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Research Article

Evaluation of Efficacy of Platelet Rich Plasma in Healing of Maxillofacial Bony Defects: A Comparative *In-Vivo* Study

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Abstract

Background and Objective: Platelet rich plasma is an autologous blood product prepared from centrifugation of blood. When activated it liberates various growth factors in large quantities which hasten hard and soft tissue healing. This study aims to evaluate the efficacy of the Platelet rich plasma in the regeneration and healing of maxillofacial bone defects and compare it with conventional healing.

Study Design: Twelve subjects diagnosed with acquired bony defects following excision of benign lesions like cysts and tumors in the maxillofacial region were treated using the conventional surgical excision of the underlying lesion. Six out of them were augmented with Platelet rich plasma gel and the other six were allowed to heal without Platelet rich plasma. The defect areas were re-examined radiologically after 1 month, 3 months and 6 months, post-operatively.

Results: The control group showed a mean density change from the 1st to 6th post-operative month 2.8 ± 7.44 and the experimental group showed a value of 37.3 ± 21.46. The mean of the difference between the values obtained at the end of 1st month and 6th month in the experimental group treated with Platelet rich plasma was fifty times the average obtained in group not treated with Platelet rich plasma. Two patients in control group showed a decrease in the defect density at 6 months as compared to the value obtained at 1st post-operative month. All the six bone defects grafted with Platelet rich plasma showed an increase in the density at the end of six months compared to the defects treated with conventional treatment.

Conclusions: Our study showed an increase in the rate of healing when autologous Platelet rich plasma was used when compared with conventional healing. We conclude that Platelet rich plasma enhances bone healing in maxillofacial defects and we recommend it use in regenerative procedures for osseous defects in maxillofacial region.

Keywords: Platelet Rich Plasma; Growth Factors; Benign Lesions; Bone Density.

Introduction

Osseous regeneration depends on a number of factors such as, the anatomical location, the patient's age, parameters such as monocortical or bicortical nature of the defect, periosteal integrity, vascular supply, and surgical technique [1-3]. The concept that whether "critical size defect" influences the bone healing, however, has become obsolete over the years.

The choice of graft for hastening the process of bone regeneration can vary from autologous (bone harvested from the ileac crest, rib, symphysis, calvarial bone), allograft (Fresh frozen bone, Freeze-dried bone allograft [FDBA] Demineralized freeze-dried bone allograft [DFDBA]), to synthetic (often made of hydroxyapatite, bio-active glass). Since reaction of the human body to these synthetic substances varies from person to person, a comparison would always be biased. The best alternative to avoid such a bias would be to use an autologous graft in the form of bone or blood product.

The transplantation of autogenous bone into the bony defect is regarded as a "gold standard" for its reconstruction [9,10]. However In most cases, two surgical procedures are necessary: one for bone harvesting and the other for its implantation. To add to this disadvantage would be the complications associated with the donor site, for example, wound infection, chronic pain, nerve injuries, functional complications, and bone fractures. Also, the outcomes in bone transplantation in the recipient site would include the probable wound infection, necrosis, and resorption, which could amount to almost 30% of loss of the incorporated graft material [11-13].

Platelet rich plasma is an autologous blood product prepared from centrifugation of blood. The effect of PRP, a concentrate of platelets has been used extensively since it was first introduced in 1998 by Marx., *et al.* in maxillo-facial reconstruction procedures. Platelet rich plasma has been a graft of controversy, and despite a large number of studies carried out on the material, the findings are conflicting and anecdotal leaving us with a lot of scope to conduct scientific experimental studies on platelet rich plasma for understanding and assessing its use in bony regenerative process in human species.

Material and Methods

Twelve patients who required treatment with surgical excision of benign lesions of maxillofacial region were included in the study conducted between the year 2009 - 2012 in a dental institute in Belgaum district in India. All twelve patients underwent surgical excision of the lesion. Six patients out of the total, included in group I, were allowed to heal conventionally. The rest of the six included in group II were augmented with PRP derived from their own blood, post excision of the lesion.

Inclusion criteria were, human subjects diagnosed with acquired bony defects such as post traumatic deformities and defects caused by excision of benign lesions of maxillofacial region requiring surgical excision, post-surgical defects measuring more than 1cmsq in area and subjects with normal platelet count. Exclusion criteria were subjects with systemic diseases or immune-compromised state, which may interfere with normal healing and subjects with a history of bleeding and other platelet disorders.

All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. patients gave their informed consent prior to their inclusion in the study.

PRP gel preparation

A standard protocol was followed for all the cases. A table top centrifugation machine and a hot water bath were used for the procedure.

An average of 20 ml of blood was drawn under aseptic conditions from the patient's ante-cubital fossa to obtain a gel volume of 6 ml. The blood drawn was immediately transferred into sodium citrate [3.2%] containing vacutainers and was shook vigorously to curtail clotting. Each vacutainer contained in it, blood volume of 2.7 ml plus 0.3 ml of anticoagulant. The vacutainers were placed in the centrifugal machine and run through a 1000 RPM for 12 minutes. At the end of 12 minutes the column of fluid obtained had three layers separated due to differential densities. The supernatant, yellowish fluid containing Platelet poor plasma (PPP), a middle layer of buffy coat containing the platelet rich concentrate and a bottom layer containing the denser red blood cells. The supernatant was discarded and the platelet rich concentrate along with a millimeter of red blood cell layer was obtained.

Preparation of autologous thrombin

2.5 ml of PRP was thoroughly mixed with 0.08 ml of 10% Cacl2. This resulted in the separation of clot from thrombin. The clot is discarded, and the thrombin is used for the preparation of PRP gel. 1 ml of autologous thrombin was used for every 6ml of PRP concentrate and was subjected to a temperature of 37 degree in a water bath for two to three minutes. The PRP prepared, was poured into a sterile petri-dish.

Surgical Technique

The patients were prepared for the surgical procedure with prior consent. The procedure was performed under local or general anaesthesia and a mucoperiosteal flap was raised to expose the pathology. A bone window with minimal bony sacrifice was created to access the lesion. The underlying lesion was completely excised from its attachments and the defect was rendered sterile. The PRP gel prepared simultaneously was placed into the defect. A water tight closure of the mucoperiosteal flap was carefully carried out. Patients were monitored during the immediate post-op period for the retention of the gel and were recalled at third post-op day and seventh post-op day.

Patients were followed up for a period of 6 months. Radiographic assessment was carried out through orthopantomography (OPG) at follow up periods of first month, third and sixth month. The rate and amount of remineralisation was assessed in terms of density of the defect using the KODAK 8000C, digital panoramic and cephalometric system on the OPG. Standard reference points at all follow-up examinations were used as a guide to delineate the area of measurement. Density was measured in horizontal and vertical plane and an average of this was taken as the final value.

Serial no.	Lesion Involved	Defect Size in millimetres		Density Measured in Hounsfield Units				
		MAX Width	MAX Height	Pre-Op Value	First Follow Up [Ist Month]	Second Follow Up [3rd Month]	Third Follow Up [6TH Month]	Percentage Increase Value
1.	Simple Bone Cyst	31.6	13.2	91.00	92.00	90.00	85.00	-07.00
2.	Odontogenic Cyst	10.6	10.8	72.00	80.00	87.00	74.00	-06.00
3.	Radicular Cyst	37.3	24.2	81.00	61.50	72.00	72.00	09.50
4.	Dentigerous Cyst	7.1	11.2	75.00	73.50	77.50	79.00	06.50
5.	Residual Cyst	14.6	15.8	78.00	76.00	80.00	82.00	O6.00
6.	Residual Cyst	18.4	12.5	60.00	72.00	78.00	80.00	08.00

Table 1: Bone Density Values in Group I [Bone Defect Only].

Serial no.	Lesion Involved	Defect Size in milimeters		Density Measured in Hounsfield Units				
		MAX Width	MAX Height	Pre-Op Value	First Follow Up [1st Month]	Second Follow Up [3rd Month]	Third Follow Up [6th Month]	Percentage Increase Value
1.	Dentigerous Cyst	15.9	14.2	78.50	50.50	56.00	80.00	29.50
2.	Periapical Cyst	20.0	18.6	81.00	71.50	76.50	94.50	23.50
3.	Dentigerous Cyst	16.1	6.6	76.50	93.00	116.50	128.00	35.00
4.	Ameloblastoma	43.5	19.8	74.00	75.00	101.00	130.50	54.50
5.	Adenomatoid Odontogenic Tumour	23.7	15.5	46.00	49.00	115.00	118.50	69.50
6.	Sialo-Odontogenic Cyst	27.3	20.3	85.00	90.50	93.00	102.00	11.50

Table 2: Bone Density Values in Group II [Bone Defect + Prp Gel].

Discussion and Conclusion

Platelet rich plasma is a form of blood clot that contains a highly concentrated number of platelets. As explained by Robert Marx and Arun Garg in their book on Dental and Craniofacial Applications of Platelet Rich Plasma, a normal blood clot that would be found on a wound contains 94% red blood cells, 6% platelets, and less than 1% of white blood cells; in contrast, a PRP blood clot contains 94% platelets, 5% red blood cells and 1% white blood cells. The granules present in platelets begin degranulating within 10 minutes of clot formation and secrete over 90% of their pre-packed growth factors within one hour which ultimately bring about osseous and soft tissue regeneration [37].

Since the time of its inception PRP has been used in various regenerative procedures such as defects around implants, periodontal bone defects [20], in guided tissue regeneration, as a soft tissue regenerator in chronic ulcers of foot [30], knee joint replacement [16], as a conducive agent in sinus lift procedures [17], mandibular reconstructive procedures [14,15] and recently PRP has been put to test in aesthetic procedures such as in conjunction with derma abrasion and facelift procedures [25].

The various growth factors released by platelet rich concentrate are Epidermal growth factor [EGF], Insulin like growth factor [IGF], Platelet derived growth factor [PDGF], Transforming growth factor (alpha and beta) [TGF], Vascular endothelial growth factor [VGF] and Platelet factor [PF-4]. Each of these growth factors have a role to play during wound healing and regeneration of soft and hard tissues. The EGF regulates cell proliferation, differentiation and survival. IGF is the key regulator of cell metabolism and growth, it stimulates the osteoblasts to proliferate and differentiate. PDGF is a major mitogen for connective tissue cells. TGF also promotes cellular proliferation and differentiation along with apoptosis. TGF has the ability to stimulate osteoblast deposition of the collagen matrix and brings about mitogenesis of osteoblast precursors at the site of wound.

VGF regulates angiogenesis. PDGF in particular activates cell membrane receptors on target cells to initiate a particular activity within the target cell, like the bone deposition by osteoblasts. The alpha granules secreted by the growth factors are rich in cell adhesion molecule vitronectin which is required for osteoconduction and osteointegration. These granules secrete their pre-packaged growth factors within 10 minutes of clot development and over 90 % of it is secreted within 1 hour. These factors immediately bind to the transmembrane receptors of osteoprogenitor cells, endothelial cells and mesenchymal cells. The fibrin and fibronectin within the clot and the vitronectin forms the initial matrix. Hence because of the increased concentration of platelets, the PRP stimulates a greater and faster initial cellular response than the normal blood clot. By 17 - 21 days there is capillary penetration into the defect and increased osteoprogenitor cells, cellular metabolism and proliferation. This forms a basal matrix for continued osseous regeneration until the osteoid has completely matured and ossified [37].

The various factors influencing osseous regeneration are the anatomical location, the patient's age, parameters such as monocortical or bicortical nature of the defect, hormonal secretions, periosteal integrity, and vascular supply. Studies on healing of maxillofacial defects which were allowed to heal without grafting showed successful radiological evidence for osseous regeneration, some of them being as large as 6cm in size. Although all these defects healed spontaneously, the time taken to heal or regenerate to 80% and more was an extended period of 24 months [1-3]. This extended period is reduced to a period of 6 months when treated with PRP gel [7,11,14,38]. Our study included defects which ranged from the smallest size measuring a maximum dimension of 10.6mm to a size of 43.5mm.

A cyst is defined by a size more than one centimetre in diameter [39]. Considering this fact, it was decided upon to restrict the defect size in our study to more than one centimetre as most of the benign intraosseous lesions are cystic in nature.

The need for various bone grafts and growth factors has arisen because of the requirement for early prosthetic rehabilitation in today's aesthetically and functionally conscious population.

Platelet rich plasma gel is a product of centrifugation of patient's own blood. It overcomes the disadvantages of autologous bone and simplifies the surgical procedure [11,12,14,18,24]. Although an additional laboratory procedure is required for the preparation of platelet gel, it can always be prepared by non-surgical personnel and thereby not increasing the surgical time. PRP extraction units like the Tubex® system which utilizes Vortex Engineering technology and PRP injection device deliver PRP gel at a faster rate without the need for manual separation of the centrifuged blood. These separator devices are expensive. However, it is possible to carry out the same procedure using the conventional table top centrifugation system which provides a PRP concentrate which is rich in 94% platelets, 5% red blood cells and 1% white blood cells. This entire PRP preparation was available for our group of patients at an affordable price.

In a retrospective study, the patients treated for benign cysts of jaw and ameloblastoma were assessed for radiographic changes seen in the interior of surgical site observed during course of bone

healing. The changes were recorded as unchanged, ground-glass, spiculed, and trabecular. The changes seen in original cystic margin were recorded as completely preserved, partly decreased in width and clarity partly absent and entirely absent. According to their data it was suggested that the optimal time for detection of normal healing in the defects through radiographic examinations was four months [40], hence in our study the follow up of patients was done at 1st, 3rd and 6th month post-operatively to assess and compare the healing of bone.

The radiological changes seen and measured at the end of 6 months are significant enough for a comparison with the corresponding control group. In the literature an average of 43.4% increase in bone density has been observed at the end of 6 months in defects healing without grafts and 69.5% in group treated with PRP [14]. Landmark study carried out by Marx et al confirmed through histomorphometry that an average of 55.1% increase in density was seen in the control group and a 74% increase in the experimental group treated with PRP [15]. An investigation on spontaneous bone regeneration of 27 cysts at 6 months, after their enucleation observed an average rise of 37% in the bone density [1].

In our study, majority of the patients, belonged to the age group which ranged from 35 - 45 yrs. In the control group where the defects were allowed to heal without PRP showed an increase in bone density with time.

Using KODAK 8000C, digital panaromic and cephalometric system, bone density was estimated in our study to compare the osseous healing in terms of density. The final increase in bone density was measured by calculating the difference between density at 6 months and density measured at the end of first month post-operatively.

The control group showed a mean density change from the 1st month to 6th month as 2.8 ± 7.44 and the experimental group showed a value of 37.3 ± 21.46. This shows an increase in the rate of healing when autologous PRP was used.

We conclude that PRP enhances osseous regeneration in maxillofacial region and recommend its application in maxillofacial surgeries.

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