filtek Z350 Xt) (**Fig.20 & 21**)

Fig. 7: Test tube containing 3.8% sodium citrate

Fig. 8: 10mL of blood being withdrawn from the patient's Cubital Vein

Fig. 9: Collected blood isbeing placed in test tube containing anticoagulant

Fig. 10: Inside of the centrifuge machine with the test tube

Fig. 11: First spin at 2400 rpm for 10minutes

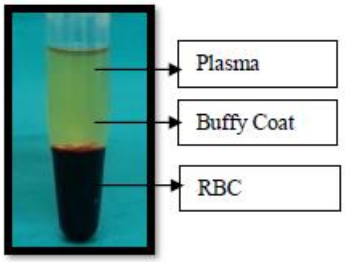


Fig. 12: RBC separated from plasma

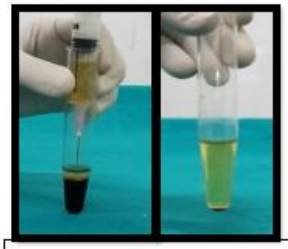


Fig. 13: Plasma and buffy coattaken into another test tube

Fig. 14: Second spin at2700 rpm for 17 minutes

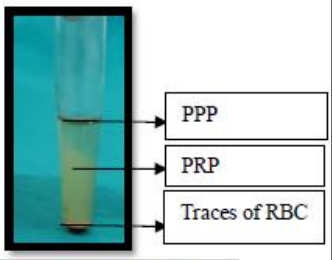


Fig. 15: Freshly Prepared PRPin the middle, with PPP at top

Fig. 16: PPP is removed

Fig. 17: Freshly Prepared PRP injected into the canal by insulin syringe

Fig. 18: PRP allowed to clot 2-3 mm below CEJ for 10minutes, visualized under Dental Operating Microscope.

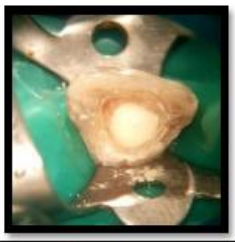


Fig. 19: Biodentine placed over PRP clot

Fig. 20: Coronal restoration with Composite over Biodentine

Fig.21: IOPAR showing composite restoration

In the Blood Clot Group intracanal bleeding was induced upto CEJ **(Fig.22)** from periapex using precurved #20 hand instrument. Waiting 10-15 minutes for the clot formation, collaplug (SRI GOPAL KRISHNA LABS PVT. LTD. MUMBAI) was placed over it for placement of3 mm of MTA (Angelus) upto CEJ. **(Fig.23)** followed by 15-20 minutes of its setting , tooth was restored with composite resin(3M ESPE Filtek Z350 Xt) **(Fig.24)**

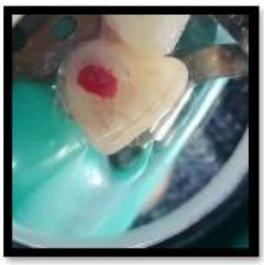


Fig. 22: Induction of bleeding

Fig. 23: Placement of 3 mm of MTA (Angelus) upto CEJ

Fig. 24: Tooth restored with composite resin

In the **Control Group** under rubber dam isolation cavit was removed **(Fig.25) ,** copious irrigation was done for washing Ca(OH)2 with 17% EDTA & Normal saline **(Fig.26)** then canal dried with paper points followed by **Obturation done with Gutta Percha upto Working length. (Fig.27)** & the tooth was restored with composite resin (3M ESPE Filtek Z350 Xt) **(Fig.28)**

Fig. 25: Cavit removed

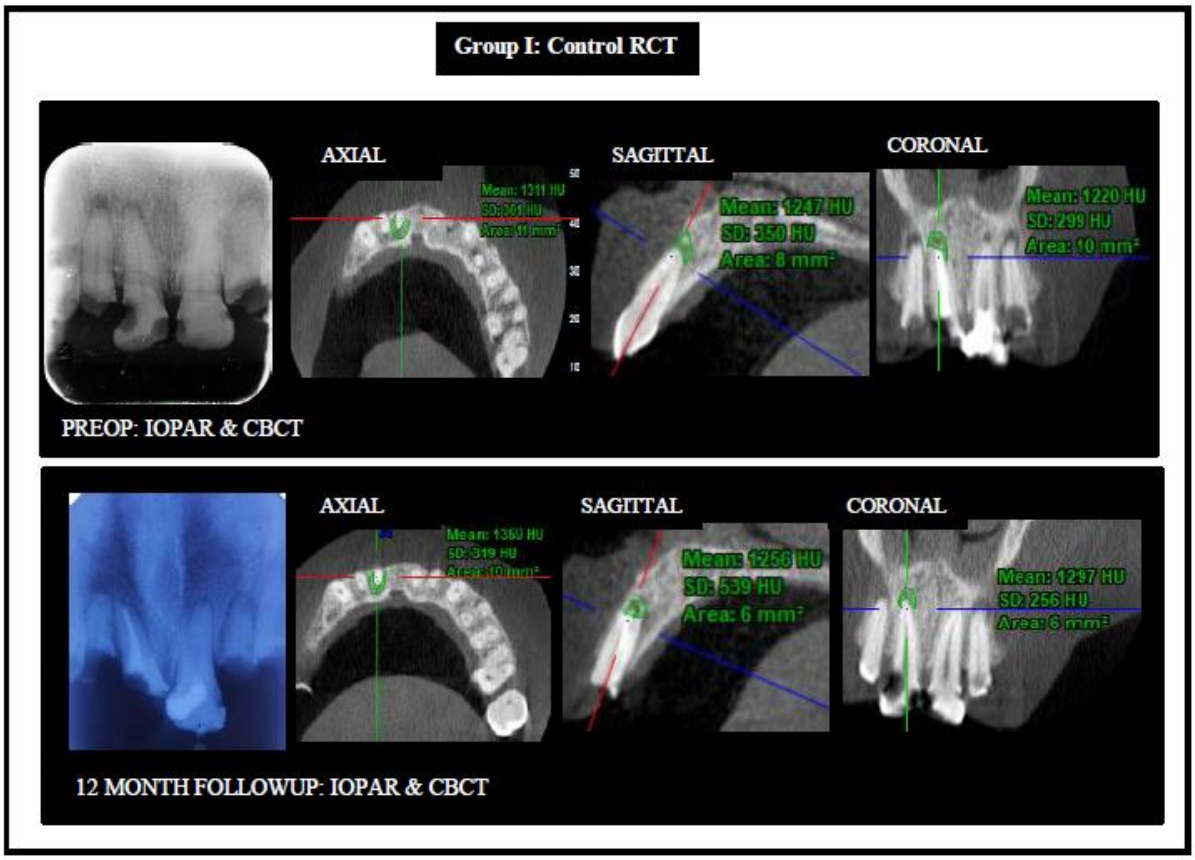
Fig. 26: Ca(OH)2 removed

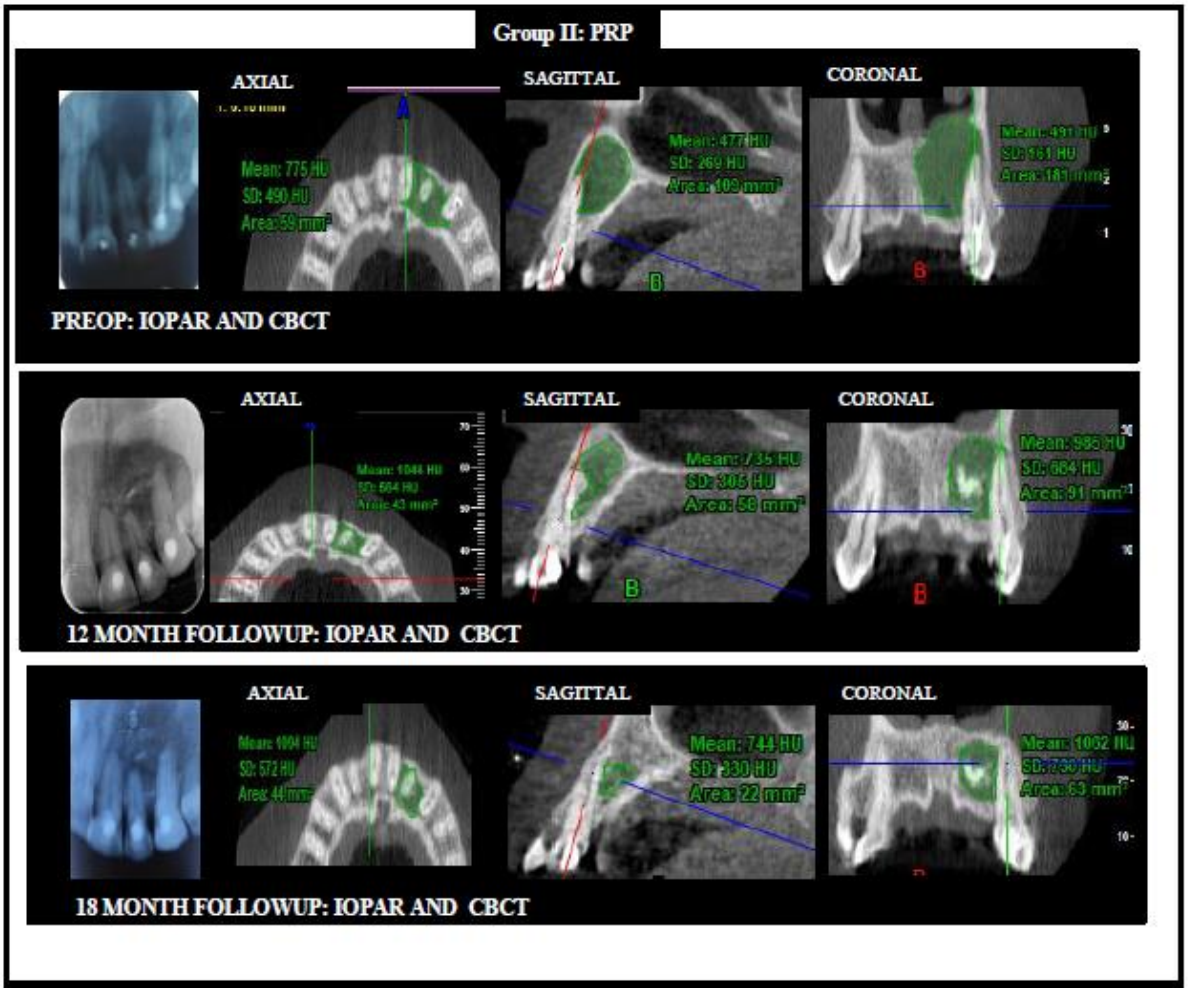
Fig.27: Obturation

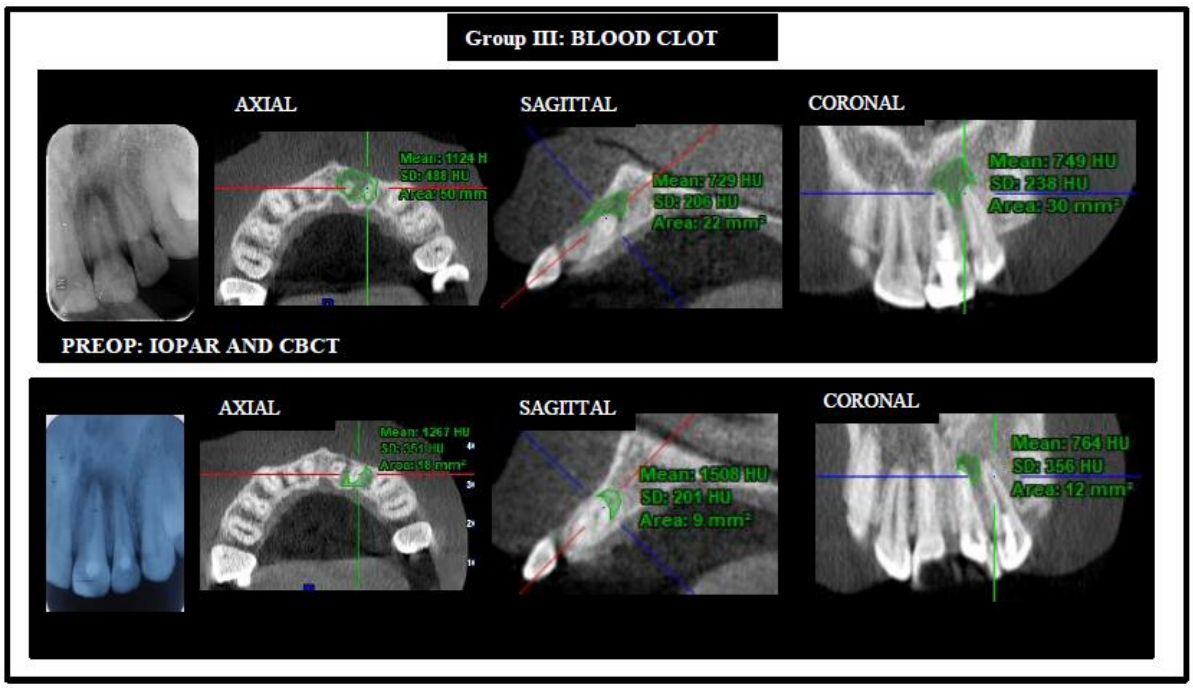
Fig.28: Tooth was restored with composite resin

The patients were recalled every three months. The teeth were assessed clinically and radiographically both through IOPAR and CBCT scans- axial, sagittal and coronal views to evaluate success of REP. Vitality test with cold (Endofrost, Coltene Whaldent. Langenau, Germany) and electric pulp test (EPT) (Confident Dental, Bangalore, India) was done.

Radiological healing assessed through IOPAR was quantitively evaluated through CBCT scans and only the data at the last follow up visit of each case was tabulated for statistical analysis. However, pulp vitality could not be found positive in any of the cases under the study.




Group II: PRP
AXIAL
SAGITTAL
CORONAL
Mean: 775 HU
SD: 490 HU
Area: 59 mm²
Mean: 477 HU
SD: 269 HU
Area: 103 mm²
PREOP: IOPAR AND CBCT
AXIAL
SAGITTAL
CORONAL
Mean: 735 HU
SD: 305 HU
Area: 58 mm²
Mean: 986 HU
SD: 684 HU
Area: 91 mm²
12 MONTH FOLLOWUP: IOPAR AND CBCT
AXIAL
SAGITTAL
CORONAL
Mean: 744 HU
SD: 330 HU
Area: 22 mm²
18 MONTH FOLLOWUP: IOPAR AND CBCT


Group III: BLOOD CLOT
AXIAL
SAGITTAL
CORONAL
Mean: 729 HU
SD: 206 HU
Area: 22 mm²
Mean: 749 HU
SD: 238 HU
Area: 30 mm²
PREOP: IOPAR AND CBCT
AXIAL
SAGITTAL
CORONAL
Mean: 1508 HU
SD: 201 HU
Area: 9 mm²
Mean: 764 HU
SD: 166 HU
Area: 12 mm²

III. Results:

31 teeth (Control n=2, PRP n=20 and BC n=9) was involved with periapical pathology were evaluated for bony healing (reduction in periapical lesion area and gain in Hounsfield unit) using CBCT and rest **21 teeth** were assessed clinically & IOPAR on the basis of elimination of clinical signs and symptoms. All teeth were clinically evaluated. There was no pain,tenderness, swelling, discharging sinus and mobility.

CHART I

Group I: CONTROL GROUP

Pt. No.	Tooth No.	Age/Sex	Last follow Up(M)	Preop				Last Follow up				% Red in A	Preop				Last Follow up				% Gain in GV
				Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
				PERIAPICAL LESION SIZE (mm^2)									GRAY VALUE (HOUNSFIELD UNIT)								
1)	11	40/F	12	11	8	10	9.6	10	6	6	7.3	23.9	1311	1247	1220	1259.3	1359	1256	1297	1304	3.5
2)	21	21/M	9	14	32	37	27.6	11	29	24	21.3	22.8	782	579	594	651.6	1045	638	625	769.3	18
3)	43	53/F	9	PRE OP & POST OP: LAMINA DURA INTACT & REGULAR PDL SPACE																	
	44																				
4)	14	20/M	9																		
5)	44	35/F	12																		
MEDIAN			9				19				14	Overall % Red				955				1037	Overall % Gain
IQR			9-12				9.6-28				7.3-21	23.12				652-1259				769-13	8.5

Ax.-Axial, Sag.-Sagittal & Cor.- Coronal views of CBCT scan Av.- Average Red.- Reduction

CHART II

Group II: PRP GROUP

Pt. No.	Tooth No.	Age Sex	Last follow Up(M)	Preop				Last Follow up				% Red in A	Preop				Last Follow up				% Gain in GV
				Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
				PERIAPICAL LESION SIZE (mm^2)									GRAY VALUE (HOUNSFIELD UNIT)								
1)	21	34/F	18	59	52	164	116.2	44	12	34	51.4	55.77	775	642	493	606.1	1094	1183	630	997.8	64.6
	22				143	220			98	102				504	523			1006	1074		
	23				109	181			22	63				477	491			744	1062		
2)	21	43/M	12	10	12	8	10	7	8	7	7.3	27.00	1053	546	681	760	1092	737	736	855	12.50
3)	11	22/F	12	15	39	40	31.3	13	34	37	28	10.54	787	534	569	630	1319	606	573	832.6	32.16
4)	21	40/F	6	14	8	14	12	6	4	12	7.3	39.17	736	449	644	609.6	1308	899	981	1062.6	74.31
	22			10	13	32	18.3	2	6	8	5.3	71.04	979	860	788	875.6	1127	1219	1319	1221.6	39.52
5)	21	25/F	9	5	4	12	7	3	3	7	4.3	38.57	1026	464	685	725	1290	1125	955	1123.3	54.94
	11			13	8	9	10	9	7	7	7.6	24.00	1373	669	959	1000.3	1476	1133	1191	1266.6	26.62
6)	11	23/F	6	73	134	232	146.3	23	76	115	71.3	51.26	499	347	361	402.3	836	500	595	643.6	59.98
7)	31	13/F	3	11	25	16	17.3	5	10	12	9	47.9	586	399	527	504	905	525	405	611.6	21.35
8)	11	28/M	3	11	12	6	9.6	5	4	4	4.3	55.21	1007	590	425	674	855	606	589	683.3	1.38
	12			22	7	11	13.3	9	5	9	7.6	42.86	914	870	424	736	980	856	753	863	17.26

Ax.-Axial, Sag.-Sagittal & Cor.- Coronal views of CBCT scan Av.- Average Red.- Reduction

CHART II

Group II: PRP GROUP contd.																					
Pt. No.	Tooth No.	Age Sex	Last follow Up(M)	Preop				Last Follow up				% Red in A	Preop				Last Follow up				% Gain in GV
				Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
9)	21	15/M	3	3	8	4	5	2	7	3	4	20	571	779	697	682.3	1298	976	1191	1155	69.2
10)	12	36/F	3	18	10	8	11.3	16	6	6	9.3	17.7	946	778	917	927.6	1033	802	1059	964.6	3.99
	11			9	5	6	6.6	3	4	4	3.6	45.4	782	711	772	755	886	754	883	841	11.3
11)	21	18/M	6	39	7	5	17	6	6	4	5.3	68.82	806	861	1017	894.6	1290	1422	1392	1368	52.9
12)	22	38/F	3	34	56	95	61.6	15	42	27	28	54.5	582	661	631	624.6	1217	1019	1115	1117	78.8
	21			5	26	8	13	3	18	7	9.3	28.46	715	502	804	673.6	1032	640	957	876.3	30.09
13)	22	25/M	9	4	30	13	15.6	4	18	9	10.3	33.97	1071	766	802	879.6	1253	667	964	961.3	9.29
14)	21	17/F	6	PRE OP & POST OP: LAMINA DURA INTACT & REGULAR PDL SPACE																	
	11																				
15)	21	24/M	12																		
16)	14	30/F	6																		
17)	14	21/F	9																		
	15																				
7)	41	13/F	3																		
9)	11	15/M	3																		
18)	13	46/F	3																		
19)	11	17/F	3																		
	12																				
12)	11	38/F	3																		
13)	21	25/M	9																		
MEDIAN			6				13				7.6	Overall % Red.				704				963	Overall % Gain
IQR			3-9				9.9-22				5.1-15	47.68				621-877				839-1131	34.6

Ax.-Axial**, Sag.-**Sagittal & **Cor.-** Coronal views of CBCT scan **Av.-** Average **Red.-** Reduction

CHART III

Group III: BLOOD CLOT GROUP																					
Pt. No.	Tooth No.	Age Sex	Last follow Up(M)	Preop				Last Follow up				% Red in A	Preop				Last Follow up				% Gain in GV
				Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.		Axial	Sagittal	Coronal	Av.	Axial	Sagittal	Coronal	Av.	
				PERIAPICAL LESION SIZE (mm^2)									GRAY VALUE (HOUNSFIELD UNIT)								
1)	11	17/F	6	6	33	17	18.6	4	11	10	8.3	55.3	826	808	684	772.6	977	772	771	840	8.72
2)	21	24/F	12	13	13	16	14	3	12	4	6.3	55	696	642	870	736	850	522	553	761	3.39
3)	22	23/M	6	75	85	138	99.3	52	75	101	76	23.4	524	669	566	586.3	711	530	631	624	6.4
4)	21	21/M	3	22	31	25	26	16	17	16	16.3	37.3	760	744	850	784.6	973	755	728	818.6	4.33
	11		3	50	22	30	34	18	9	12	13	61.7	1124	729	749	867.3	1267	1508	764	1179.6	36
5)	21	40/F	3	No apical periodontis preoperatively it developed postoperative which revealed in CBCT scan at last followup visits: Considered failure																	
6)	21	23/M	6	4	27	9	13.3	3	12	4	6.3	52.6	440	389	480	436.3	850	522	553	641.6	47
7)	21	14/F	3	4	10	6	6.6	3	4	4	3.6	45.4	1190	738	930	952.6	1323	724	940	995.6	4.5
8)	22	18/M	12	39	25	52	38.6	12	9	17	9.3	75.9	806	852	759	805.6	1269	889	1442	1200	48.9
9)	11	19/F	3	5	8	5	6	4	5	3	4	33.3	1442	932	1382	1252	1467	1252	1582	1433.6	14.5
10)	11	40/F	PRE OP & POST OP: LAMINA DURA INTACT & REGULAR PDL SPACE																		
11)	22	20/F																			
	11																				
MEDIAN			6				19				7.3	Overall % Red.				785				840	Overall % Gain
IQR			3-6				10-36				3.9-14	44.17				661-910				701-1190	18.08

Ax.-Axial**, Sag.**-Sagittal & **Cor.**- Coronal views of CBCT scan **Av.**- Average **Red.**- Reduction #- **REP Failure**

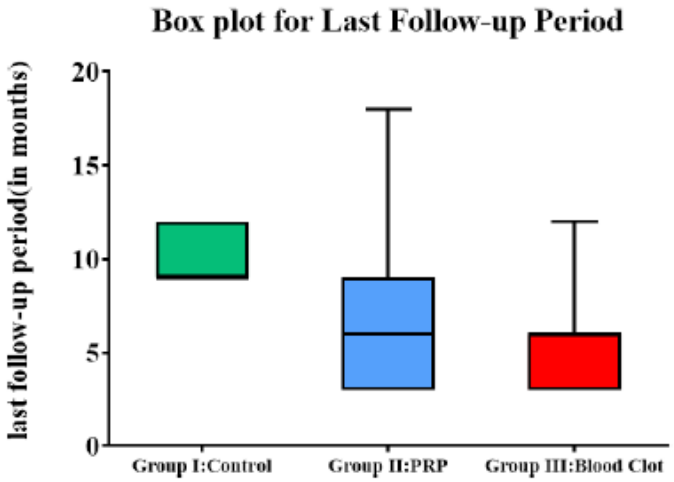
On comparison of the last follow-up periods between the three groups it was seen that medianof follow up period in this study was 9 months (range 9-12), 6 months (range 3-9) and 6 months (range 3-6) in Control, PRP and BC group respectively. Kruskal-Wallis test provided very weak evidence of a difference (P=0.06) between the mean ranks of last follow-up periods between the three groups, implying **no association between the last follow-up periods and the effect of it on the treatment outcomes. (Table 1, Box Plots)**

TABLE 1: COMPARISON OF LAST FOLLOW-UP PERIODS BETWEEN THE THREE GROUPS

Groups	Number (n)	Median	IQR	H Value	P value
Group I: Control	5	9	9-12	5.78	0.06
Group II: PRP	19	6	3-9		
Group III: Blood Clot	9	6	3-6		

n: number of patients

Median and IQR is calculated for all observations



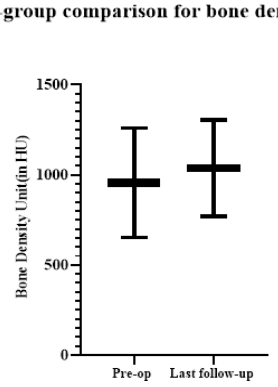
Wilcoxon signed rank test was carried out to evaluate the differences between the preop and post op values for **bone densityunits** and it generated the following results **(Table 2, Box Plots)**

i. Control: no statistically significant difference (P=0.18)
ii. PRP**:** **highly statistically significant** difference (P<0.001)
iii. Blood Clot: **highly statistically significant** difference (P=0.008)

TABLE 2: COMPARISON OF THE BONE DENSITY UNITS BETWEEN THE BASELINE AND THE FOLLOWUP MEASUREMENTS FOR EACH GROUP

Descriptive statistics	**Preop**	**Last followup**	**Z value**	**P value**
Group I: Control	**(n=2)**	**(n=2)**		
Median	955	1037	1.34	0.18
IQR	652-1259	769-1304		
Group II: PRP	**(n=18)**	**(n=18)**		
Median	704	963	3.72	<0.001
IQR	621-877	839-1131		
Group III: Blood Clot	**(n=9)**	**(n=10)**		
Median	785	840	2.67	0.008
IQR	661-910	701-1190		

n:number of tooth
Median and IQR is calculated for all observations



Wilcoxon signed rank test was carried out to evaluate the differences between the preop and post-op values for **periapicallesion size** and it generated the following results

i. Control: no statistically significant difference (P=0.18)

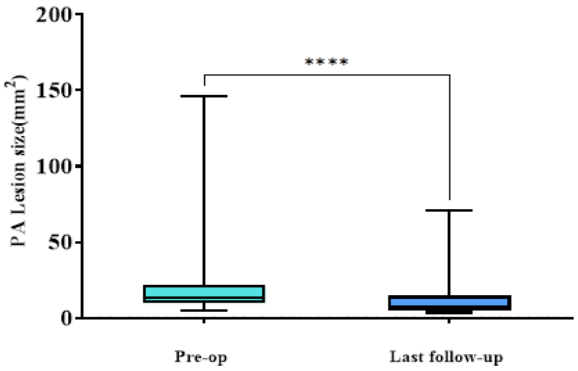
ii. PRP: **very strong evidence of a statistically significant difference** (P<0.001)

iii. Blood Clot: **strong evidence of a statistically significant difference** (P=0.013) **(Table 7, Box Plots)**

TABLE 3: COMPARISON OF THE PERIAPICAL LESION SIZE BETWEEN THE BASELINE AND THE FOLLOWUP MEASUREMENTS FOR EACH GROUP

Descriptive statistics	Preop	Last follow up	Z value	P value
Group I: Control	**(n=2)**	**(n=2)**		
Median	19	14	-1.34	0.18
IQR	9.6-28	7.3-21		
Group II: PRP	**(n=18)**	**(n=18)**		
Median	13	7.6	-3.72	<0.001
IQR	9.9-22	5.1-15		
Group III: Blood Clot	**(n=9)**	**(n=10)**		
Median	19	7.3	-2.67	0.008
IQR	10-36	3.9-14		

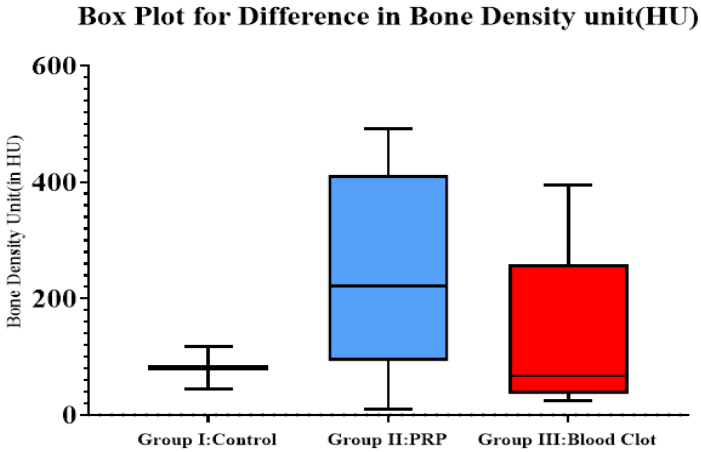
Box plot for Intra-group comparison for lesion size of Group II:PRP



Kruskal-Wallis test provided very weak evidence of a difference (P=0.15) between the mean ranks of bone density gainbetween the three groups. **(Table 4 & Box Plots)**

TABLE 4: COMPARISON OF THE BONE DENSITY OF THE LESIONS OF THE THREE GROUPS AT LAST FOLLOW UP PERIOD WITH RESPECT TO THE PREOPERATIVE AREA

Groups	Number (n)	Median	IQR	H value	P value
Group I: Control	2	81	45-118	3.78	0.15
Group II: PRP	18	222	93-412		
Group III: Blood Clot	9	67	36-259		

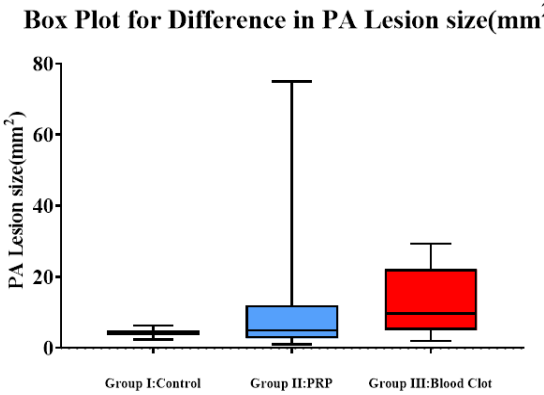


Kruskal-Wallis test provided very weak evidence of a difference [P=0.36] between the mean ranks of lesion size reduction between the three groups. **(Table 5 & Box Plots)**

TABLE 5: COMPARISON OF THE DIFFERENCE OF RADIOLUCENT AREA OF LESIONS OF THE THREE GROUPS AT LAST FOLLOW UP PERIOD WITH RESPECT TO THE PREOPERATIVE AREA

Groups	Number (n)	Median	IQR	H value	P value
Group I:Control	2	4.3	2.3-6.3	2.03	0.36
Group II: PRP	18	5	2.7-12		
Group III: Blood Clot	9	9.7	5-22		

n:number of tooth



IV. Discussion:

The present study aimed to **compare the outcome of conventional root canal treatment (CRCT) with that of REPs on the clinical and radiographic healing of nonvital mature teeth with periapical lesion.** The null **hypothesis was that REPs and CRCT show no significant differences in terms of clinical and radiographichealing evaluated through IOPAR & CBCT.**

CRCT procedure was performed as per standard protocol[4]. In Regenerative Endodontic Procedure (REP) **NaOCl in high concentrations, are known to denature the dentin-derived growth factors[5]. 2.5% NaOCl** & **17% EDTA** has a positive effect on the survival and differentiation of stem cells subsequently cultured in contact with the conditioned dentin [5,6]**. Therefore in the present study 2.5% NaOCl & 17% EDTA as prime**

irrigating solution is used. According to AAE 2018 guidelines on REP 1- 5 mg/ml of TAP & calcium hydroxide are suggested. As preparation of TAP at this concentrations is difficult to prepare and its total removal from the canalis also not guaranteed hence Ca(OH)2 was used in the present study.

Several reports [7,8,9,10] have been documented the successful outcome of REPs using Blood Clot, PRP & PRF as scaffold. Blood clot induction from periapex has advantage of being inexpensive, patient friendly and periapically-induced BC bring stem cell population into canal space[11]. **In the present study out of total 52 teeth, 13 teeth in 11 patients were evaluated for REP using BC as scaffold**. There were some case reports[12,13,14,15,16,17,18] which showed that bleeding was induced by overinstrumenting the canal with hand files to stimulate the migration of stem/progenitor cells. It demonstrated the resolution of clinical signs and symptoms with complete periapical healing of **mature permanent teeth with apical periodontitis** after regenerative endodontic treatment.

PRP as a scaffold in REP has the advantage of increased concentrations of growth factors that can **attract stem cells present in the apical tissues (vital pulp cells, periodontal ligament, apical dental papilla, bone marrow) and even from periapical lesions**. Moreover PRP provokes proliferation of human dental pulp cells and **increases the protein expression of osteoprotegerin (OPG) and alkalinephosphatase (ALP) activity** thereby increases the speed of hard and soft tissue healing. There are studies[19,20] in which it had been reported that PRP alone resulted in effective scaffold formation in successful revascularization. **In the present study 33 teeth in 19 patients out of total 52 teeth were evaluated for REP using PRP as scaffold**

In this present study patients with **different age groups** were included. They were matched for their **age and gender** in the three allocated groups which were also matched for the **type of teeth treated**. The follow up period varied case to case in different groups, but it was seen that there was **no association between the last follow up periods & the effect of it on the treatment outcome. However the studies of outcome analysis[21,22,23,24,25]reported to have no significant difference in efficacy of BC & PRP as scaffold in regenerative endodontics.**

On **clinical examination**, all the teeth were **asymptomatic** with no tenderness, swelling or discharging sinus. Pulp sensibility test with cold and EPT of experimental group was negative. Similar findings were also seen in the study conducted by **Arsalan et al (2019)[26]** where in all the teeth under study **(CRCT & REP) remained asymptomatic at the followup visits.**

32 teeth (2 in control, 20 in PRP , 10 teeth in BC groups) were evaluated **qualitatively by IOPAR &** also evaluated **through CBCT** to **quantify** the decrease in size of the lesion and increase in the Hounsfield unit. IOPAR evaluation revealed healing of periapical pathology in **all the 32 teeth** and its quantitative assessment **through CBCT** in terms of **decrease in lesion size and increase in bone density** were seen **significantly high** within the **PRP** & **BC** mediated REP group **than control group in which conventional RCT was done**. Study conducted by **Shah and Logani (2012)[27]** reported **significant decrease** in lesion size assessed through CBCT & IOPAR following REP using **BC as scaffold** .In the same way **Saoud et al (2014)[28]** also revealed healing of apical pathology through REP using BC as scaffold in necrotic mature teeth.

At the end of follow up visits the **bone density gain and lesion size reduction was seen different in 3 groups but this difference was not significant in the present study.**Similar findings was also obtained in the study of **Alagl et al (2017)[29]** where in outcome of REP with PRP & BC was compared.

BC mediated REP studies of **Saoud et al (2016)[30], Arsalan et al (2019)[31], Emre Nagas et al (2018)[32], Abou Samra et al (2018)[33], Nageh et al (2018)[34], Paryani et al (2013)[35]**, evaluated through IOPAR only, provided ample evidence of healing of periapical pathology justifying feasibility of REP in treatment of mature permanent tooth with necrotic pulp with apical periodontitis. **In the present study the IOPAR assessment of healing of periapical pathologies both in CRCT & REP groups were also validated with CBCT observations.** But

search literature failed to provide existence of such study of comparing and evaluating healing of periapical pathologies through CRCT and REP in mature teeth assessed with the help of CBCT and IOPAR except a study of **Arsalan et al (2019)[36]** where in outcome of CRCT and REP evaluating through IOPAR only.

Qingan Xu et al (2018)[37] successfully treated a mature non vital premolar with pulpitis (without periapical

pathology) through REP. **In the present study 20 teeth with pulpitis & without periodontitis that include both in control & experimental group showed intact lamina dura and regular PDL space** in IOPAR & CBCT scan till the last follow up visit depicting success in CRCT & REP, except in one tooth in **BC group** in which CBCT revealed apical periodontitis at 3 month follow up visit indicating failure.

Patients are kept on follow up visits, on passage of time the pulp sensibility test may appear positive. It is also documented that false negative results of pulp sensibility test may be obtained in presence of the capping material MTA deeper into the root canal and poor/delayed development of neurovasculature of newly grown vital tissue[38,39].

V. Conclusion:

Within the limitation of the present study it can be concluded , REP has potential as a treatment option for necrotic mature teeth with or without periapical pathology using Blood Clot, PRP and PRF as scaffold and therefore can be used as an alternative to conventional root canal treatment.

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