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Assessment of Role of Platelet Rich Plasma for Healing of Diaphyseal Long Bone Fracture in Lower Limb: A Randomised Control Trial

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ABSTRACT
Recovery of long bones diaphyseal injuries is frequently hampered, resulting in delayed union and nonunion. PRP treatments are autologous or allogenic products, containing more platelets than normal whole blood. PRP ease of preparation, bio safety, and adaptability as well as their lower cost, have promoted its therapeutic usage for bone repair and bone regeneration. PRP act as an artificial hematoma for enhancing osteogenic efficacy, resulting in diaphyseal repair. Optimal healing potential involves an interplay of bio mechanical and biological factors. This review is to assess the role of PRP for treating of surgically managed diaphyseal long bones fractures by comparing it with a control group and to evaluate the ratio of cortex with callus and time of fracture to unite. This study will help to reduce chance of non union and delayed union of long bone fractures and can be helpful in treatment of patients having comorbidities. Aim of this study was to evaluate the role of platelet rich plasma in bone healing radiologicaly diaphyseal long bone fracture in lower limb.

INTRODUCTION

Trauma, no matter how it occurs, is generally accepted as the leading cause of musculoskeletal damage. Accidents involving falls from heights, motor vehicle crashes, gunshot wounds and sports injuries have all been named as the major causes of injury in the world[1].

Though bone tissue has the ability for spontaneous repair following injuries, its regenerative capacity is restricted by a variety of variables, including age, fracture type and genetic bone disorder[2,3]. Lower limb fractures constitute a significant clinical challenge for orthopaedic surgeons[4]. These fractures involve many components of the limb architecture i.e. soft tissue damage, bone injury and possible vascular compromise makes it a difficult pose for orthopaedic surgeons to treat[5].

A number of intra-medullary devices are available and still the standard treatment for long bone fractures, due to good results obtained in several studies[6-8].

The micro environment surrounding a fracture site has gotten a lot of attention nowadays due to advances in molecular biology and genetics. Though the entire pathophysiology of fracture healing is not fully known, modulation of the microenvironment surrounding the fracture site using growth factors has yielded encouraging outcomes[9].

Platelets are the natural storage channels for different growth factors and cytokines found in the fracture hematoma, which initiates and regulates the fracture healing process[10]. The bioactive factors produced by PRP can participate in the processes of neovascularization, tissue remodelling and inflammatory control, leading to the concept of utilising PRP for tissue repair[11,12]. PRP is a blood product with a higher concentration of platelets than normal, resulting in a significant number of bioactive compounds in physiologic proportions[13]. Platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) and transforming growth factor (TGF) are the primary growth factors generated by platelets, which play a role in blood coagulation, soft tissue repair and bone mineralization[14,15]. Members of the TGF-b superfamily, including as TGF-b1, are particularly interesting platelet-released growth factors. Mesenchymal stem cells produce BMPs, which work in combination with TGF-b1 to promote chondroblastic and osteoblastic development, as well as the creation of new bone matrix[16-18]. Furthermore, evidence for PRP anti-microbial effects potential have been suggested by several studies which are highly desirable in relation to a surgical bone application[19,20].

PRP is gaining popularity and working wonder in Orthopaedics. Due to its easy preparation protocols, biosafety and flexibility, as well as their lower cost, have all promoted the therapeutic use of PRP for the promotion of tissue repair and bone regeneration[8].

Thus, the aim of this present study was to evaluate the role of platelet rich plasma in bone healing radiologically in diaphyseal long bone fracture in lower limb. We hypothesize that PRP can create an artificial hematoma effect and help fractures heal faster by supplying vital growth factors in fractures which were treated with intramedullary nailing.

MATERIALS AND METHODS

A prospective Randomised control trial of 58 patients with fracture in lower limb, admitted at our tertiary level health care institute was conducted. The study was carried out between from 2019-2021. All adult patients having diaphyseal long bone fractures of lower limb (less than 3 weeks old), treated surgically were included in the study. Patients with pathological fractures, having open fractures and any factor that affects fracture healing were excluded from the study. There were 44 male and 10 female in the study.

Patients unfit for autologous donation (platelet count $<130\times10^9$/L) and patients with thrombocytopenia were also excluded from the study. An informed consent has been obtained from all the participants for inclusion and the study was authorized by the institutional ethical committee.

The Patients were randomly allocated to two groups using a computer generated sequence of random numbers, i.e. Control and Case. In intervention group i.e. Case group, platelet rich plasma was injected at the fracture site, under the guidance of image intensifier while in control group, these patients PRP injections were not given following surgical fixation.

Platelet Rich Plasma was made by Method[21] During procedure aseptic conditions were maintained. Approximately 34-42.5 mL whole blood in 8.5 mL acid citrate dextrose (ACD) Tubes was collected by venipuncture from anterior cubital vein. Then blood was centrifuged using a soft spin @3000 rpm for 3 minutes. After that supernatant plasma containing platelets were transferred into another sterile tube of 10 mL (without anticoagulant). Then tubes were centrifuged again at a hard spin @4000 rpm for 15 minutes to obtain a platelet concentrate.

The lower 1/3rd was PRP and upper 2/3rd was platelet poor plasma (PPP). At the bottom of the tube, platelet pellets were formed. Platelet poor plasma was discarded and required PRP was suspended in a minimum quantity of plasma (2-4 mL) by gently shaking the tube. This freshly prepared PRP (3 mL) were injected at the fracture site on 10th day after surgical fixation by using an 18G sterile needle.

Follow-ups: After injection proper sterile dressing was done and a patient was discharged. Patients were

prescribed Tab. Tramadol 50 mg sos and other relevant medications. Follow up was done monthly till 3 months from the first injection and then at 6th and 9th month.

Radiological assessment: Radiological assessment will be done at each follow up by determining cortex to callus ratio on AP and lateral radiographs of the fractured bone and were compared with control group. Radiological caliper measurement of radiographs was done to determine the callus to cortex width. Maximal callus width divided by average width of the 2 cortices in close proximity to the fracture line gives the cortex to callus ratio[22]. All the radiographs were taken on the same X ray machine.

Statistical analysis: At the end of the study the data including radiological assessment by cortex to callus ratio and time to union was collected and analyzed by appropriate statistical tests including chi square test, Kruskale Wallis test and ANOVA. For all tests, probability less than 0.05 were considered as significant.

RESULTS

Result showed that statistical tests found no significant difference between the distribution of patients in two groups with respect to age, sex, side of fracture and type of fracture (Table 1).

The mean cortex to callus ratio was calculated for the selected groups at all follow up nine months. Case group had better cortex to callus ratio than control group at all nine month and this difference was statistically significant at on 2nd ($p = 0.0230$), 3rd ($p<0.0001$) and 6th ($p = 0.0363$), month of follow up. In other months (1st and 9th month) though study group had better cortex to callus ratio, but this difference was not statistically significant ($p>0.05$). On comparing these groups for radiological union for the two groups by ANOVA test and were 7.85±0.66 and 8.33±0.67 months, for case group and control respectively. Statistically significant ($p = 0.0110$) difference was observed for the two groups. The study population of our study had any allergic reaction, infection or any other complication due to PRP application (Table 2).

Table 1: Demographic data of patients in the study

Group	n	Mean Age (year)	Male/Female	Side of Fracture (right/left)
Control	27	32.44±15.47	21/6	14/13
Case	27	30.70±11.26	23/4	18/9

DISCUSSION

Recovery of long bones diaphyseal injuries is frequently hampered, result in slow union and non-union. Scaffolds, growth factors, cell therapies and systemic pharmaceutical interventions have all been recommended as adjunctive therapies to surgical surgery in order to improve recovery. In orthopaedics, new research has been focused on developing new materials s that may enhance bone healing, alone or in combination with other graft materials. Autografts are considered as the gold standard for enhancing fracture healing but their usage is limited by donor-site morbidity and limited supply[22-25].

PRP treatments are autologous or allogeneic products containing more platelets than normal whole blood. PRP's ease of preparation, bio-safety and adaptability, as well as its inexpensive, have promoted its therapeutic usage for bone repair and bone regeneration. However, because to the inconsistent results, there is still a need for more study into the osteogenic efficacy of PRP. The current prospective study seeks to assess the effect of PRP in radiographic long bone diaphyseal repair. We postulate that applying PRP to long bone fractures improves repair by supplying artificial hematoma and different growth factors.

Among gender distribution, there was a male predominance in both group 21 (77.8%) and 23 (85.2%) patients in case and control groups respectively while female were accounted for 6 (22.2%) and 4 (14.8%) patients which is in correlation with other study Singh et al.[8] (13.1 and 19.0).

The majority of injured side in our study was right side with 14/18 patients (51.9%/ 66.7%) and left side in 13/9 patients (48.1%/ 33.3%) in case and control group respectively. Among bone involvement shaft femur was affected in majority and were in 21 (77.8%) and 15 (55.6%) in case and control group respectively while both bone were affected in 6 (22.2%) and 12 (44.4%) respectively. In majority fracture of SOF right side accounted for about 40.8% and 37.0% in case and control group respectively. Similarly surgical procedure CRIF with FIN was used in majority and were in 21 (77.8%) and 15 (55.6%) in case and control group respectively while CRIF with TIN were affected in 6 (22.2%) and 12 (44.4%) respectively. Our findings are concordant with previous human or animal model studies[8,10,26,27] where most commonly used fracture model was femur fracture (Fig. 1 and 2).

Table 2: Comparison of cortex to callus ratio and time to union of groups by Chi-square test during 9 months of follow-up

Cortex to Callus Ratio	1st Month	2nd Month	3rd Month	6th Month	9th Month	Time to union (months)
Control	1.064±0.016	1.07±0.01	1.2±0.01	1.32±0.02	1.34±0.02	7.85±0.66
Case	1.056±0.023	1.06±0.02	1.18±0.02	1.31±0.02	1.33±0.03	8.33±0.48
P a	0.114	0.0230*	<0.0001*	0.0363*	0.1146	0.0110*

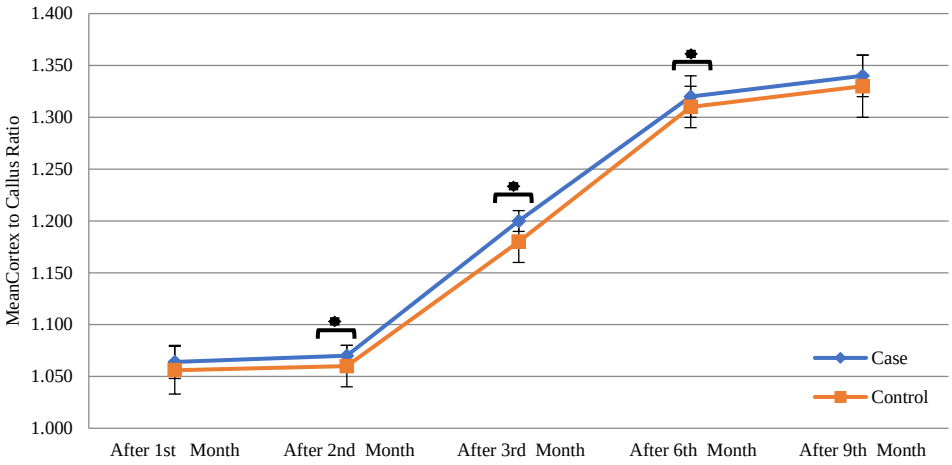

Fig. 1: Comparison of cortex to callus ratio of groups by ANOVA during 9 months of follow-up (*Statistically significant, p<0.05)

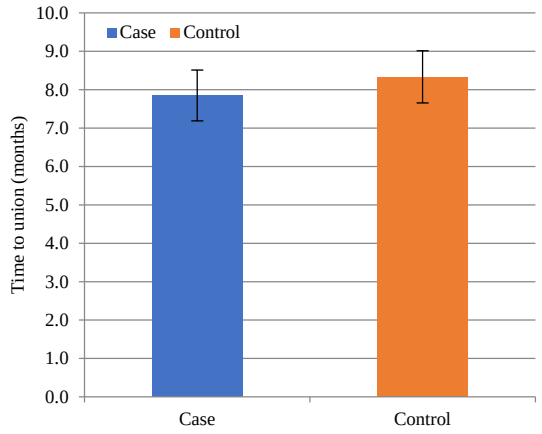

Fig. 2: Comparison of time to union between the two groups

The cortex to callus ratio was measured for the two groups at all nine months of follow up. After 1st, 2nd, 3rd, 6th and 9th month cortex to callus ratio were 1.0641±0.015, 1.0719±0.012, 1.2041±0.013, 1.3241±0.016 and1.3431±0.016 for case group respectively while in control group these were 1.0556±0.022, 1.0607±0.020, 1.1815±0.018, 1.3124±0.022 and 1.3336±0.025, respectively. Group case had better cortex to callus ratio than group control at all nine month and this difference was statistically significant at 2nd ($p = 0.0230$), 3rd ($p<0.0001$) and 6th ($p = 0.0363$), month. In other months though study group had better cortex to callus ratio and the differences were not statistically significant. Time to union was compared between two groups. These were 7.8519±0.66 and 8.3333±0.67 months' time of union for case and control group respectively and were has statistically significant difference.

Similar finding were also reported by Singh *et al*.[8] in their studies where they found statistically significant difference in 3rd and 4th month in cortex to callus ratio while in another study conducted by Gawande *et al*.[10] no such statistically significant difference were reported. In our study time to union was found not concordant with other studies reported by Gawande *et al*.[10], Calori *et al*.[9] and Singh *et al*.[8] where they reported not statistically significant difference. These contradictory results with cortex to callus ratio and time to union may be because of otherwise union rate differences due to different surgical procedures.

The research and clinical evidence in the literature on PRP's healing capabilities are contentious. Anitua[14], Marx *et al*.[15], Schlegel *et al*.[28] and Thorwarth *et al*.[29] all reported that PRP had a favourable impact on bone repair. Other investigations, however, including Froum *et al*.[30], Shanaman *et al*.[31], Raghoebar *et al*.[32] and Fuerst *et al*.[33] found no such favourable impact of PRP on bone repair.

The rationale for these contradicting outcomes for PRP insufficiency in other research might be that the efficiency of the growth factors unleashed by PRP is insufficient to promote bone growth in lesions with limited regeneration ability. Therapeutic and animal investigations that demonstrated good results for PRP were mainly conducted in well vascularized cancellous bone deformities with an abundance of precursor cells[15,34]. PRP was also employed in several trials in conjunction with autografts[15] or a composite with an extra intrinsic osteogenic benefit[28,34].

Present study has its own limitation. The number of subjects in each group was small. Future randomized controlled studies needs to be conducted

in a larger population to standardize the procedure, PRP dose, platelet concentration to have a better result.

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