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RESEARCH ARTICLE

EVALUATION OF CERVICAL CROWN/ROOT FRACTURE FOLLOWING PRF REVASCULARIZATION VERSUS ROOT CANAL TREATMENT OF NON-VITAL ANTERIOR PERMANENT TEETH WITH CLOSED APEX (RANDOMIZED CLINICAL TRIAL).

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Abstract

This study was conducted to test the efficacy of using the second generation of platelet concentrates; platelet rich fibrin (PRF) in the revascularization process. As PRF was known to contain growth factors which help in angiogenesis, neurogenesis, and dentinogenesis. Twenty four patients with thirty maxillary permanent mature central incisors with closed apex showing signs/symptoms of pulp necrosis were selected from the outpatient clinic of endodontic department, Cairo University. An approval from the ethic committee and an informed consent from the patients were taken. Patients were randomly divided into two groups; Control Group: Root canal obturation (number of teeth=15), Intervention Group: Revascularization using PRF (number of teeth=15). The incidence of cervical crown/root fracture was evaluated clinically after 2 weeks, 1, 3, 6, 9, and 12 months postoperatively. Both groups showed 100% clinical success as complete resolution of the signs and symptoms were observed through the follow up period. No cervical crown or root fracture occurred in both groups after 12 months follow-up period.

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Introduction:-

Preservation of the natural dentition remains a primary objective in endodontic practice. When the pulp is diseased or necessitates removal for restorative reasons, it can be replaced with an artificial filling material. Esthetics, form and function are not primarily dependent upon a vital pulp after root maturation, but there are disadvantages to conventional root canal treatment. It has been suggested that endodontically treated teeth dry out over time and that the dentin in endodontically treated teeth undergoes changes in collagen cross-linking. Therefore, it has been suggested that endodontically treated teeth are more brittle and may fracture more easily than non- endodontically treated teeth (1). Some clinicians believe that a post should be placed into the root after endodontic treatment to strengthen or reinforce it. Some studies, however, point out that posts do not strengthen teeth, but instead that the preparation of a post space and the placement of a post can weaken the root and may lead to root fracture (2). So keeping tooth vital is important in decreasing the incidence of tooth fracture, and preserving the proprioceptive mechanism.

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Several case reports have documented revascularization of necrotic pulp in root canal systems by disinfection followed by establishing bleeding into the canal system via over instrumentation. Regenerative endodontic therapy was limited to pulp revascularization of immature teeth; until Shah & Logani (3) were the first to attempt pulp revascularization in mature teeth followed by several case reports by (4-6). Their results were promising; relieving clinical signs and symptoms, periapical healing, and some cases showed regained tooth sensitivity. The development of the second generation Platelet concentrate known as Choukroun's Platelet Rich Fibrin (PRF) which is totally autologous in nature, very simple technique and inexpensive. PRF contains platelets, growth factors, and cytokines that might enhance the healing potential of both soft and hard tissues (7).

Subjects and Methods:-

Twenty four patients (fifteen teeth in the intervention group and fifteen teeth in the control group) aged between 18 years to 40 years old were recruited from the outpatient clinic of the Endodontic Department, Faculty of Oral and Dental Medicine, Cairo University, in the duration between 2015- 2016. The protocol of the trial was approved by the Ethics committee, Faculty of Oral and Dental Medicine, Cairo University. The treatment procedures and the aim of the study were thoroughly explained for all the patients.

The patients were asked about their dental and medical history; medically compromised patients were excluded intentionally. Patients were asked to follow the general instructions and to sign a printed informed consent that explains the aim of the study and obligates the patient to inform the time if the tooth was fractured after root canal obturation or PRF revascularization and to return it at the specific time. Also obligates him/her for a follow up period for clinical and radiographic examination every 3 months for at least 12 months. Inclusion criteria for the study was: Patient's age between 18-40 years with no sex predilection, Medically free, suffering from necrotic pulp in maxillary Central incisors permanent teeth with closed apex associated with or without periapical radiolucency, no response of vital pulp with the electric pulp tester, with normal occlusion and bite, and occlusal contact with the opposing. Patients were excluded who suffer from Malocclusion or deep bite or overjet, Patients reporting bruxism or clenching or TMJ problems, pregnant females or patients having systemic disorder, Patients with non- carious lesion affecting tooth structure such as (tooth wear, attrition, abfraction). Also teeth that have no occlusal contact, extra coronal restorations, greater than grade I mobility, pocket depth greater than 3 mm were not included in the study. The Thirty Permanent Central incisors in 24 patients were randomly divided into two groups (n = 15). The sequence generation for the patients' numbers was done using computer software found on <http://www.random.org/> by a co-investigator. The table was kept with the thirty numbers which were generated and distributed randomly in a table on an Excel sheet. The allocation sequence was generated by the centre of evidence based dentistry, Faculty of Oral and Dental Medicine, Cairo University. The table was kept and only accessed by a co- investigator where the operator doesn't know the group into which the patient will be enrolled. Folded numbered papers were packed in opaque sealed envelopes and dragged by the patients.

After diagnosis and acceptance of the patient who satisfied the eligibility criteria, the Patients were given anesthesia, access cavity preparation and working length determination were done. Then the operator opened the envelope, and the participant dragged a folded number from the sealed envelope. After that, the operator called the co-investigator and informed him about the patient's number to know if the patient will be enrolled in the intervention or control group. Double blinding was implemented in this study by the assessor and the statistician.

Full history of chief complaint was taken including; intensity, quality, onset, duration, location, course, initiating and relieving factors of pain. The included patients were complaining of mild or moderate dull pain with or without fistula tract. Other patients were complaining of moderate to severe pain with intraoral swelling and pain on biting and percussion. Some Patients were complaining from tooth discoloration due to previous trauma. The clinical diagnosis of necrotic pulp with symptomatic or asymptomatic apical periodontitis was confirmed by a negative response to electrical pulp tester.

At the first visit in the control group, the tooth was anaesthetized by buccal infiltration technique using 1.8 – 3.6 ml of 2% lidocaine with 1:100,000 epinephrine local anesthetic solution. An access cavity was performed, The tooth was isolated with rubber dam and negotiation of the canals was done using stainless steel hand k- files size # 15. Working length was determined using an electronic apex locator, then confirmed with intraoral periapical radiograph to be 0.5-1 mm shorter than radiographic apex. Mechanical preparation of root canal was done by step-back technique. The canals were thoroughly irrigated between two successive files using 1.5% sodium hypochlorite. This action was repeated until the apical area was prepared at least three sizes larger than the initial file, where the

master apical file (MAF) is reached, which was K-file # (40-80). Master cone with 2% taper was selected corresponding to the same size of the master apical file and confirmed by periapical radiograph using the bisecting angle technique. The canal was dried with paper points. Double antibiotic paste (DAP) was prepared by grinding one tablet of metronidazole (500 mg) and one tablet of ciprofloxacin (500 mg), which were then mixed with saline to form a homogenous paste of reasonable creamy consistency. This mix is then injected into the canal to a level just below the cemento-enamel junction (CEJ). A cotton pellet was placed and the cavity was temporarily sealed by glass ionomer cement. At the second visit, the participants were recalled after 3 weeks from the first visit for completion of root canal treatment. The rubber dam was placed, temporary filling was removed using high speed handpiece, and re-irrigation of root canals was done with 20 ml 17% EDTA for 1 minute, followed by saline irrigation. Then the canals were dried using paper points and obturated by cold lateral compaction technique using selected master cone, auxiliary gutta percha cone no. 25, and ADSEAL resin-based root canal sealer. The patient was recalled after 2 days to restore the tooth using a glass ionomer cement base and resin composite restoration.

At the first visit for the Intervention Group, The same steps were done as that for the control group till determining the working length. Mechanical preparation of root-canals was done by standardized technique. The canals were thoroughly irrigated between two successive files using 1.5% sodium hypochlorite. The Standardized technique steps included; determining working length and selecting initial apical file size (25-60), then doing circumferential filing to increase the apical construction 3 files sizes greater than initial apical file size, reaching apical canal preparation to K-file size no #40-80. The rest of the steps of root canal disinfection were the same as that of control group mentioned above. At the second visit, the participants were recalled after 3 weeks from the first visit for completion of root canal treatment. A plain anesthesia 1.8 ml 3% mepivacaine buccal infiltration was administered. The rubber dam was placed, temporary filling was removed using high speed handpiece, and re-irrigation of root canals was done with 20 ml 17% EDTA for 1 minute followed by saline irrigation. The canals were dried using paper points. Intentional over-instrumentation 2-3 mm past the apex into periapical region was done with K-file # 20-40 to induce bleeding near the apical foramen to a level below CEJ. The file was gently given 2-3 clock-wise turns and then withdrawn by giving counter-clockwise rotation. Excess blood reaching the pulp chamber was dried using small cotton pellet hold with tweezers. A 5 mL sample of whole venous blood was drawn from the patient's forearm (right median cubital vein) by the clinic nurse. The blood sample was then transferred into a test tube without anticoagulant and centrifuged immediately using a tabletop centrifuge at 3000 revolutions per minute (RPM) for 12 min. Three distinct layers were formed in the tube: platelet-poor plasma at the top, platelet-rich fibrin clot (PRF) in the middle and red blood cells at the bottom. Then the freshly prepared PRF membrane was fragmented, and the fragments placed incrementally in the canal using a hand plugger and a finger spreader size 40 up to the level of the CEJ. A 3-mm-thick layer of White MTA was placed directly over the PRF matrix. A moist cotton pellet was placed over the MTA, and tooth was temporized using Temporary filling. The patient was recalled after 2 days to remove the cotton pellet, and the tooth was restored using a glass ionomer cement base and resin composite restoration.

The evaluation of cervical crown / root fracture outcome was binary, the patient was requested to notify if there is any crown or root fracture happened to the teeth included in the study was done at the intervals of follow up periods at 2 weeks, 1 month, 3 months, 6 months, 9 months and 12 months.

Results:-

Clinical and radiographic evaluation results showed that there was not any cervical crown/root fracture through the follow up period in both groups. Chi square test revealed that the difference between both groups was not statistically significant. Moreover, the difference between different stages within the same group was not statistically significant, $P > 0.05$. (Table 1, figure 1)

Table 1:- Number and percentage of cases showed yes/no cervical crown/ root fracture through the follow up period in both groups.

Groups		2 weeks	1 month	3 months	6 months	9 months	12 months	X ²	P value
Control	Yes	0	0	0	0	0	0	0	1 ^{ns}
	No	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)	15 (100%)		
Intervention	Yes	0	0	0	0	0	0	0	1 ^{ns}
	No	15	15	15	15	15	15		

		(100%)	(100%)	(100%)	(100%)	(100%)	(100%)		
X^2		0	0	0	0	0	0	----	
P value		1 ^{ns}	1 ^{ns}	1 ^{ns}	1 ^{ns}	1 ^{ns}	1 ^{ns}		

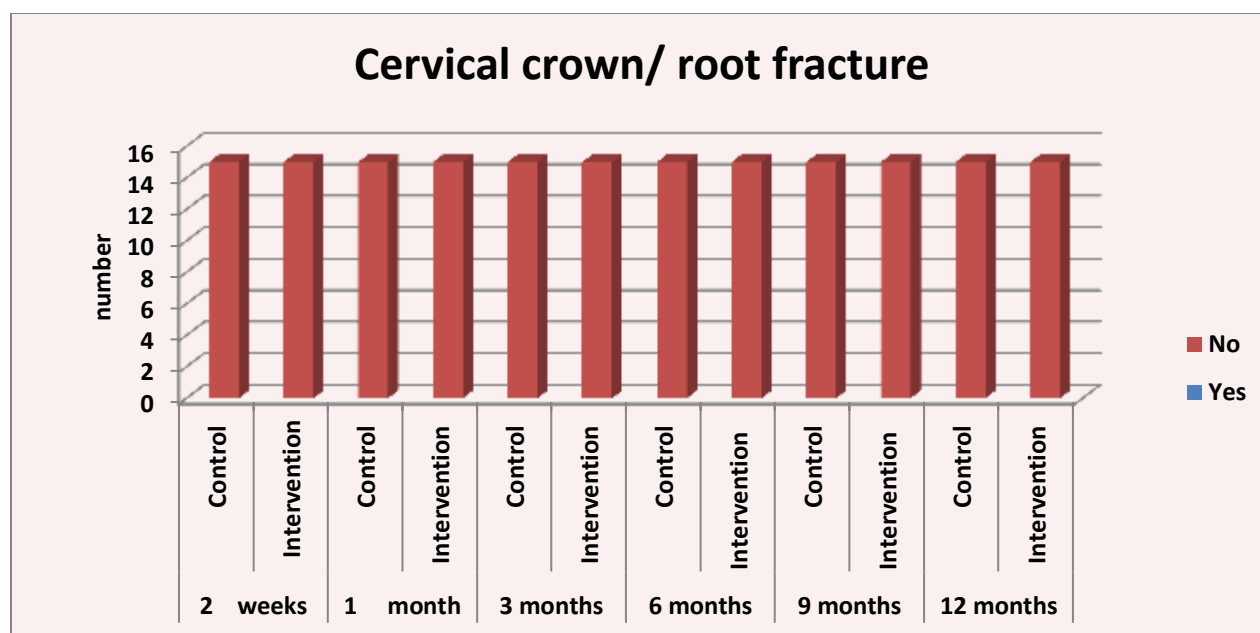


Figure 1:- Bar chart represents crown/root fracture with time in both groups.

Discussion:-

The main objective of root canal therapy is to minimize the number of microorganisms and pathologic debris in root canal systems to prevent or treat apical periodontitis. The process of chemo-mechanical debridement of the root canal systems has been described as the removal of all of the contents of the root canal systems before and during shaping. The infection of the root canal system is a polymicrobial infection, consisting of both aerobic and anaerobic bacteria. In mature teeth, disinfection is carried out with a combination of mechanical instrumentation, irrigation, and the placement of an intracanal medicament.

The rationale of cleaning and shaping should be based on the ability to thoroughly remove the tissue, necrotic debris, and bacteria and not a specific obturation technique. Dentin removal appears to be the primary method for decreasing microorganism numbers. However, it may not be possible to remove bacteria that are deep in the tubules regardless of the technique.

The larger preparation sizes have been shown to provide adequate irrigation and debris removal as well as significantly decreasing the number of microorganism (8). It appears to be a relationship between increasing the size of the apical preparation, canal cleanliness and bacterial reduction. Thus master apical file reached ISO K-file no (40-80) in the control group.

In the PRF group, the apical foramina were prepared to reach ISO K-file # 60-80 for effective root canal disinfection as mentioned previously. A pilot study was done on an extracted maxillary central incisor before the beginning of the research to see if the use of PRF could be used as a revascularization method in mature teeth concerning the manipulation and the possibility of its insertion inside the canal. It was concluded that as long as increasing the size of canal preparation to ISO file # 60-80, the easier and the more possibility of inserting PRF inside the canal.

In human Regenerative endodontic treatment studies of mature permanent teeth with necrotic pulps and apical periodontitis, (9) enlarged the apical foramen to a #35 K-file, where they explained that the size of # 25 hand K-file is approximately 0.25 mm in diameter, and the average size of human cells ranges from 10–100 μ m. Therefore, there is no reason why blood-borne cells and other cells including mesenchymal Stem Cells (MSCs) and thin blood

vessels could not migrate into the canal from the periapical area through the apical foramen with a diameter of 0.25 mm.

In the present study, the canal disinfection protocol was the same for all subjects and started at the first visit where irrigation and intracanal medicaments were used. Copious irrigation with 20 ml of 1.5% NaOCl for 5 minutes with a needle with closed end and side-vents, to minimize the possibility of extrusion of irrigant into the periapical space. Although Concentration of 5.25 % NaOCl is a tissue solvent and antimicrobial agent, but it has a proteolytic action on the dentin collagen matrix; decreasing the elastic modulus and flexural strength of human dentin, while a 0.5% solution does not (10). Moreover, The reduction of intracanal microbiota is not any greater when 5.25% NaOCl is used as an irrigant as compared to 0.5% (11).

Concerning the PRF revascularization group, 5.25% NaOCl concentration was found to be cytotoxic to stem cells in the apical tissues and decrease odontoblastic differentiation (12). NaOCl was then flushed away from the root canal with saline in an attempt to reduce any lingering toxicity that can reduce the regeneration responses as recommended by American Association of Endodontists 2016 (13-14). Based on the current available evidence, there is no significance for using hypochlorite solutions at concentrations over 1.5 %.

Double antibiotic paste (DAP) that consists of only ciprofloxacin and metronidazole eliminating minocycline was used in the present study. Because case reports and studies have shown that minocycline causes visible crown discoloration (15- 16), and significant reduction in tooth fracture resistance (17-18); as it causes demineralization of intertubular dentin by binding to calcium ions via chelation to form an insoluble complex and become incorporated into the tooth matrix causing the discoloration (19) and reduction in phosphate/amide ratio and microhardness of dentin. Also the acidity of TAP (PH = 2.9) is more than that of DAP (PH = 3.4) having a more demineralization effect on the intertubular dentin, causing significant reduction in phosphate/amide ratio and decrease in the dentin microhardness and fracture resistance (20-21).

Anesthesia was given at the second visit. A plain anesthesia 3% mepivacaine was used in the PRF group to facilitate inducing the bleeding. Washing the antibiotic paste from the canal of both groups was done by gently filling the root canals and using copious and gentle irrigation with 20 ml 17 % EDTA for 1 minute. Exposure of root dentin to EDTA for extended periods might also decrease its modulus of elasticity and flexure strength, leading to increased risk for root fracture (20). Also 17 % EDTA needle irrigation showed completely removal of antibiotic paste from root canals (21).

For the PRF group, a solution of 17% EDTA was used as its chelating effect promotes the release of dentin-derived growth factors that were previously embedded into dentin during the process of dentinogenesis. These growth factors such as transforming growth factor –beta1 (TGF-b), dentin sialoprotein (DSP), platelet derived growth factors (PDGF), vascular endothelial growth factor (VEGF), placenta growth factor (PlGF) and fibroblast growth factor (FGF2), and very low concentrations of epidermal growth factor (EGF); have been shown to stimulate proliferation, survival, and differentiation of dental stem cells.(22)

In the control group, the obturation was done using number # 45-80 gutta-percha master cones in combination with an epoxy resin-based sealer (ADSEAL). Cold lateral compaction technique was used due to its simplicity, easiness, low cost, predictability, and controlled placement. Also a recent study showed that root canal preparation and filling techniques had no influence on the fracture resistance of extensively damaged teeth (22).

Components needed for successful regenerative endodontics include absence of intracanal infection, a good coronal seal to prevent reinfection, a physical scaffold to promote cell growth and differentiation, as well as signaling molecules for the stimulation of stem cells. The file size used passing beyond the apical foramen by 2-3 mm for irritating the periapical region to induce bleeding was not specifically recommended (24). In the current study a sterile stainless steel H-file size 20-40 was used to induce bleeding by irritating the periapical area.

Sources of stem cells for pulp revascularization according to an article by Hargreaves et al in 2013 (23) could be Periodontal ligament stem cells (PDLSCs), bone marrow mesenchymal stem cells (BMSCs), inflammatory periapical progenitor cells (IPAPCs), and some surviving dental pulp stem cells around the root apex may participate in pulp regeneration.

PRF, a second-generation platelet concentrate, has been developed that overcomes the limitations associated with the use of Platelet rich plasma PRP. The classic PRP production protocol requires blood collection with an anticoagulant, two-step centrifugation, followed by artificial polymerization of the platelet concentrate using calcium chloride and bovine thrombin. Unlike its counterpart, PRF preparation eliminates the excessive process of adding anticoagulants or bovine thrombin making it similar to centrifuged natural blood.

Furthermore, a highly resistant and elastic membrane of fibrin is obtained, which does not dissolve quickly after application (24); allowing cellular migration, cytokine enmeshment, and slow continuous release of cytokines such as platelet derived growth factor (PDGF), transforming growth factor b1 (TGFb1), Fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) from 7 days (27) to 28 days achieving peak level at 14th day coinciding with cell in growth (25); so it directs more efficiently stem cell migration, proliferation, differentiation and supplements the angiogenesis (26).

Lastly, the presence of leucocytes and cytokines along with small amounts of lymphocytes in PRF can play a significant role in the self-regulation of inflammatory and infectious phenomenon. Thus we used PRF as a scaffolding material in an infected necrotic mature tooth for pulpal regeneration and tooth revitalization as it satisfies many criteria of an ideal physical scaffold.

MTA was placed directly over the PRF to obtain a coronal seal. Freshly mixed MTA has a soft consistency and may be applied without pressure. In the presence of PRF, placement of MTA at the proper position; exactly 3mm below CEJ was easier and more comforting during the clinical work. MTA needs to be placed at the level of the cervical constriction or even a little more coronally (27). A more coronal placement of MTA will potentially achieve hard tissue formation in the critical cervical area of the tooth which is where catastrophic fractures appear to occur (28).

Clinical and radiographic follow up assessments were done every 3 months for 1 year. Clinical assessment regarding pain and swelling/fistula showed no recurrence in all subjects. In the current study all the cases of control group and PRF revascularization technique, were clinically successful where there was resolution of apical periodontitis and elimination of clinical signs/symptoms.

In the current study all the cases of both groups showed no cervical crown/ root fracture for the follow-up periods up to 12 months, this could be explained by the following possible reasons:

The first reason is the criteria of tooth selection that was restricted to intact maxillary central incisors teeth or teeth with minimal loss of tooth structure, so that conserving as much as possible of tooth. According to (29) access cavity preparations in sound maxillary central incisors affects tooth stiffness by only 5%, and the removal of dentin at the wall of the canal without extensive alteration of the root canals' outline by manual widening resulted in minimal tooth deformation when using a light force. In fact, the largest reduction in tooth stiffness results from excessive access preparation, especially the loss of marginal ridges (30).

The second reason is that no included teeth in the study had class V or cervical decay in the tooth structure, as when chewing forces act on tooth, it experiences flexing or bending. The bending-associated stress distribution in a column-like structure is subjected to an eccentric load. The column tends to bend, resulting in compressive stress on one side and tensile stress on the other side. These stresses are highest at the outer aspect and diminish to zero toward the center of the cross section. In a tooth under compressive force, the maximum stress resulting from bending is predominantly observed at the cervical aspect of the root (cervical dentin). The maximum stress/bending stress reduces notably toward the apical region of the root (31). This decreased stress distribution in the middle and apical third of the root was attributed to the shape/angulation of the tooth and its interaction with the supporting bone. Thus the cervical root dentin and its relationship with the supporting alveolar bone is crucial for a stable stress distribution from the root to the supporting bone (32).

The third reason is that the literature does not support a widely held belief that attributes particular weakness or brittleness to non vital dentin. Interestingly, comparisons between vital and non vital dentin of contralateral teeth demonstrated no or only minor differences in microhardness values and dentin modulus of elasticity after periods ranging up to 10 years after treatment (34, 35). The loss of pulpal vitality is accompanied by a slight change in tooth moisture content. This loss of moisture (9%) is attributed to a change in free water but not in water bonded to the organic and inorganic components (36). This alteration was associated with a slight change in values for the Young

modulus of elasticity. However, no decrease in compressive and tensile strength values was associated with this change in water content (31). Only one study showed no difference in moisture content between vital and non vital teeth (35). No difference in collagen cross-linkage was found between vital and non vital dentin (35). Thus, non vital teeth undergo minor changes in physical characteristics.

The fourth reason is that the irrigants used in this study was with low concentration and for short duration. 1.5% concentration NaOCl was used as irrigant, as the use of NaOCl with concentrations over 2% having more deleterious effects on the physical properties of dentin (36). The use of 17% EDTA was only for 1 minute, as exceeding this duration has the potential for causing excessive dentinal erosion and changing the dentin physical properties (37).

The fifth reason is that the obturation was done in the control group using cold lateral compaction technique with resin based sealer and gutta percha. It is believed that resin root canal sealers bond to root dentin can increase the fracture resistance of endodontically treated teeth, and strengthens the compromised tooth structure, which has been subjected to chemical and mechanical preparation (38).

The sixth reason is that some teeth regained sensitivity in the intervention group; indicating the presence of vital tissues with sensory fibers inside the canal. An important advantage of retaining pulp vitality appears to be the continued function of proprioceptive mechanisms and the protective avoidance of excessive occlusal forces during mastication.

The seventh reason is that both groups had Composite resins final restoration, which exhibit great potential to reinforce and strengthen the remaining tooth structure (39-40).

Our results showed a 100% survival rate of teeth in both groups. For the control group our results support the finding of Landys Borén (39) who concluded in a systematic review that approximately 80% of the teeth treated at this specialist clinic in endodontics survived at least for 10 years. Moreover it was in coincide with Ng et al (40) , who concluded that available data support the common opinion among clinicians that tooth survival is likely to be influenced by the strength and integrity of remaining tooth tissue.

Our results also showed no cervical crown/root fracture in the intervention group, which supports the findings of (5,6) who did not report any tooth fracture in their case reports

Conclusion:-

Root canal disinfection was the key for the treatment success. Platelet rich fibrin (PRF) revascularization was a successful regenerative endodontic treatment modality in mature permanent Incisors with closed apex. A 12 months follow up was enough to monitor clinical and radiographic evidence of healing but not enough to detect cervical crown or root fracture.

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