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ORIGINAL ARTICLE

Role of autologous platelet-rich plasma in treatment of long-bone nonunions: a prospective study

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Abstract

Purpose Fracture union is a complex biological process, which depends upon several systemic and local factors. Disturbance of any of these factors may lead to nonunion of the fracture. These nonunions have a huge impact on quality of life as well as socioeconomical aspects. The platelets on activation release a number of growth factors and differentiation factors, which play important role in fracture healing. This study aimed to look for efficacy of platelet-rich plasma in the treatment of established fracture nonunions of long bones.

Methods A total of 94 patients with established nonunion of long bone (35 tibia, 30 femur, 11 humerus, 4 radius, 12 ulna, 2 with both radius and ulna) were included in this study. We injected 15–20 ml of autologous platelet-rich plasma (>2,000,000 platelets/μl) under image intensifier at each nonunion site. The fracture union was evaluated clinically and radiologically regularly at monthly interval till 4 months.

Results Eighty-two patients had their fracture united at the end of 4 months. Thirty-four patients showed bridging trabeculae on X-rays at the end of 2 months, while 41 patients showed bridging trabeculae at the end of third month. Twelve patients did not show any attempt of union at 4 months and were labeled as failure of treatment. There were no complications.

Conclusion Platelet-rich plasma is a safe and effective treatment for the treatment of nonunions. More studies are needed to look into molecular mechanism of this fracture healing acceleration by platelet-rich plasma.

Keywords Nonunion · Platelet-rich plasma (PRP) · Femur · Tibia · Humerus · Radius

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Introduction

Nonunion remains a significant source of morbidity and has a significant impact on quality of life. Autologous cancellous bone is considered to be the gold standard for the treatment of nonunions [1, 2]; however, the limited supply and donor-site morbidity associated with autogenous bone graft have led the researchers to explore newer alternatives like allograft, bone substitutes, demineralized bone matrix, growth factors, cell therapy and gene therapy which have their own set of advantages and disadvantages [3–6].

Recently, platelet-rich plasma (PRP) has been a breakthrough in the stimulation and acceleration of bone and soft tissue healing. The platelets on activation release a number of growth factors and differentiation factors like platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin and thrombospondin-1, which play an important role in intracellular signaling pathways that induce the production of proteins needed for the regenerative processes, such as cellular proliferation, matrix formation, osteoid production and collagen synthesis, required for fracture healing [7–13].

Keeping in view the important role of platelets in fracture healing, the present study aimed to evaluate the efficacy of autologous platelet-rich plasma in the treatment of nonunion of fractures of long bones.

Materials and methods

The study was funded by Indian Council of Medical Research (ICMR) and was conducted at the Department of Orthopedics, All India Institute of Medical Sciences, New Delhi, over a period of 27 months (October 2008–January 2011) prospectively. After documenting the successful results of the procedure, more patients were treated with this method over the next 3 years. Ethical committee clearance was obtained before starting the study. A total of 94 patients were included in this study.

The inclusion criteria were:

1. Patients with clinical and radiological signs of nonunion of the long bones,
2. Stable internal fixation/or stable reduction with plaster immobilization,
3. Acceptable alignment of fracture fragments,
4. More than 90 % contact between the fracture fragments.

The nonunion was defined as a fracture of duration more than 6 months after injury or time from last fracture site operation, which had not shown progressive evidence of healing process throughout the past 3 months [14, 15].

The exclusion criteria were:

1. Gap nonunions,
2. Presence of local or systemic infection,
3. Pseudarthrosis,
4. Patients unfit for autologous donation (platelet count $<130 \times 10^9$/l, age >60 years, hypofibrogenemia, patient on medicines known to influence platelet function like aspirin),
5. Patient unwilling to give written informed consent.

A total of 94 patients with established nonunion of long bone (35 tibia, 30 femur, 11 humerus, 4 radius, 12 ulna, 2 with both radius and ulna) were treated by platelet-rich plasma (PRP). Their area-wise distribution is given in Table 1. Sixty-six patients were males, and 28 were females. Seventy-one patients had previously undergone open reduction and internal fixation, while 23 patients were being treated by closed reduction and plaster application.

A total of 100 ml of autologous whole blood was aspirated from cubital vein with all aseptic precautions in each patient on the morning of surgery, and PRP was prepared in the blood bank facility within the hospital and transferred to operation theater immediately thereafter for injection. PRP preparation involved a series of centrifugation and separation cycles to concentrate platelets without inducing their premature activation. Quality control of platelet concentrate in all cases confirmed the platelet count of a minimum of 2,000,000/μl and leuko-reduction. We injected 15–20 ml of autologous platelet-rich plasma in each

Table 1 Long bones and their region affected by nonunion treated by PRP injection in this study

S. no.	Bone affected	Patients
1	*Femur*	
	Proximal	10
	Shaft	12
	Distal	8
	Total	30
2	*Tibia*	
	Proximal	6
	Shaft	14
	Distal	15
	Total	35
3	*Humerus*	
	Proximal	0
	Shaft	8
	Distal	3
	Total	11
4	Ulna	14
5	Radius	6

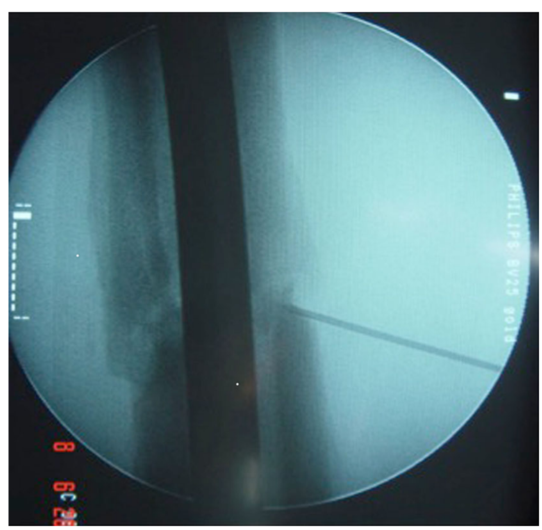

Fig. 1 PRP was injected at nonunion site under image guidance with the help of an 18-gauge needle

patient under image intensifier using a 14G intravenous cannula or a bone marrow biopsy needle (Fig. 1).

All the patients were assessed clinico-radiologically for healing of fracture at monthly follow-up till 3 months. The absence of localized tenderness, abnormal mobility and pain was considered clinical criterion for union. Radiological union was assessed with the help of anteroposterior, lateral and two oblique X-rays at each visit. Radiographic union was considered when at least three cortices out of four showed continuity on anteroposterior and lateral views [16].

Results

Eighty-two patients had their fracture united at the end of 4 months. Thirty-four patients showed bridging trabeculae on X-rays at the end of 2 months, while 41 patients showed bridging trabeculae at the end of third month. Additional seven patients showed union at the end of fourth month (Figs. 2, 3). Twelve patients (five femur, four tibia, one humerus, one with radius, one with both radius and ulna) did not show any attempt of union at 4 months and were labeled as failure of treatment. The two tibial fractures, which did not unite, had been treated conservatively in plaster cast, and the fracture was in the distal one-third of tibia. Another two tibial fractures and humeral fracture were treated by open reduction and plate fixation and were in mid-shaft. The all five femur fractures, which failed to unite, were located in the middle one-third and had been previously treated using an interlocking nail. One radius fracture, which failed to unite, was present in the proximal one-third of the shaft of radius and had been previously treated using a plate. One patient with both radius and ulna fracture was previously treated with open reduction and plating for both bones. These 12 patients required a revision surgery with stable fixation and autologous iliac cancellous bone grafting.

The average time between injury and platelet injection was 9.1 months (range 7–24 months). In the patients where the fracture united, it was observed that the platelet injection had been given within 2–4 months of diagnosis of nonunion. However, in the 12 patients where the fracture failed to unite, it was seen that the platelet concentrate had been injected 12 months or later after the diagnosis of nonunion. In one patient with tibia fracture, there was a broken implant with instability too. There were no complications seen associated with platelet injection in any patient.

Discussion

Despite continuous advances in the treatment of long-bone fractures, disturbances of healing processes remain a difficult challenge. Approximately 10 % of treated fractures require further surgical procedures because of impaired healing [17, 18]. The preferred management of delayed union and nonunion is to provide the essential elements for bone formation. The autologous cancellous bone remains the gold standard of treatment.

As compared to classic open grafting techniques, percutaneous administration of substances with osteoinductive and osteogenic properties offers the advantage of decreased

Fig. 2 Preoperative radiographs of a subtrochanteric nonunion femur with dynamic condylar screw in situ (**a**), 12 weeks post-PRP injection radiographs showing good union (**b**)

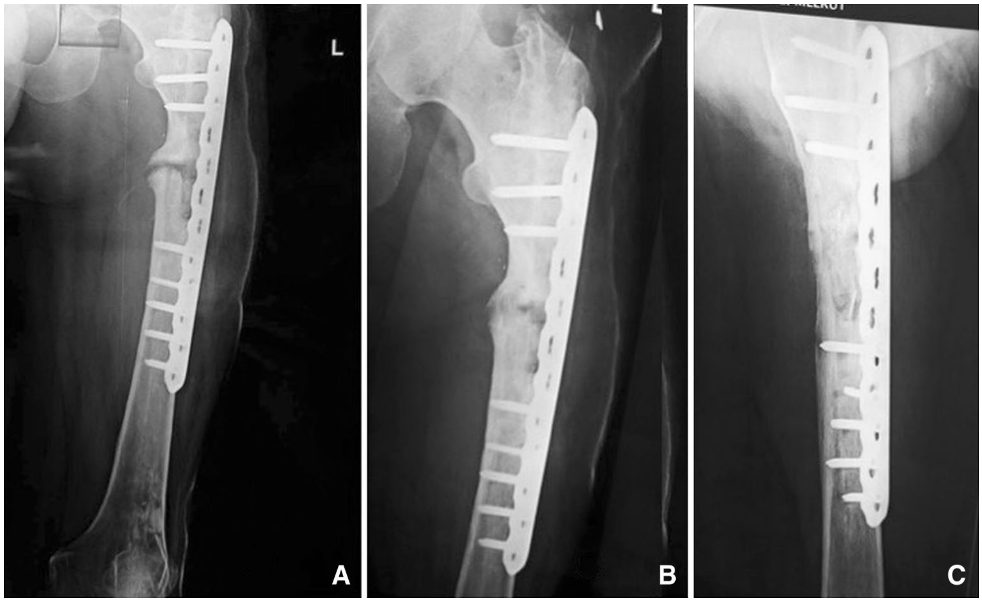

Fig. 3 Preoperative radiographs of nonunion proximal femur with locking plate in situ, (**a**) 12 weeks post-PRP injection radiograph, (**b**) 16 weeks post-PRP injection radiograph showing solid union (**c**)

morbidity as well as decreased costs and hospitalization time [14, 17]. The local application of bone marrow and bone morphogenetic protein-7 has been shown to be capable of increasing bone defect healing [19–21]. While BMP is prohibitively expensive, bone marrow aspirate injection has been used with some success in India [21]. Although bone marrow has both osteoinductive and osteogenic properties, several authors have shown that the level of osteoprogenitor cells in the bone marrow aspirate is highly variable [22–24].

The numerous growth factors present in platelets such as platelet-derived growth factor, transforming growth factor-β, insulin-like growth factors I and II, epidermal growth factor play an important role in bone healing. The desired platelet concentrations can be achieved more reliably; moreover, autologous platelets have been demonstrated to promote tissue healing in other clinical situations [25–30]. In nonunion, platelet concentrate might stimulate the prematurely terminated bone healing processes [13, 31].

The working definition of platelet-rich plasma (PRP) is a volume of autologous plasma that has a platelet concentration of 1,000,000 platelets/μl in a 5-ml volume of plasma [17]. Exogenous application of platelet-associated growth factors have been shown to stimulate proliferation of osteoblasts in culture [26, 32] and has been demonstrated to stimulate the recruitment and proliferation of osteoblasts in defects in skulls of rabbits and to augment the healing of tibial fractures in rats in a dose-dependent manner [33, 34]. More importantly, the application of autologous platelet-rich plasma used as source for growth factors has been demonstrated clinically in maxillofacial and mandibular defects as well as lumbar fusion with promising results [8, 35–38].

However, there is paucity of evidence to support the effectiveness of platelet-rich plasma in achieving union in cases with nonunion. Gandhi et al. [39] 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. Bielecki et al. [40] 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 nonunion 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.

Most authors have used platelet-rich plasma in their investigations [13, 41]. However, they have used a combination of PRP and thrombin in a dose ranging from 1000 to 5000 units [12, 37, 42, 43]. After activation of clotting system by thrombin, a series of proteolytic reactions is initiated in PRP that ultimately results in platelet degranulation and the conversion of soluble fibrinogen into insoluble fibrin. Moreover, thrombin itself stimulates fibroblast proliferation and synthesis of type IV collagen and active mediators such as NF-kappa-B [12, 37, 39, 40]. We believe that sufficient thrombin is produced due to local trauma while thoroughly infiltrating the fracture site with a needle. There are, in addition, issues related to the availability and cost of thrombin. The bovine source of thrombin has attendant transmissible risks [44], and it is unacceptable to Hindus due to the religious sentiments. Hence, we chose to inject platelets without thrombin.

We used 20 ml of PRP arbitrarily in the absence of standard recommendations. We used very high concentration of platelet (nearly 5 times the normal values) and very high volumes (20 ml), hitherto unreported in the literature. We believe it was necessary as in nonunion, the process of healing has halted completely and the local growth factor levels are abysmally low. No previous trials have used such high doses of platelets, and we believe that this could be the possible explanation for the high rates of union that could be achieved in our 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 [45, 46], the optimum dose of PRP for nonunion is not described and consensus is lacking regarding the production and characterization of PRP, but our study does demonstrate the efficacy of this method by currently used doses in nonunion of long bones in the setting of mechanical stability without bone gaps or voids. Moreover, the method was proven to be safe without any adverse effects.

Our study has a few limitations. Firstly, it is not a randomized study; secondly, 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 [47]. Ultrafiltration to prepare PRP may be a more scientific and efficient method [40]. Thirdly, we are not able to conclude whether the method will be effective in the presence of bone defects as we selected only fractures with near total contact between the fracture fragments. One recent article has stressed upon proper and careful selection of patients for effective outcome of PRP in the treatment of nonunion [48].

Even though our study is not a randomized study, yet a large sample size of 82 patients out of 94 patients achieving a complete union is compelling evidence supporting this technique. The administration of platelet-rich plasma into the nonunion site of long-bone fractures aided in their union. These encouraging results warrant further research in the form of randomized controlled trials to better elucidate the findings of this study.

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Compliance with ethical standards

Conflict of interest None.

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