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SYNERGISTIC OSTEOGENIC POTENTIAL OF HUMAN MESENCHYMAL DENTAL PULP STEM CELLS AND PLATELET-RICH PLASMA ON REPAIR OF ANTERIOR MAXILLARY BONE DEFECT

Abeer Kamal *, Nesrine khairy ** and Dina Sabry ***

ABSTRACT

Purpose: The aim of the present study was to evaluate **synergistic osteogenic potential of** human dental pulp mesenchymalstem cells (hDMSCs) and platelet-rich plasma (PRP) in enhancement of bone regeneration in anterior maxillary bone defects**.**

Patients and methods: Eighteen patients (10 males and 8 females), were selected and divided equally into three groups. Group I included patients (4 male and 2 female) having anterior maxillary cyst and impacted or supernumerary teeth in another place indicated for surgical removal for production of mesenchymal dental pulp stem cells, group II and III included patients (3 male and 3 female in each) having anterior maxillary cyst. The cyst was enucleated and the space left were filled by hDMSCs with PRP in group I. in group II the cavity was filled by PRP only. In group III the cystic cavity left with no filling material and considered as control group. Cone Beam Computed Tomography (CBCT) was performed preoperatively, one month and six months postoperatively for comparison of bone density intra group and inter groups.

Results: Comparison of bone density between the three groups at one month post-operative period showed that group II recorded highest and significant bone density. At six months post-operative period the three groups showed significant increase in bone density However group I (hDMSCs with PRP) showed highest mean increase in bone density.

Conclusions: hDMSCs can provide an osteogenic cell source for new bone formation and the PRP improves and retains their differentiation capacity due to possible synergisticosteogenic potential between PRP and stem cells.

KEYWORDS: Dental pulp stem cell, Platelet rich plasma, Bone regeneration, Bone defect.

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INTRODUCTION

Surgical anterior maxillary bony defect created after cystic enucleationconstitute a major problems in oral and maxillofacial surgery field. Several trials have been exerted by the use of bone substitutes that may have one or all of these biological mechanisms including osteoinduction, osteoconduction, osteogenesis, or osteopromotion. These trials encountered a lot of limitations. Increased risk of infection, extrusion of grafting materials , immunologic reactions and exposure to secondary operative side have been reported as a limitations to these procedures[1-6]. Stem cells are promising bone building material. Dental pulp stem cells (DPSCs) are newly promisingtechnology for stem cell biology and regenerative medicine. It is multi-potent cells capable of differentiation along multiple lineages [7]. It have the remarkable potential for multi-lineage differentiation capacity including osteoblast [8], cartilage [9], adipocyte[10], muscle [11], hepatocyte [12], and neurons [13]. Therefore, an improved comprehension of the cellular and molecular mechanisms, which modulate self-renewal and differentiation properties of DPSCs, could be pursued to bring forth future progress in regenerative medicine.

It has been reported that DPSCs have higher neurogenic, angiogenic, and bone regeneration compared with bone marrow and adipose stem cells. The main advantage of DPSCs is it's immunologically privileged.[14-16]Dental pulp stem cells have been presented to differentiate into numerous cell types, comprising osteoblast-like cells that secrete abundant extracellular matrix and build a woven bone in vitro. The bone differentiation of DPSCs has been well confirmed in vitro and in vivo, [17-19] and confirmed by specific bone markers in the newly formed bone [20,21]. Dental pulp stem cells can be attained from discarded permanent teeth including impacted third molars, supernumerary teeth, displaced teeth or orthodontically unnecessary teeth. Exfoliated deciduous teeth could be an excellent source of cells for banking of stem cells[22, 23].

Dental pulp stem cells can be collected easily with little ethical concerns and harvested in a minimally invasive and safe manner. Many studies have been demonstrated that DPSCs could betransplanted with a different type of scaffold and exhibit bone-like structure. The types of scaffold included,hydroxyapatite / tri-calcium phosphate (HA/TCP)[24–26], collagen [20, 27], nanofiber hydrogel [28], HA nano-hydroxyapatite/ collagen/ poly (L-lactide) (nHAC/PLA) [29], and platelet-rich plasma (PRP) [30]. Critical size bone defects could be repaired by the use of DPSCs [26, 31]. Dental pulp stem cells show greateramountof bone than bone marrow and periosteal stem cells. It was considered as avaluable source forbone tissue engineered around dental implants [32]. It has been described that DPSCs can be used for therapeutic purposes as the repairofcraniofacial bone[20, 33].An in vivo study showed that human DPSCs generated both osteoblasts and endotheliocytes, and eventually formed a bone-like structure with an integral blood supply similar to that of human adult bone in immunocompromised rats[34]. Zheng et al [35]reported that stem cells from miniature pig deciduous teeth were able to regenerate bone to repair critical-size mandibular defects in a swine model. In a clinical study, DPSCs and collagen sponge scaffold formed a biocomplex that could completely restore mandibular bone defects in patients [20].

Platelet-rich plasma PRP was first defined in 2007 as a preparation of platelets concentrated in a small volume of plasma. It is essential for bone growth and regeneration. [36] The contribution of blood derived platelets to the bone healing process is thought to be based on the growth factors GFs present in PRP. The following GFs are reported to be presented in platelet aggregates include: platelet-derived growth factor (PDGF), transforming growth factors (TGF),vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), insulin growth factor-1 (IGF-1), and basic fibroblast

growth factor (bFGF), as well as three blood proteins known to act as cell adhesion molecules for osteoconduction (fibrin, fibronectin, and vitronectin) [37–40]. Consequently, platelet gel biotechnology has evolved in the field of regenerative surgery.

Platelet rich plasma is widely used in various maxillofacial fields. It is an autologous preparation, using the patient's own blood in a significantly small quantity. It is safe with no risk of infections, disease transmission, immunogenic reactions or any other adverse effects which exist with allografts or xenografts. It is easily available and not time-consuming for both the patient and the clinician [41]. The major effects of PRP are resulting from platelet-derived growth factor PDGF, an important protein for hard- and soft-tissue healing. Itstimulatechemotaxis, mitogenesis and replication of stem cells at the site of tissue injury. This leads to formation of bone matrix and angiogenesis by stimulating vascular endothelial growth factor VEGFto accelerate soft-tissue healing due to neo-vascularization. Platelet-derived growth factor stimulates fibronectin production for cellular proliferation and migration during healing, including osteoconduction and hyaluronic acid, and help in promoting wound contraction and remodeling [42].

Review of literature reported that human dental pulp stem cells have efficiently capable to enhance bone regeneration in oral and maxillofacial area. It was planned to assess the synergistic osteogenic potential of human dental pulp stem cells and platelet-rich plasmain anterior maxillary bone defect. The result of this study was hopedto augmentthe process of bone regeneration with optimum quantity.

PATIENTS AND METHODS

Eighteen patients (10 males and 8 females) suffering from anterior maxillary cyst were selected. They were chosen from those attending the outpatient clinic of the Oral and Maxillofacial Surgery department, Faculty of Dentistry, Cairo University, Egypt. The sample were **divided into three groups**. Group I: comprised 6 patients having maxillary cyst and impacted or supernumerary teeth in another place and need surgical removal for production of dental pulp stem cells DPSCs, Group II and III included6 patients in each having anterior maxillary cyst.

Ethical approval for this study was obtained from the ethical committee and informed written consent was performed. The age of patients ranged from 20 to 43years with a mean of 36.5 years. All patients were healthy according to American Society of Anaesthesiologist (ASA1)[43] without any contraindication for minor oral and maxillofacial surgery and local or general anesthesia. Exclusion criteria included cyst less than $3cc^3$ and more than 4 cc^3[44],patients who is medically compromised, those with acute infection and pregnant females. Clinical examination and preoperative cone beam computed tomography scan were performed for proper diagnosis. Cone Beam Computed Tomography scan CBCT (Scanora 3D Soredex Finland 85kv 15Ma) was used to determine the position of impacted and/ or supernumerary teeth figure (1) for group I, the extension, size, position and assessment of radiodensity of the cyst for all groups, and also for comparison between the three groups pre and postoperatively.

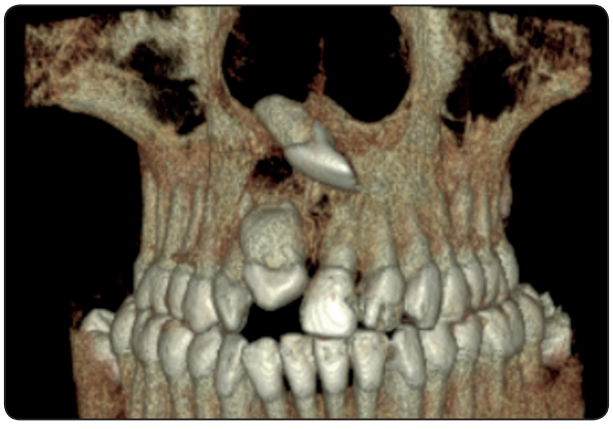

Fig. (1) 3D reconstructed CBCT radiograph showing supernumerary tooth (group I)

For group I, the impacted tooth or the supernumerary tooth was removed surgically two weeks before cystic enucleation under local anesthesia(articaine HCL 4% with 1: 100,000 vasoconstrictor -Septanest SP, Septodont pharmaceutical Industries, France). The tooth was preserved in phosphate buffer saline PBS until the isolation and culture of mesenchymal cells from human dental pulp tissues was obtained. The process of isolation and culture usually take about two weeks. The patients returned and the maxillary cyst was enucleated. (Figure 2. 3 a & b) The bony cavity was filled by isolated dental pulp stem cell with platelet rich plasma PRP as a scaffold.

Isolation and culture of mesenchymal cells from human dental tissues (two weeks before cystic enucleation):

All the process was performed at Stem cell Lab., Faculty of medicine, Cairo University, Egypt. According to the technique described by Di Benedetto et al.[45]Human dental derived mesenchymal stem cellshDMSCs were harvested from the attached dental pulps separated from impacted or supernumerary tooth. The dental tissues were digested in a solution of 0.1U/ml collagenase type II (Sigma) for 60 min. at 37°Cfollowed by centrifugation at 500 xg for 5 minutes in phosphate buffer saline. Cells debris was removed by passing digested tissue through a 40 mm nylon cell strainer (BD FalconTM, BD Biosciences, Franklin Lakes, NJ, and USA) and, dental cells were expanded in vitro. hDMSCs were dissociated on confluence using a 0.25 %(w/v) trypsin-EDTA (Gibco). Cell pellets was obtained by centrifugation at 500xg for 5 minutes. Cells were then re-cultured in Dulbecco's modified eagle's medium (ADMEM) supplemented with 10% PBS, 100U/ml penicillin and 100 mg/ml streptomycin at 37˚C in a humidified atmosphere of 5% CO_2. Culture medium was changed twice a week and passages were expanded three times for further analysis and characterization. Figure 2.

Preparation Platelet rich plasma (at the time of cystic enucleation):

According to the technique described by Dhurat et al [46] whole blood specimen was collected using acceptable medical procedure to avoid hemolysis. Thirty cc venous blood was withdrawn by venipuncture from the same patient in 4 GEL-VAC PRP tubes supplemented with 0.2% citrate phosphate dextrose (CPD) as an anticoagulant. The blood was centrifuged firstly using a hard spin at 3750 rpm for 10 min to separate erythrocytes. The supernatant plasma containing platelets with mononuclear cells (MNCs) was transferred into another sterile tube (without anticoagulant). The tube was centrifuged secondly at a lower speed 3,400 rpm for 5 min (a hard spin) to obtain a platelet concentrate. The lower 1/3 rd. was PRP and upper 2/3 rd. was platelet-poor plasma (PPP). At the bottom of the tube, platelet pellets was formed. PPP was removed and the platelet pellets was resuspended a minimum quantity of plasma (2-4 mL) by gently shaking the tube or by using long needle. The PRP was transformed into gel form to act as scaffold by hot water path. Figure 2.

For Group II, the cyst was enucleated and the resulting cavity was filled by only PRP. **Group III,** the cyst was removed and the cavity left to heal without adding any filling material (control group).

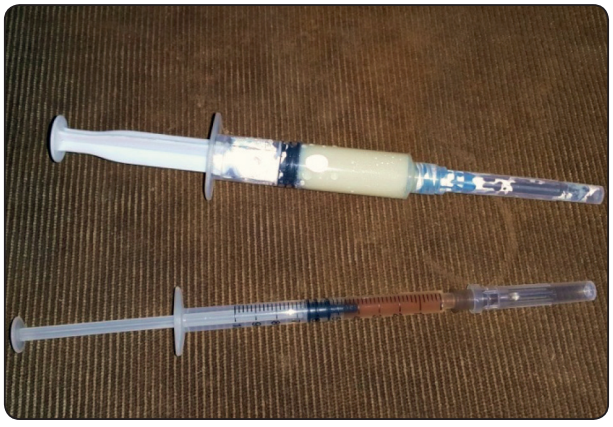

Fig. (2) prepared (hDMSCs) and gel form PRP

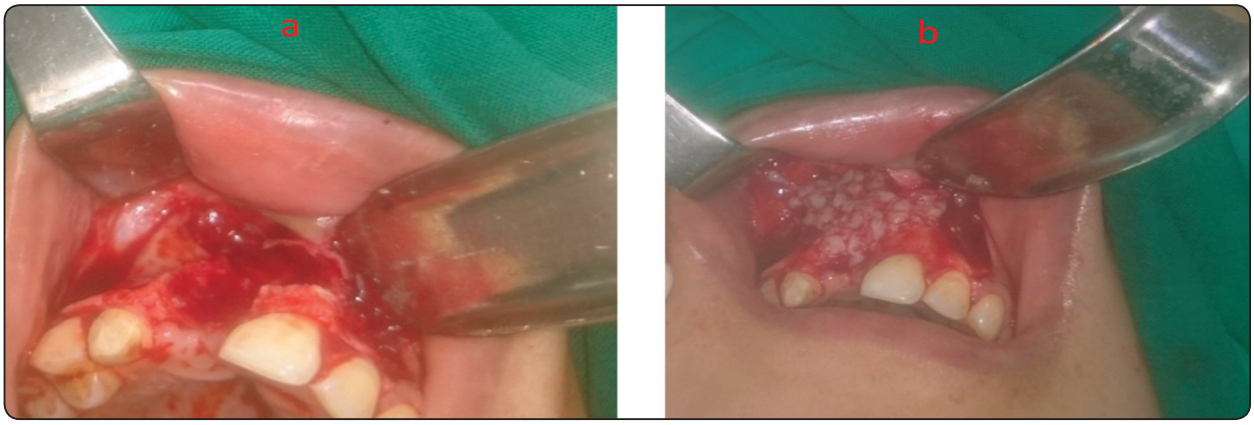

Fig. (3) a. bone defect after cystic enucleation.b. cavity filled with (hDMSCs) and PRP

Cone beam computerized tomography was performed at three time interval for each patients in the three groups, preoperatively, one month postoperative and 6 months postoperatively. Bone density was measured at different area of the cystic cavity. Comparisons were performed to detect the changes in bone densityinter- group and intra-groups as following:

Inter-groups comparison:

a) Comparison of bone density between the three groups at one month postoperatively by the use of CBCT

b) Comparison of bone density between the three groups at six months postoperatively by the use of CBCT

Intra-group comparison (effect of time on bone density in each group)

Statistical analysis was performed using a commercially available software program (SPSS 19; SPSS, Chicago, IL, USA. 2016) to compare the mean of CBCT recorded at the start of the study and in the different observation times. As data was parametric, significance of the difference was evaluated using one way analysis of variance (ANOVA test).The level of significance was set at $P < 0.05$.

RESULTS

Healing in the post-operative course was uneventful. No inflammation was detected nor pain with slight swelling improved within 3 days. No wound dehiscence was detected.

Inter-group comparison: ***(Table 1. Figure 4)***

a) Comparison of bone density between the three groups at one month postoperatively by the use of CBCT showed that group II recorded highest and significant bone density, group I and III showed decrease in bone density. (Table 1. Figure 4)

b) Comparison of bone density between the three groups at six months postoperatively by the use of CBCT showed that the three groups exhibited significant increase in bone density with p value 0.038. However group I (stem cell with PRP) presentedwith highest mean increase in bone density. (Table 1. Figure 4)

Intra-group comparison (effect of time on bone density in each group)

In group I (Stem cell and PRP), the greatest mean value was recorded 6 months post-operatively (416.21 ±236.56), whereas the least mean value was recorded at 1 month post-operatively, with a significant difference between the group ($P<0.0001$). (Table 2.Figure 5, 6, 7).

In group II (PRP), the greatest mean value was recorded 6 months post-operatively (506.33 ±133.49), whereas the least mean value was recorded at 1 month post-operatively, with a significant difference between group (P<0.0001). (Table 2. Figure 5, 8, 9).

In group III (Control), the greatest mean value was recorded 6 months post-operatively (204.79 ±99.24), whereas the least mean value was recorded at 1 month post-operatively, with a significant difference between group (P<0.0001). (Table 2. Figure 5).

TABLE (1) Comparison of bone density between the three groups at 1m and 6m postoperatively

	Gp	Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		F	P value
					Lower Bound	Upper Bound		
1 M	I	-799.8	208.7	512.1	-1,859.1-	259.55	2.413	**.047***
	II	269.64	128.55	46.65	173.13	366.15		
	III	-551.1	132.0	353.5	-1,282.4-	180.28		
6M	I	962.55	308.6	614.1	-307.87-	2232.98	1.828	**.038***
	II	99.75	37.61	13.80	71.20	128.30		
	III	166.37	51.28	30.88	102.49	230.25		

Significance level p<0.05, *significant

TABLE (2) Intra group comparison (effect of time on bone formation)

Gp		Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		F	P value
					Lower Bound	Upper Bound		
I	1 M	-30.33	45.19	9.23	-49.42-	-11.25-	58.797	<0.0001*
	6M	416.21	236.56	48.29	316.32	516.10		
II	1 M	183.0	94.33	19.26	143.17	222.83	68.459	<0.0001*
	6M	506.33	133.49	27.25	449.96	562.70		
III	1 M	-0.370	69.18	14.12	-29.6-	28.84	41.449	<0.0001*
	6 M	204.79	99.24	20.26	162.89	246.70		

significant at p<0.05

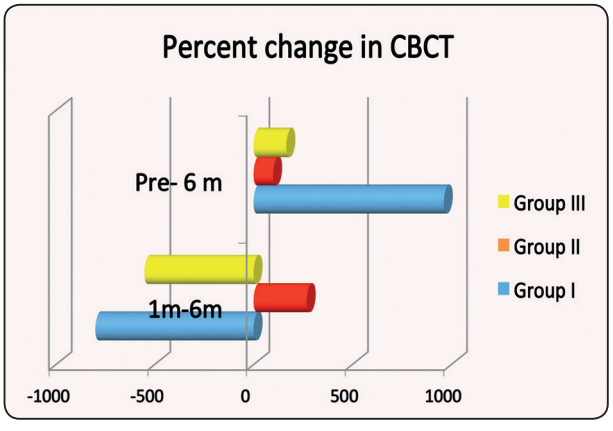

Fig. (4) Bar chart showing bone density changesin CBCT in different groups at each interval

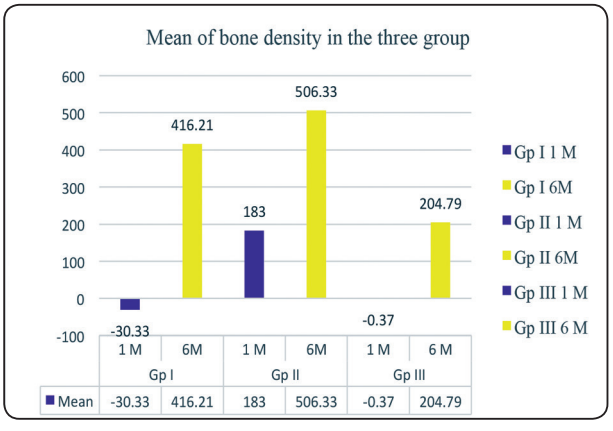

Fig. (5) Column chart showing CBCT results in different observation times within the same group

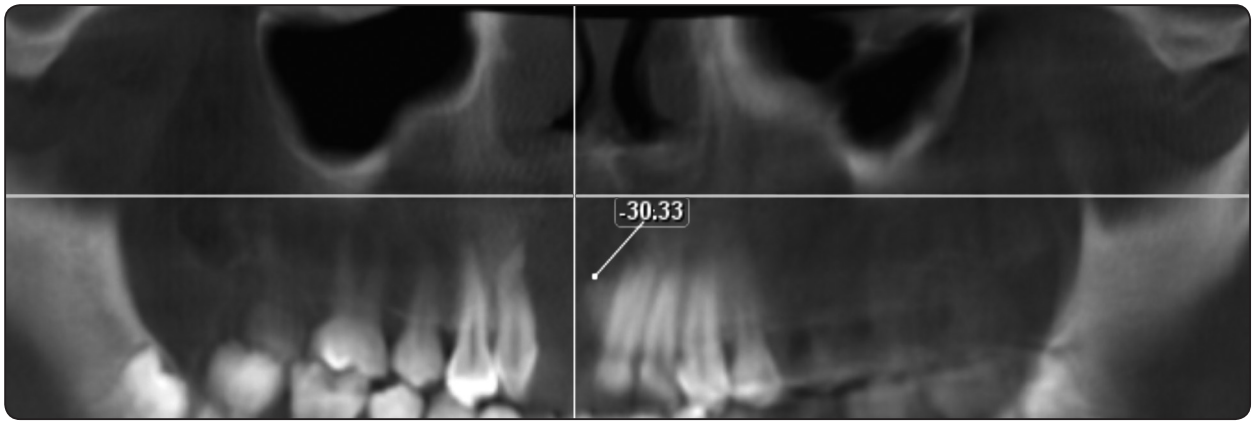

Fig. (6) CBCT radiograph showing mean bone density in group I (Stem cell and PRP) 1 m postoperatively

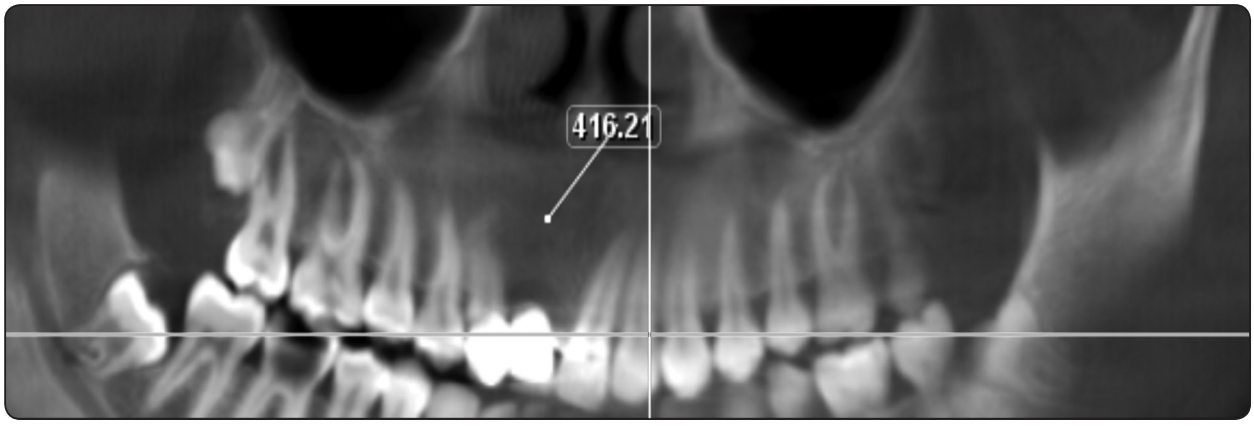

Fig. (7) CBCT radiograph showing mean bone density in group I (Stem cell and PRP) 6 m postoperatively

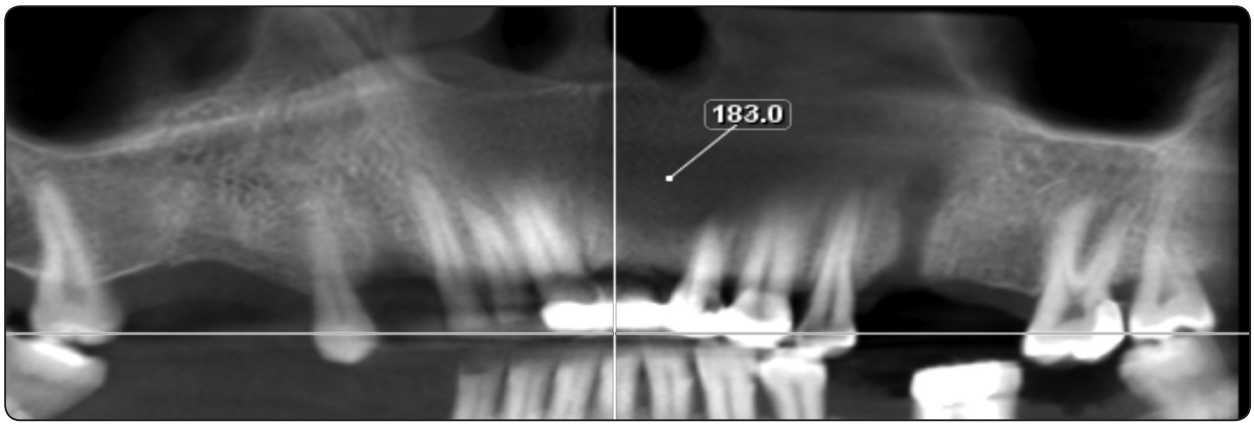

Fig. (8) CBCT radiograph showing mean bone density in group II (PRP) 1 m postoperatively

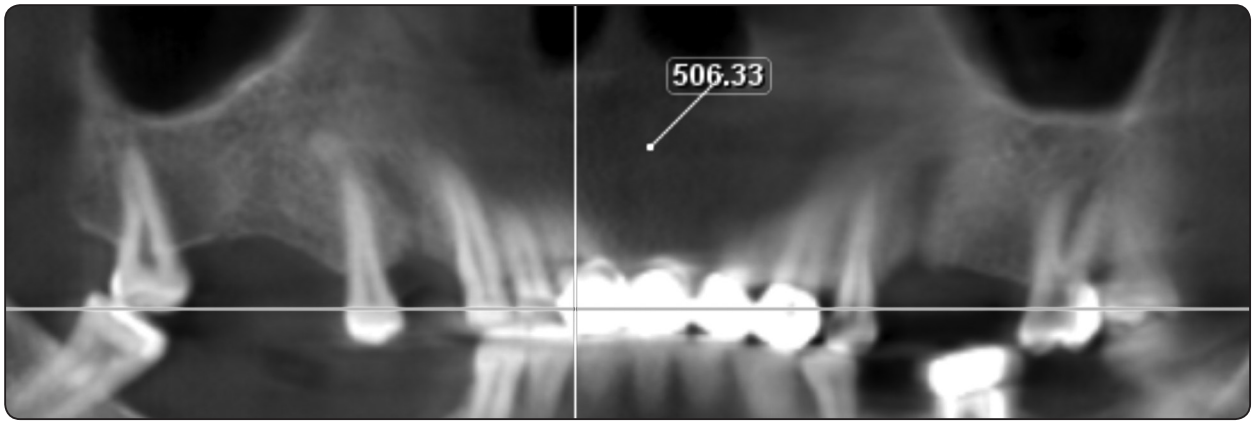

Fig. (9) CBCTradiograph showing mean bone density in group II (PRP) 6 m postoperatively

DISCUSSION

Stem cells induce bone regeneration and is considered a recent modalities in maxillofacial surgery field and their use were widely studied. Their synergistic osteogenic potential with different scaffolds has not received the same attention. Dental pulp stem cells are simply available source of stem cell with great amount of cells, and superior to other types of stem cells. It is easily collected from discarded teeth with little ethical concerns .It is harvested in a minimally invasive and safe manner,easyto bank from teeth that are lost naturally during child hood or removed surgically due to impaction and cryopreserve. The success of dental pulp stem cells depends on its induction power which is represented by resistance to stress, secretion of cytokines and growth factors. This opinion is in agreement with Misako Nakashima et al, [16] Tatullo et al[47] and Gharaibeh et al [48]. Indeed the obtained results will augment the basic knowledge needed to improve the field of maxillofacial surgery. It is hoped that the synergistic osteogenic potential of dental pulp stem/progenitor cells and platelet rich plasma will promote unlimited healingof bone defects with optimum quality.

The present study included three groups with anterior maxillary bone defect, group I (stem cell and PRP) has been designed to evaluate possible synergisticosteogenic potential of dental pulp stem cells with PRP and used as empirical group. Group II (PRP) was designed for comparison as platelet rich plasma widely used material for bone regeneration. Group III (control group) was designed to evaluate and monitor bone healing without any interventions.

Cone beam computerized tomography has been utilized in the present study to measure radiodensity being reliable, reproducible, accurate and noninvasive quantitative monitoring methodof bone defect healing. It is a widely accepted low radiation dose imaging modality for preoperative planning providing precise anatomical information,higher image resolution with less radiation dose and exposure time than the conventional clinical multidetector CT. The radiation exposure of a typical dental examination with CBCT is reported to be only one third compared to multidetector CT. The 3D image produced by CBCT allows for detailed morphological analysis of bone. This idea was supported by Kröpil et al[49], Buyuk et al [50]and Kim[51].

Inactivated gel form platelet rich plasma without adding bovine thrombin have been utilizedin the present study for the followings, to decrease the possible influencing factors of calcium and thrombin, no need for activation of PRP being activated by exposure to collagen leading to release

of growth factors, the gel form PRP act as scaffold without adding bovine thrombin, and inactivated PRP increases demineralized bone matrix osteoinductivity in vivo, thisconceptwas supported bymany authors.[37, 52-55]

Significant increase in bone density in the present study has been detected at one month post-operativelyin group II (PRP), this finding is in agreement with Fernandes et al [56], Ruktowski et al [57] and Celio-Mariano et al [58]. Thisobservation can be attributed toearly promotive osteogenic potential of bone regeneration by PRP. This early effect is attributed to short lifespan of the platelet due to early dissolution of fibrin and growth factors. This explanation is in concordance with many authors [56-62]. Decrease bone density at six months post-operatively in group II (PRP) can be attributed to the fast degradation rate of the fibrin and the dissolution of PRP. Platelet rich plasma itself cannot induce new bone formation because it is not osteoinductive[37]. This results is in agreement with the study of fernandes et al [56] with recommendation to use and deliver the PRP via a carrier which can degrade slowly, so as to release the PRP with its content of growth factors in a sustained manner.

In spite of significant increase of bone density at six months post-operatively in all the three groups, the greatest mean value of increase was recorded in group I (stem cell & PRP).This observation is ascribed to possible synergisticosteogenic potential between PRP and stem cells. ThePRP improves the aggregation and cohesiveness of bone substitutes,it helps bone regeneration, it modifies the properties of mesenchymal stem cells (MSCs) when seeded on scaffold, and its growth factors could provide a nutritive environment to the MSCs. The success of MSCs depend on its induction power represented by its resistance to stress, secretion of cytokines and growth factors. This opinion is in agreement with many authors. [63-68]Human dental pulp mesenchymal stem cellscan provide an osteogenic cell source for new bone formation and the PRP improves and retains their differentiation capacity.[69-71]

The present study concluded that human dental pulp mesenchymalstem cellscan provide synergisticosteogenic potential with PRP for new bone formation in anterior maxillary bone defect. The PRP improves and retains the differentiation capacity of human dental pulp mesenchymal stem cells by acting as scaffold. The present study concluded that human dental pulp stem cells recommended for bone regeneration in medium size bone defect with PRP. Further study is recommended on larger size bone defect in different intraoral sites.

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