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Original article

To evaluate the role of platelet-rich plasma in healing of acute diaphyseal fractures of the femur

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ABSTRACT

Purpose: New research is focusing on the use of autologous growth factors to increase the effect of bone fracture healing while decreasing the amount of healing time for the patient. Platelets have been demonstrated to be the natural storage vessel for several growth factors and cytokines that promote blood coagulation, tissue repair, and the process of bone mineralization. The present study aims to evaluate the role of platelet-rich plasma (PRP) in healing of acute femoral shaft fractures radiologically. We hypothesize that it provides artificial hematoma and releases various growth factors.

Methods: This prospective randomized study was carried out in 72 patients of traumatic fracture of the femoral shaft operated with interlocking nails (closed or open). Patients were divided into two groups: study group A ($n = 33$) treated with intramedullary nailing & PRP injection/gel application in the same setting; and control group B ($n = 39$) treated with intramedullary nailing without PRP application. Both groups were further divided into two subgroups. Study group included subgroup A1 ($n = 14$) operated with closed intramedullary nailing and PRP injection at the fracture site under radiological control, and subgroup A2 ($n = 19$) operated with open intramedullary nailing and PRP gel along with fibrin membrane application at the fracture site; while control group included subgroup B1 ($n = 16$) operated with closed intramedullary nailing, and subgroup B2 ($n = 23$) operated with open intramedullary nailing. Radiological assessment of fracture healing was done by measuring the cortex to callus ratio every month till union at 6 months.

Results: Measurements of mean cortex to callus ratio revealed significant difference between the groups A & B at third and fourth months. Measurements of mean cortex to callus ratio did not reveal significant difference between the subgroups at first and sixth months. A statistically significant difference was observed between subgroups A1 & B2 and B1 & B2 at the second month; between subgroups A1 & B2, A2 & B2 and B1 & B2 at the third month; and between subgroups A1 & B2 at fourth and fifth months.

Conclusion: PRP has no effect on femoral shaft fracture healing treated with closed intramedullary nailing. However, PRP and matrix scaffold provided by fibrin membrane may provide an artificial hematoma effect in the initial phase of healing in open or failed closed intramedullary nailing.

Introduction

With the increased incidence of road side accidents and high velocity trauma these days, femoral fractures are very frequently encountered by any orthopedic surgeon. Intramedullary nailing has proven to be a gold standard treatment modality for diaphyseal femur fractures.[1] Intramedullary nailing has many theoretical and practical advantages compared with other treatments for femoral shaft fractures. Femoral nailing gives predictable realignment of bone, rapid healing and early functional use of the limb.[1,2]

With the development made in the field of molecular biology and genetics, much attention has been recently placed on the healing environment at the molecular level. Despite the often contradicting evidence regarding the exact pathophysiology of bone repair, a complete understanding of this cellular process is becoming clearer, and manipulation of the local fracture environment by application of growth factors has been considered a treatment option from which positive results have been reported.[3] New research is focusing on the

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use of autologous growth factors to increase the effect of bone fracture healing while decreasing the length of healing time for the patient. Platelets have been demonstrated to be the natural storage vessel for several growth factors and cytokines that promote blood coagulation, tissue repair, and the process of bone mineralization.[4–6] The bone regenerative effect of platelet-rich plasma (PRP) is modulated by growth factors such as platelet-derived growth factor, insulin-like growth factor, and transforming growth factor (TGF)-β.[7,8] Platelet released growth factors of particular interest include the members of the TGF-β superfamily such as TGF-β1. In conjunction with TGF-β1, BMPs are released from mesenchymal stem cells and trigger chondroblastic and osteoblastic differentiation as well as the production of new bone matrix.[4,9,10] As a result, autologous PRP, consisting of a concentrated suspension of platelets in a limited amount of plasma, has gained an increasing reputation as a successful fracture healing therapy.[11,12]

The easy preparation protocols, biosafety and versatility of platelet-rich preparations and their reduced cost have encouraged their therapeutic use for stimulation of tissue healing and bone regeneration. But because of the conflicting results, there is still need for further research regarding the osteogenic potency of PRP. The present prospective study aims to evaluate the role of PRP in fracture healing radiologically in patients with acute fractures of femoral shaft treated with intramedullary nailing. We hypothesize that PRP application enhances the fracture healing in fresh femoral shaft fractures by providing artificial hematoma and various growth factors.

Materials and methods

General data of patients

This prospective study was carried out on 72 patients (69 males and three females) with acute diaphyseal femur fractures admitted at our tertiary level health care institute, between 2011 and 2013. Inclusion criteria of the study were: (1) age of 18–60 years, (2) acute closed femoral shaft fractures, and (3) minimum follow-up of six months. Patients with open fractures, head injuries, pathological fractures, ipsilateral femoral fractures of proximal & distal segments (i.e. AO type 31 & 33), ipsilateral tibial fractures and fractures associated with bone disorders were excluded from the study. Patients unfit for autologous donation (platelet count $<130 \times 10^9$/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 local ethical committee and performed in accordance with the ethical standards of the 1964 Declaration of Helsinki as revised in 2000.

After detailed history and examination documentation, Anteroposterior and lateral radiographs including full extent of femur from hip joint to knee joint were obtained. This helped with fracture classification and preoperative planning. Fractures were classified according to AO and Winquist and Hansen classification. As per AO classification, 33 fractures were type 32A, 38 type 32B and 1 type 32C. According to Winquist and Hansen classification, 33 fractures were type I, 31 type II, 7 type III and 1 type IV. Patients were subjected to all relevant preoperative investigations and were taken up for surgery as soon as he/she became fit for anesthesia.

Grouping and treatment

Surgery was performed under spinal/general anesthesia. Interlocking nailing (closed or open) was done in all patients. Open nailing was done only in patients in whom closed nailing failed or C-arm image intensifier was not available. Patients were randomly allocated to one of the two groups using a computer generated sequence of random numbers, as follows: study group A ($n = 33$) treated with intramedullary nailing & PRP injection/gel application in the same setting, and control group B ($n = 39$) treated with intramedullary nailing without PRP application. Both groups were further divided into two subgroups depending upon if closed or open intramedullary nailing was done. The study group comprised of subgroup A1 ($n = 14$) operated with closed intramedullary nailing and PRP injection at the fracture site under radiological control and subgroup A2 ($n = 19$) operated with open intramedullary nailing and PRP gel along with fibrin membrane application at the fracture site. While the control group comprised of subgroup B1 ($n = 16$) operated with closed intramedullary nailing, and subgroup B2 ($n = 23$) operated with open intramedullary nailing. The demographic data of the patients in different subgroups has been shown in Table 1.

Preparation of platelet-rich plasma (PRP) and PRP gel

PRP was prepared in Department of Blood Transfusion using the standard preparation techniques on the day of application from the patient's own blood under aseptic conditions using Cryfuge 6000i (Thermofisher Scientific, Germany). A total of 70 ml of blood was drawn from the antecubital vein. Blood was anticoagulated with citrate phosphate dextrose adenine (CPDA) with a ratio 1:9 to the blood. After 10 min centrifugation at 2000 rpm, the blood was layered in three basic components: red blood cells, platelets, and platelet-poor plasma (PPP). Because of the different sediment coefficients, the red blood cells were at the lowest level, the platelets were in the middle and the PPP was at the top. Red cells layer was drawn from the tube. The remainder was agitated for few seconds and underwent a second centrifugation at 2800 rpm for 10 min. The blood was then centrifuged into two layers; the supernatant was PPP while the lower layer was concentrated platelets. About three quarters of the supernatant was collected as PPP in separate vials and was used to make autologous thrombin. The residual was PRP (approximate 12–14 ml). PPP and 10% calcium gluconate was mixed (0.2–0.5 ml calcium gluconate/ml of PPP) and the solution was kept at room temperature for 12–15 min. Autologous thrombin gets settled at the bottom of the vial and was collected after removing the fibrin membrane formed at the top of the vial.

PRP was activated by addition of autologous thrombin (0.2 ml/ml of PRP) and subsequently calcium gluconate (0.2 ml/ml of PRP). This activated PRP was taken in a syringe and injected at the fracture site under radiological control in closed intramedullary nailing cases. In

Table 1
Demographic data of patients in the study.

Subgroup	*n*	Mean age (yr)	Male/Female	Fracture side (right/left)	Mode of trauma		
					RTA	Fall	Others
A1	14	29.93	13/1	7/7	14	0	0
A2	19	31.11	19/0	12/7	17	2	0
B1	16	34.12	14/2	9/7	15	0	1
B2	23	32.13	23/0	13/10	22	1	0
p value	>0.05	>0.05	>0.05	>0.05	>0.05		

Note: RTA means road traffic crash.

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