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Role of PRP injection in delayed union and non union of long bone fractures

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ABSTRACT

Background: The evidence for PRP osteogenic potential has been suggested by several in vitro studies, i.e. PRP addition in culture medium promoted the proliferation and differentiation of human mesenchymal stem cells (MSCs) and the effect of PRP on osteogenic differentiation was also seen on human adipose derived stem cell (ADSC). We aim to study the role of PRP in accelerating bone healing potential at the fracture site in cases of delayed union or non union of long bones.
Materials and Methods: The present study was conducted from JUNE 2018 to JUNE 2019 where 20 patients with delayed/non union of long bone fractures were treated with PRP injection. The patients were assessed for callus formation over radiological examination.
Results: In our study 65 percent of the patients benefitted from PRP therapy and showed good callus formation and 35 percent did not benefit from the treatment and required further operative intervention. The pre procedure VAS score in patients with delayed union was 3.5 $\pm$ 1.3 and non union group was 2.8 $\pm$1.4 which reduced to 1.2$\pm$0.3 and 1.1$\pm$0.3 respectively.
Conclusion: The use of PRP in treatment of non union and delayed union of long bone fractures has shown good results with no complications, it is a promising new modality which needs prospective, randomised, placebo-controlled, multicentric-studies with a much larger sample size to clarify the results and better understanding of the effects of PRP in fracture healing.

1. Introduction

Delayed fracture union is absence of callus formation 4 months after injury where as nonunion is considered after 6 months of injury without any progression towards union. Bone grafting is well accepted method of treatment of nonunion of long bone fractures. These grafts act as scaffolds which provides biomechanical strength, autologous and allogenic graft are commonly used.

Nonunion of long bone fractures is still a serious complication after infection in compound fractures. It poses tremendous economic burden upon the society. Several methods & modalities of treatment have been devised to bring about early union including biodegradable implants, BMP-7 and PRP injection.

PRP is autologous blood product containing very high concentration of platelets which provided PDGF- Platelet derived growth factor, VEGF- Vascular Endothelial growth factor, FGF- fibroblast growth factor. These factor are responsible for repair and granulation tissue formation. Substances released from activated platelets affect and induces intra cellular signaling pathways responsible for production of protein, required for fracture healing.

Preparation of PRP concentrate requires two step procedure. In first step blood is divided into platelet rich and cell rich components. Second step involving centrifugation at high speed to reduce to desired volume of plasma containing $>$ 10 lac platelets/ml of plasma.

Platelet-rich plasma (PRP) is a concentration of blood platelets suspended in a small volume of plasma. The first

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author who tried to define platelet-rich plasma was Marx, who suggested the concentration of 1 million platelets in 1μl of plasma (i.e. a level 4-5 fold higher than that found in the peripheral blood).[1]

However, Anitua et al. wrote about a platelet count of 300,000 in 1 μl of plasma, which he named plasma rich in growth factors (PRGF). The definition of platelet-rich plasma remains controversial.[2]

The first information about the use of platelet-rich plasma during cardiac surgery was published in 1987 by Ferrari et. Al. At present, platelet-rich plasma is used in medical fields such as maxillofacial surgery, cardiac surgery, orthopaedics, sports medicine, dentistry, aesthetic medicine and general surgery. Platelet rich plasma is obtained by centrifuging the patient's whole blood. As a result, blood cells are divided into three fractions: platelet poor plasma, platelet-rich (Buffy coat) and red blood cells.[3]

The next step is to separate the red blood cells from platelet-poor plasma and the buffy coat fractions. The other fractions are centrifuged using various speeds and times of spin. Platelets are isolated in a small volume of plasma.

There are several industrial methods of obtaining PRP from the patient's blood which differ in terms of centrifugation parameters, type of blood containers or the anticoagulants used.

Numerous studies have been conducted, but the best method of PRP preparation is yet to be determined.

By definition, delayed fracture union is the absence of clear radiographic signs of callus formation, four months after the injury, whereas non union is considered absence of callus even six months after the injury. Toivanene et al. recommended opting for surgical procedure if the fracture shows no clinical or radiographic signs of bone union after only 6 weeks of functional brace use. Others recommend surgery at 10-12 weeks after the injury and even suggest a treatment change if there is a strong suspicion of nonunion in two consecutive radiographs showing no callus formation at 6 and 8 weeks.[4]

The evidence for PRP osteogenic potential has been suggested by several, in vitro-studies i.e. PRP addition in culture medium promoted the proliferation and differentiation of human mesenchymal stem cells (MSCs) and the effect of PRP on osteogenic differentiation was also seen on human adipose derived stem cells (ADSCs). Furthermore, PRP can improve cell chemokinesis and chemotaxis through cytoskeleton reorganization and accelerate cell migration, thus influencing osteoblast like cells mobility, anti-microbial effects have also been suggested. However, besides the beneficial role in terms of proliferation and differentiation, as well as cell migration and protection towards microbial contamination, in-vitro studies have also shown controversial results on PRP potential to favour bone healing.[4] in certain studies.

We aim to study the role of PRP in accelerating bone healing potential at the fracture site in cases of delayed union or non union of long bone fractures.

2. Materials and Methods

The present study was conducted in the department of orthopaedics at SRMS-IMS, Bareilly on 20 patients having delayed/non union of long bone fractures from June 2018 To June 2019 after obtaining approval from hospital ethics committee.

2.1. Inclusion criteria

1. All cases of long bone fractures treated conservatively or operatively showing no / poor signs of union.
2. Patients above 18 years of age.

2.2. Exclusion criteria

1. Pathological fractures.
2. Old neglected fractures.
3. Old fractures with implant failure.
4. Patients with uncontrolled diabetes, hepatic or renal impairment disorders.
5. Presence of local or systemic infection.
6. Patients unfit for autologous donation (platelet count 130 x 10^3/I, age $>$ 60 years, hypofibrogenemia, patient on medicines known to influence platelet function like aspirin).

All protocols and procedures applied in this study are as per guidelines of ethics committee of this institution.

All the patients with delayed or non union of fractures were seen either at casualty or orthopaedics outpatient department. They were assessed for vascular and neurological status. Anteroposterior and lateral radiographs were taken respectively.

A total of 20 patients with established nonunion of long bone (8 tibia, 2 femur, 4 humerus) and 6 patients with delayed union of bone (1 radius, 1 both bone forearm, 3 tibia, 1 femur) were treated by platelet-rich plasma (PRP) injection. 16 patients were males, and 4 were females. Ninety percent (18 patients) had previously undergone open/closed reduction and internal fixation, while 10 percent (2 patients) were being treated by closed reduction and plaster application.

2.3. Technique

1. PRP KIT (Dr. PRP KIT, FDA approved).
2. PRP centrifuge (REMI PRP Plus Centrifuge).
3. 20 ml and 10 ml disposable syringes.
4. Butterfly needle (18 G).
5. Acid cit rate dextrose (ACD) anticoagulant solution.
6. Vacuum vacutainer and adaptor.
7. Sterile latex gloves.

8. Sterile cotton gauze pieces.
9. Savlon, betadine solution, spirit.

2.4. Preparation of the venipuncture site and blood sample collection

1. Proper vein was selected and the venipuncture site was cleaned with sterile cotton swabs soaked in savion, spirit and betadine in a sequential manner.
2. 2 ml of ACD anticoagulant was collected in a 20 ml disposable syringe.
3. Through butterfly needle connected to the hub of the 20 ml syringe containing anticoagulant, 18 ml venous blood was drawn from selected vein and dispensed in the PRP kit through the upper injection port.

2.5. PRP separation procedure

1. PRP centrifuge was pre-cooled at 2500 RPM for 15 minutes.
2. Program for selection was selected through the 'Separation' icon displayed on the centrifuge screen.
3. PRP kit was placed in the centrifuge along with an appropriate balance and separation started.
4. 1^{st} centrifugation was carried out at 2,700 RPM for 12 minutes.
5. On completion of separation, the PRP kit was taken out from the centrifuge and the height of the separated boundary (buffy coat) was adjusted by pulling and adjusting the knob up or down.
6. The plasma and RBC layer was blocked completely by adjusting and fastening the knob clockwise.

2.6. Concentration procedure

1. The concentration procedure was started with the 2nd centrifugation at 3200 RPM for 6 minutes.
2. After completion of centrifugation, the upper silicone lid of PRP kit was removed and through 10 ml sterile, disposable syringe, platelet poor plasma was removed leaving behind almost 4 to 5ml of the' product (PRP).
3. PRP in the kit was mixed thoroughly but gently before administration [Precautions were taken to maintain sterility throughout the preparation procedure].

2.7. Quality validation of the PRP

1. Platelet count of the PRP was performed by automated haematology analyzer.
2. The final product was considered acceptable if the final platelet concentration is 3 to 5 times the baseline platelet count of the patient.

2.8. Application of PRP

Patient was placed under the fluoroscopic guidance and the site of delayed or non union was localized.

A 26 gauge 1.5 inch needle was used to inject 4 ml concentrate of PRP for large bones and 2 ml for small bones at the fracture site following standard aseptic precaution protocol.

PRP injection was instilled every 4 weeks with a maximum of 4 injections.

3. Results

The mean age of the patient was 38.1 $\pm$ 12.98 years range (18-80) years. A total of 14 patients met with road traffic accident, 5 patients suffered injury due to fall ana 1 patient due to assault trauma. One patient who had tibia fracture also had associated abdominal injury and 4 patients were also diagnosed with severe osteoporosis. None of the patient in this study developed any complications due to the PRP injection therapy.

There were 6 patients in the delayed union group amongst which 5 patients were treated with open reduction technique and 1 patient was treated with closed reduction and casting. There were 14 patients in the non union group amongst which 13 patients were treated with open reduction and 1 patient was treated conservatively.

In the delayed union (Figure 1) group 5 patients who underwent PRP therapy showed good union and one patient did not benefit from the treatment so bone grafting was done and eventually the fracture united in12-6 weeks. There were 14 patients in the non union (Figure 2) group in which 8 patients showed fracture union and 6 patients did not benefit from the treatment. Amongst these 6 patients, 4 patients had revision surgeries where the previous implant was removed and plating along with bone graft was done. In 2 patients even though fixation was adequate there was no signs of union so bone grafting was done to help unite the fracture. They were also suffering from severe osteoporosis. The mean union time was 12 weeks (range 4 to 16 weeks) in our study.

In our study 65 percent of the patients benefitted from PRP therapy and showed good callus formation and 35 percent did not benefit from the treatment and required further operative intervention.

The pre procedure VAS score in patients with delayed union was 3.5 $\pm$1.3 and non union group was 2.8 $\pm$ 1.4 which reduced to 1.2 $\pm$ 0.3 and 1.1 $\pm$ 0.3 respectively.

4. Discussion

There have been many advances for treating long bone fracture but delayed or non union present their own difficult challenges. Approximately 10% of treated fractures require further surgical procedures because of impaired healing." The preferred management of delayed union and nonunion is autologous cancellous bone grafting which still remains the gold standard of treatment.

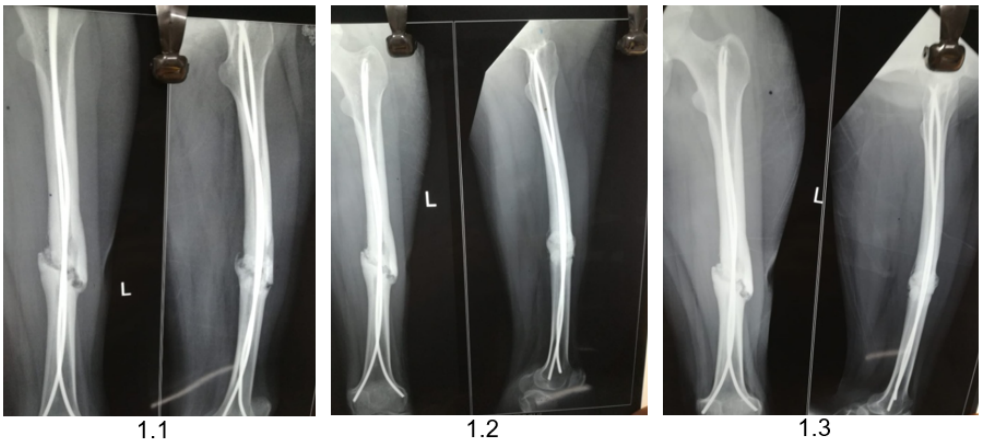

Fig. 1: 14 year old female 11 months after operation, treated by intramedullary TENS Nailing; **1.1:** Hypertrophic nonunion in a patient of fracture femur; **1.2:** 4 weeks after PRP injection; **1.3:** 8 weeks after PRP injection

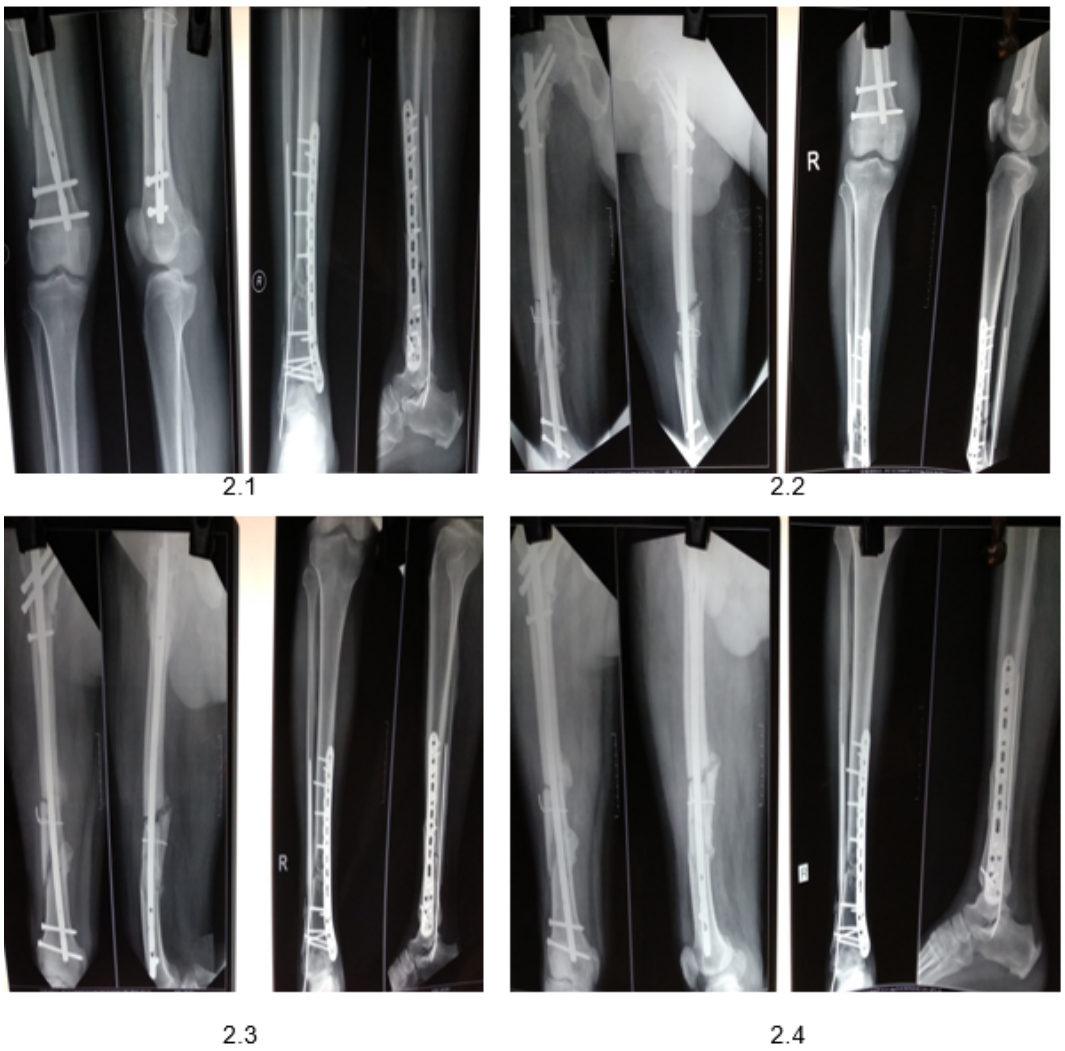

Fig. 2: Sub-tronchentric fracture with splint segmental fracture of femur + fracture both bone leg treated by recon nail & MIPPO plating in tibia – 9 months post op case (Non-union); **2.1:** Post op case at 9^{th} month; **2.2:** 4 Weeks following PRP injection; **2.3:** 8 Weeks following PRP injection; **2.4:** 12 Weeks following PRP injection

As compared to classic open grafting techniques, percutaneous administration of substances with osteo-inductive and osteogenic properties offers the advantage of decreased morbidity as well as decreased costs and hospitalization time.[5] The local injection of bone marrow and bone morphogenetic protein-7 has been shown to be capable of increasing bone defect healing.[6] While BMP is prohibitively expensive, bone marrow aspirate injection has been used with some success in India.[7] Although bone marrow has both osteo-inductive and osteogenic properties. Several authors have shown that the level of steoprogenitor cells in the bone marrow aspirate is highly variable.[8]

In nonunion, platelet concentrate might stimulate the prematurely terminated bone healing process.[9] Three different methods can be used to obtain PRP automatic machines and commercial kits with double spin rotation, single spin rotation and manual PRP separation and selective blood filtration (plateletpheresis). Anitua reported that a platelet count over 300,000/ul in PRP is effective.[10]

Gandhi et al. measured the levels of PDGF and TGF beta in fracture hematoma of patients who had sustained fresh fractures of foot and ankle. They observed the absence of these factors in cases of nonunion. On application of PRP, these fractures united at an average of 8.5 weeks.[11]

Bielecki et al. used percutaneous injection of autologous platelet-rich gel in nonunion and delayed union. They could achieve union in all cases of delayed union and in only 13 of 20 cases of non union at an average of 10.3 weeks after the injection. It was observed that the healing could be achieved only in cases where the average time from initial operation to injection was 11 months.[12] Our results are comparable to results found in these study.

Although the majority of reported studies describe the use of PRP having soft tissue healing potential and chondrogenic potential of activated peripheral blood stem cells,[13] the optimum dose of PRP for nonunion is not described and consensus is lacking regarding the production and characterization of PRP. In our study we used 4 ml of PRP in long bones and 2 ml in small bones.

Our study has a few limitations. Firstly, we used centrifugation for concentrating platelets, which may lead to the fragmentation of the platelets and early release of growth factors and cytokines, reducing bioactivity.[14] Ultrafiltration to prepare PRP may be a more scientific and efficient method. Secondly our study did not have any control placebo group and thirdly the size of the study is very small to get statistically significant results.

5. Conclusion

The use of PRP in treatment of non union and delayed union of long bone fracture has shown good results with no complications. It is a promising new modality which needs prospective, randomised, placebo- controlled, multicentric studies with a much larger sample size to clarify the results and better understanding of effects of PRP in fracture healing.

6. Source of Funding

None.

7. Conflict of Interest

None.

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