Chinese Journal of Traumatology 20 (2017) 39–44

Contents lists available at ScienceDirect

Chinese Journal of Traumatology

journal homepage: http://www.elsevier.com/locate/CJTEE

Original article

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

Roop Singh [a, *], Rajesh Rohilla [a], Jeetesh Gawande [a], Paramjit Kumar Sehgal [b]

[a] Department of Orthopaedic Surgery, Paraplegia & Rehabilitation, Pt. B.D. Sharma PGIMS, Rohtak 124001, Haryana, India
[b] Department of Blood Transfusion, Pt. B.D. Sharma PGIMS, Rohtak 124001, Haryana, India

ARTICLE INFO

Article history:
Received 19 July 2015
Received in revised form
12 March 2016
Accepted 20 March 2016
Available online 20 January 2017

Keywords:
Platelet-rich plasma
Femoral fractures
Fracture fixation
Intramedullary

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.

© 2017 Daping Hospital and the Research Institute of Surgery of the Third Military Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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

* Corresponding author. Fax: +91 1262 21308.
E-mail address: drroopsingh@rediffmail.com (R. Singh).

Peer review under responsibility of Daping Hospital and the Research Institute of Surgery of the Third Military Medical University.

http://dx.doi.org/10.1016/j.cjtee.2016.03.007

1008-1275/© 2017 Daping Hospital and the Research Institute of Surgery of the Third Military Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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.

open intramedullary cases, the fibrin membrane prepared from the PPP was dipped in liquid PRP. At the end of nailing, the PRP was activated as above and was allowed to stand for 12–15 min at room temperature till it transformed into PRP gel, which was applied locally at the fracture site intraoperatively. The fibrin membrane was then used to cover and contain the PRP at the fracture site.

Follow-up

All patients had perioperative antibiotic prophylaxis. Patients were advised appropriate passive and active exercises for early rehabilitation. Stitches were removed on the first follow-up at 2 weeks. Thereafter patients were followed up at monthly intervals for a minimum of six months. Final evaluation was done at six months.

Radiological assessment

Radiological assessment was done at monthly intervals by determining cortex to callus ratio on AP and lateral radiographs of the fractured femur. 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 gave the cortex to callus ratio.[13] Radiological union was defined as the presence of a bridging callus in three out of four cortices in AP and lateral views. Painless full weight bearing on the affected limb was the physical requirement for union.

Statistical analysis

At the end of the study the data was collected and analyzed by appropriate statistical tests including Fisher's exact test, chi square test, unpaired *t*-test, Kruskal–Wallis test and ANOVA. For all tests, probability less than 0.05 were considered significant. Post-hoc power analysis for the groups A and B by taking the mean and variance values, and the effect size of 0.4233, the POWER came out to be 84.6% or 85%.

Results

Fisher's exact test and unpaired student *t*-test did no reveal statistically significant difference between groups A and B with respect to age, sex, side of fracture and type of intramedullary nailing (closed or open). The difference in distribution of patients in the four subgroups was not statistically significant with respect to age, sex, side of fracture, mode of trauma, AO classification, Winquist and Hansen classification and time of follow-up.

The mean time (months) taken by the patients of subgroups A1, A2, B1 and B2 to achieve a painless weight bearing was 3.21 ± 0.80, 3.47 ± 0.95, 3.25 ± 0.96 and 3.73 ± 0.98, respectively (statistically nonsignificant). Table 2 shows mean cortex to callus ratio in groups A and B and difference between two groups was not statistically significant at first, second, fifth and sixth months. But mean cortex to callus ratio was much higher in group A as compared to group B at third and fourth month (both $p < 0.05$).

Table 2
Mean cortex to callus ratio in two groups during 6 months of follow-up.

Group	Mean cortex to callus ratio					
	1 month	2 month	3 month	4 month	5 month	6 month
A ($n = 33$)	1.005	1.082	1.182	1.289	1.346	1.417
B ($n = 39$)	1.003	1.070	1.156	1.263	1.324	1.419
p value	0.478	0.087	0.004[a]	0.023[a]	0.064	0.843

[a] Indicates the difference between two groups is statistically significant.

On applying ANOVA test, no significant difference was observed at the first and six month of follow-up. We got interesting results in the second, third, fourth and fifth months (Table 3). A statistically significant difference was observed between subgroups A1 & B2 ($p = 0.009$), B1 & B2 ($p = 0.037$) at the second month while between subgroups A1 & B2, B1 & B2, A2 & B2 at the third month. Though subgroup A2 had a lower mean cortex to callus ratio than subgroup A1 and B1, the difference was statistically insignificant ($p = 0.082$ and $p = 1.000$, respectively). With time going, subgroup A2 failed to retain the significant lead in cortex to callus ratio over subgroup B2. At the fourth and fifth months, only statistical significant difference was observed between subgroups A1 & B2 ($p = 0.002$ and $p = 0.024$ respectively), which seemed to be decreasing as compared to previous months. The increase in the cortex to callus ratio in the four subgroups over a period of 6 months is shown in Fig. 1.

Radiological union was not seen in any patient until the start of fourth month. At the end of six months all the patients had union. Detailed distribution of union time in each subgroup is shown in Table 4. On comparing these subgroups by ANOVA test, no significant difference was observed between any subgroups in any month. No patient in the study group had any sort of infection, allergic reaction or any other complications due to PRP application. Two typical cases are shown in Figs. 2 and 3.

Discussion

Many new modalities of treatment including PRP are under research to promote bone regeneration. PRP has been shown to stimulate osteoblast proliferation in vitro and to enhance bone repair, presumably because of the high levels of autologous growth factors. PRP supplemented with fibrin glue to obtain a platelet gel might confine growth factor secretion to a chosen site.[14] Another advantage of PRP is its versatility, that is, it further permits local delivery of growth factors nonoperatively by infiltrating the fracture site with activated liquid plasma.[15,16] Addition of calcium gluconate promotes the gradual formation of native thrombin, mimicking the physiologic clotting process and enabling a more

Table 3
Comparison of cortex to callus ratio of four subgroups by ANOVA test.

Subgroup	Cortex to callus ratio											
	2 month			3 month			4 month			5 month		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
A1 ($n = 14$)	1.022–1.142	1.091	0.028	1.142–1.252	1.200	0.030	1.207–1.368	1.306	0.050	1.257–1.418	1.360	0.045
A2 ($n = 19$)	1.045–1.128	1.076	0.029	1.123–1.229	1.171	0.031	1.206–1.346	1.277	0.041	1.249–1.427	1.336	0.054
B1 ($n = 16$)	1.036–1.137	1.085	0.034	1.124–1.292	1.186	0.046	1.205–1.356	1.285	0.049	1.259–1.408	1.343	0.050
B2 ($n = 23$)	1.037–1.102	1.061	0.017	1.109–1.186	1.136	0.022	1.185–1.328	1.248	0.042	1.244–1.395	1.312	0.041
A1 vs. B2	$p = 0.009$[a]			$p = 0.000$[a]			$p = 0.002$[a]			$p = 0.024$[a]		
A2 vs. B2	$p = 0.389$			$p = 0.006$[a]			$p = 0.259$			$p = 0.613$		
B1 vs. B2	$p = 0.037$[a]			$p = 0.000$[a]			$p = 0.077$			$p = 0.294$		

[a] Means the p value is <0.05.

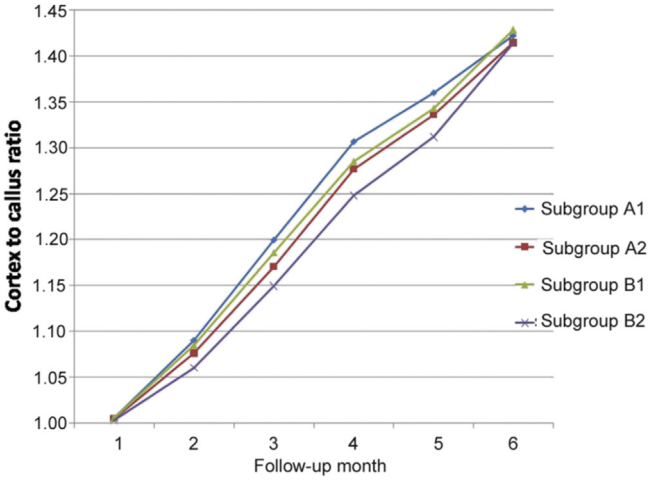

Fig. 1. Increase of cortex to callus ratio in 4 subgroups during 6 months of follow-up.

Table 4
Radiological union of four subgroups.

Subgroup	Radiological union		
	4 month	5 month	6 month
A1	6 (42.86)	12 (85.71)	14 (100)
A2	7 (36.84)	14 (73.68)	19 (100)
B1	7 (43.75)	13 (81.25)	16 (100)
B2	7 (30.43)	14 (60.87)	23 (100)

Note: no significant difference was observed between any subgroups in any month by ANOVA test (Sum of squares = 1.340, df = 3, $F = 1.200$, $p = 0.316$).

sustained release of growth factors.[17] The present study aimed to evaluate the role of PRP in biologic enhancement of healing in acute femoral shaft fractures radiologically.

In the present study the mean cortex to callus ratio was higher in group A as compared to group B in all months (except sixth month) but the difference was only statistically significant in third and fourth month. This finding suggests that PRP application enhances fracture healing to some extent. It also points towards the short term action of PRP in osteogenesis. Furthermore interesting findings were noted in mean cortex to callus ratio between the four subgroups. In the second month, the mean cortex to callus ratio was highest for subgroup A1, followed by subgroup B1. Both of the two subgroups were operated with closed technique. The intervention used (PRP) in the subgroup A1 had given it an additional advantage over subgroup B1, but failed to produce a statistically significant difference ($p = 1.000$). When these two subgroups were compared to subgroup B2, statistically significant difference was observed ($p = 0.009$ and $p = 0.037$, respectively), which probably reflects the advantage of closed nailing over open reduction and internal fixation.

At third month subgroups A1 & B1 were comparable to each other ($p = 1.000$), but continued showing a statistically significant difference in the cortex to callus ratio when compared to subgroup B2 ($p = 0.000$ and 0.000, respectively) reflecting the advantage of closed reduction over open reduction. Subgroups A1 & B1 had a better mean cortex to callus ratio when compared to subgroup A2 but the difference was statistically insignificant ($p = 0.082$ and 1.000, respectively). But when subgroup A2 was compared to subgroup B2 (both operated with open reduction technique)

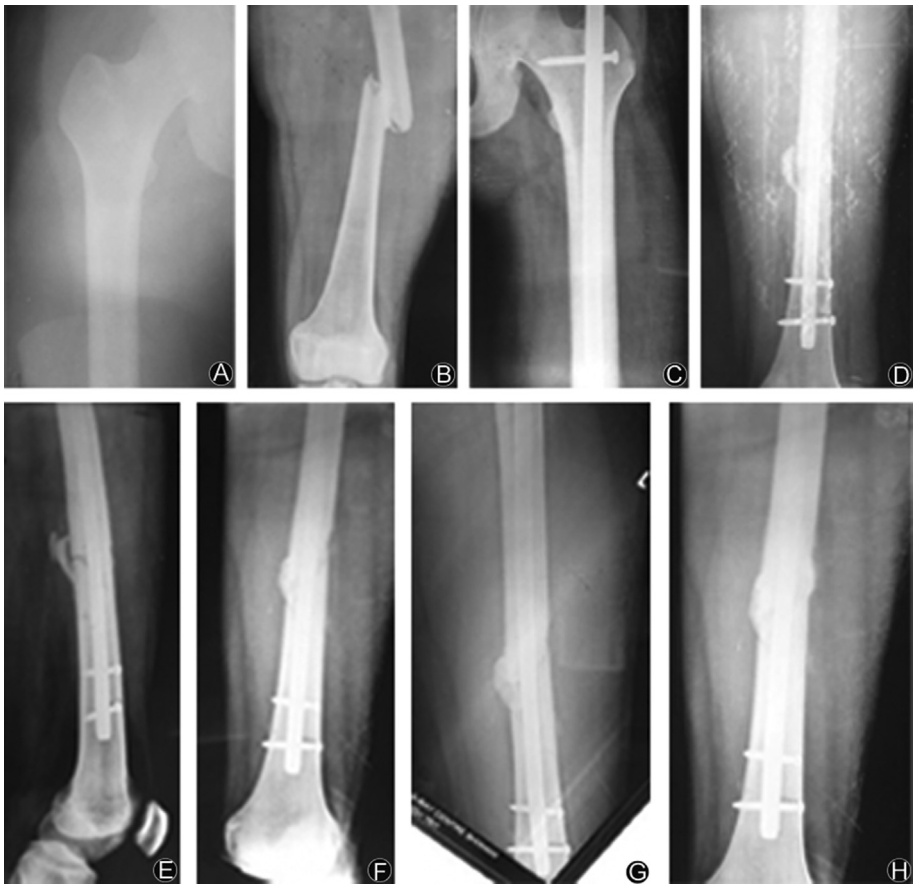

Fig. 2. X-ray images of a 32 year male with AO type 32B1.2 fracture treated by open intramedullary nailing with PRP gel and fibrin membrane application. A, B: Preoperative radiographs; C, D: Postoperative radiographs; E, F: Radiographs at 4 months show union with cortex to callus ratio of 1.234; G, H: Final follow-up radiographs at 6 months show union with cortex to callus ratio of 1.378.

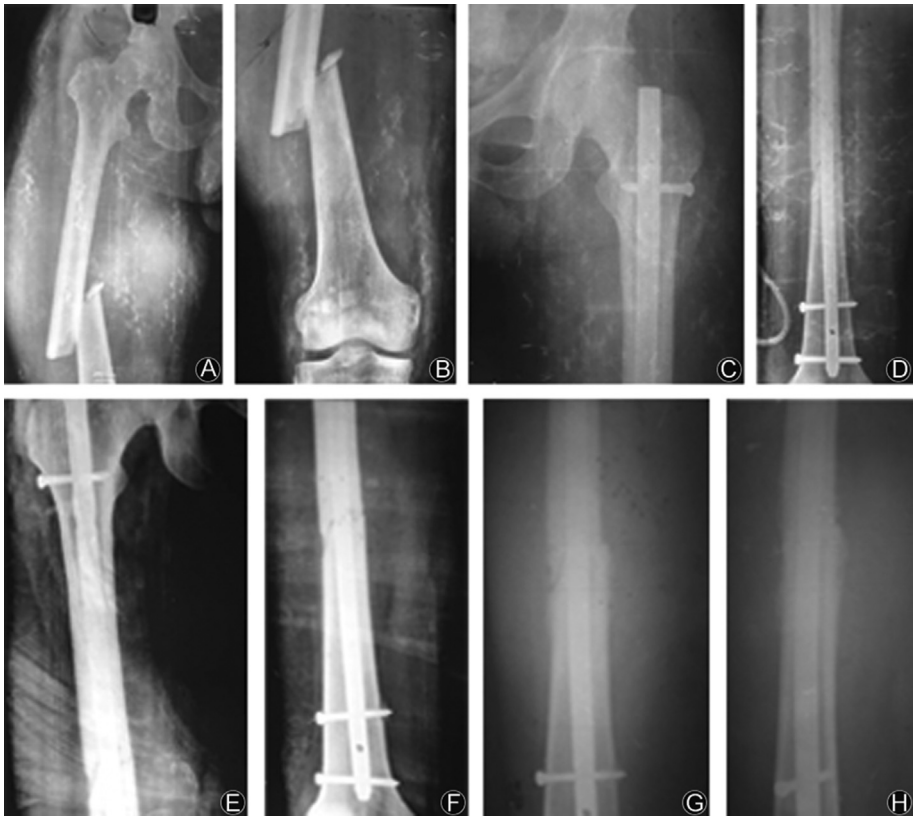

Fig. 3. X-ray images of a 36 year male with AO type 32A2.2 fracture treated by closed intramedullary nailing. A, B: Preoperative radiographs; C, D: Postoperative radiographs; E, F: Radiographs at 4 months show union with cortex to callus ratio of 1.307; G, H: Final follow-up radiographs at 6 months show union with cortex to callus ratio of 1.418.

statistically significant difference ($p = 0.006$) was observed which supports the positive effects of PRP and fibrin membrane in fracture healing in open intramedullary nailing. The initial lag period in the callus formation in subgroup B2 could be explained by the difference in the operative technique, complete loss of fracture hematoma, soft tissue damage during surgery and more blood loss. Subgroup A2 was comparable to subgroup B2 in these aspects but had an advantage of PRP and fibrin membrane which would have given it an artificial hematoma effect and growth factors right at the fracture site, which explains the significant difference between the two subgroups.

At fourth month, subgroups A1 & B1 were found to be comparable to each other ($p = 1.000$) and showed no statistical significant difference in the mean cortex to callus ratio when compared to subgroup A2 ($p = 0.377$ and 1.000, respectively). Subgroup B2 showed a rapid increase in cortex to callus ratio in this month, to make up the initial lag. However, subgroup A2 failed to retain the significant lead over subgroup B2 ($p = 0.259$) which points towards the short term action of PRP in osteogenesis. Only statistically significant difference was observed between subgroup A1 & B2 ($p = 0.002$), indicating advantage of closed reduction.

At fifth month subgroups A1 & B1 were found to be comparable to each other ($p = 1.000$) and showed no statistical significant difference in the mean cortex to callus ratio when compared to subgroup A2 ($p = 0.984$ and 1.000, respectively). Subgroup B2 was comparable to subgroup A2 & B1 with no much difference in mean cortex to callus ratio ($p = 0.613$ and 0.294, respectively). Only significant difference was observed between subgroup A1 & B2 ($p = 0.024$) which seemed to be decreasing as compared to previous month.

Human studies have reported varying effects of PRP on fracture healing.[3,14,18,19] Dallari et al[14] studied the effect of PRP in healing of tibial osteotomy in genu varum patients. They showed a significantly higher rate of osseointegration in groups treated with PRP or PRP plus stromal cells than in the control group. They concluded that adding a platelet gel combined with bone stromal to lyophilized bone chips increases the osteogenic potential of lyophilized bone chips and may be a useful tool in treatment of patients with massive bone loss. Sanchez et al reported that PRP enhanced the healing of non-hypertrophic nonunions of long bones.[18] Whereas Mariconda et al[19] failed to show the clinical usefulness of PRP in long bone nonunion treated by external fixation. Calori at al[12] compared the efficacy of rhBMP protein 7 and PRP in bone healing in nonunions. They concluded that the rhBMP protein 7 was superior to PRP in fracture healing of long bones.

The clinical and experimental data in the literature regarding the osteogenic potential of PRP are controversial. A number of authors report a positive influence of PRP on bone regeneration.[7,8,20,21] However, other clinical[22–24] and experimental[25–27] studies demonstrate no effect of PRP on bone defect healing. Why are the results in the literature so controversial? The reason for the failure of PRP in other studies might be that the potency of the growth factors liberated by PRP is too weak to induce bone formation in defects with low regenerative capacity. Clinical and animal studies that found positive effects for PRP were mostly performed in well-vascularized cancellous bone defects where an abundant presence of precursor cells can be assumed.[8,28] Some studies also used PRP in combination with autografts[8] or a matrix with an additional intrinsic osteogenic effect.[20,28]

When considering the fibrin matrix, if degradation of a scaffold does not align with the rate of bone regeneration, healing may be impaired by either a lack of a scaffold, or an excessive volume of intact scaffold. Fibrin supports angiogenesis by providing a matrix scaffold which supports cell migration and provides chemotactic

activity. The structure of a fibrin clot may affect its ability to perform as a suitable scaffold for cellular attachment,[29] while the binding of thrombin and growth factors to the fibrin fibers also support healing as a standby release mechanism during primary clot degradation.[30,31] In subgroup A2, we used fibrin membrane to act as scaffold and retain various platelet derived factors.

Several reasons have been proposed responsible for the controversial results of PRP clinical and experimental outcome. It was assumed that PRP alone cannot induce bone formation but can support osteogenesis in the presence of precursors cells.[20,22] This may explain the success of PRP observed in cancellous defects or in combination with autogenous grafts. However, this limitation will prevent PRP from becoming an attractive alternative for the reconstruction of major diaphyseal defects with low regenerative potential. One may argue that the lack of effect of PRP might also result from an insufficient platelet concentration. The growth factor content of PRP depends on the technique used for platelet concentration and the final thrombocyte count. Only few authors have investigated concentration-dependent effects. Schlegel et al[20] found somewhat better results with higher (6.5-fold compared to normal blood) than with lower platelet concentrations (4.1-fold) on bone regeneration in skull defects of minipigs. Whereas, Weibrich et al[32] assessed the effect of platelet concentration in PRP on peri-implant bone regeneration in rabbits and concluded that only significant difference in bone regeneration was seen with intermediate platelet concentrations [(2–6) × the concentration in whole blood i.e., 503,000–1,729,000 platelets/ml PRP]. They concluded that at lower concentrations, the effect was suboptimal, while higher concentrations might have a paradoxically inhibitory effect. The subclinical result in the present study might be due to inadequate PRP dose or suboptimal platelet concentration.

Present study has its own limitation. The number of subjects in each subgroup was small and we used only single dose of PRP. Future randomized controlled studies needs to be conducted in a larger population to standardize the procedure, PRP dose and platelet concentration to have a better result.

In the light of findings of the present study, we conclude that 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 initially in open or failed closed intramedullary nailing. This effect of PRP fades off in later follow-up months with no difference in fracture union at six months. The results obtained here do serve as a preliminary research and future controlled studies with larger sample size are warranted to accept or refute the osteogenic properties of PRP in acute fracture healing.

References

1. Bostman O, Varjonen L, Vainionpaa S, et al. Incidence of local complications after intramedullary nailing and after plate fixation of femoral shaft fractures. *J Trauma*. 1989;29:639–645.
2. Loomer RL, Meek R, De Sommer F. Plating of femoral shaft fractures: the Vancouver experience. *J Trauma*. 1980;20:1038–1042.
3. Calori GM, Tagliabue L, Gala L, et al. Application of rhBMP-7 and platelet-rich plasma in the treatment of long bone non-unions: a prospective randomised clinical study on 120 patients. *Injury*. 2008;39:1391–1402. http://dx.doi.org/10.1016/j.injury.2008.08.011.
4. Roldan JC, Jepsen S, Miller J, et al. Bone formation in the presence of platelet-rich plasma vs. bone morphogenetic protein-7. *Bone*. 2004;34:80–90.
5. Sanchez AR, Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. *Int J Oral Maxillofacial Implants*. 2003;18:93–103.
6. Tischler M. Platelet rich plasma. The use of autologous growth factors to enhance bone and soft tissue grafts. *N Y State Dent J*. 2002;68:22–24.
7. Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. *Int J Oral Maxillofacial Implants*. 1999;14:529–535.
8. Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85:638–646.
9. Kim CS, Kim JI, Kim J, et al. Ectopic bone formation associated with recombinant human bone morphogenetic proteins-2 using absorbable collagen sponge and beta tricalcium phosphate as carriers. *Biomaterials*. 2005;26:2501–2507.
10. Xu SW, Yu R, Zhao GF, et al. Early period of fracture healing in ovariectomized rats. *Chin J Traumatol*. 2003;6:160–166.
11. Simman R, Hoffmann A, Bohinc JR, et al. Role of platelet-rich plasma in acceleration of bone fracture healing. *Ann Plast Surg*. 2008;61:337–344. http://dx.doi.org/10.1097/SAP.0b013e318157a185.
12. Calori GM, D'Avino M, Tagliabue L, et al. An ongoing research for evaluation of treatment with BMPs or AGFs in long bone non-union: protocol description and preliminary results. *Injury*. 2006;37:S43–S50.
13. Sano H, Uhthoff HK, Backman DS. Correlation of radiographic measurements with biomechanical test results. *Clin Orthop Relat Res*. 1999;268:271–278.
14. Dallari D, Savarino L, Stagni C, et al. Enhanced tibial osteotomy healing with use of bone grafts supplemented with platelet gel or platelet gel and bone marrow stromal cells. *J Bone Jt Surg Am*. 2007;89:2413–2420.
15. Anitua E, Sanchez M, Orive G, et al. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. *Biomaterials*. 2007;28:4551–4560.
16. Anitua E, Sanchez M, Orive G, et al. Delivering growth factors for therapeutics. *Trends Pharmacol Sci*. 2008;29:37–41.
17. Tsay RC, Vo J, Burke A, et al. Differential growth factor retention by platelet-rich plasma composites. *J Oral Maxillofacial Surg*. 2005;63:521–528.
18. Sanchez M, Anitua E, Cugat R, et al. Nonunions treated with autologous preparation rich in growth factors. *J Orthop Trauma*. 2009;23:52–59. http://dx.doi.org/10.1097/BOT.0b013e31818faded.
19. Mariconda M, Cozzolino F, Cozzolino A, et al. Platelet gel supplementation in long bone nonunions treated by external fixation. *J Orthop Trauma*. 2008;22:342–345. http://dx.doi.org/10.1097/BOT.0b013e318172cea5.
20. Schlegel KA, Donath K, Rupprecht S, et al. De novo bone formation using bovine collagen and platelet-rich plasma. *Biomaterials*. 2004;25:5387–5393.
21. Thorwarth M, Rupprecht S, Falk S, et al. Expression of bone matrix proteins during de novo bone formation using a bovine collagen and platelet-rich plasma (prp)–an immunohistochemical analysis. *Biomaterials*. 2005;26:2575–2584.
22. Froum SJ, Wallace SS, Tarnow DP, et al. Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: three bilateral case reports. *Int J Periodontics Restor Dent*. 2002;22:45–53.
23. Shanaman R, Filstein MR, Danesh-Meyer MJ. Localized ridge augmentation using GBR and platelet-rich plasma: case reports. *Int J Periodontics Restor Dent*. 2001;21:345–355.
24. Raghoebar GM, Schortinghuis J, Liem RS, et al. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? *Clin Oral Implants Res*. 2005;16:349–356.
25. Aghaloo TL, Moy PK, Freymiller EG. Investigation of platelet-rich plasma in rabbit cranial defects: a pilot study. *J Oral Maxillofacial Surg*. 2002;60:1176–1181.
26. Fuerst G, Gruber R, Tangl S, et al. Effects of fibrin sealant protein concentrate with and without platelet-released growth factors on bony healing of cortical mandibular defects. An experimental study in minipigs. *Clin Oral Implants Res*. 2004;15:301–307.
27. Fuerst G, Reinhard G, Tangl S, et al. Effect of platelet-released growth factors and collagen type I on osseous regeneration of mandibular defects. A pilot study in minipigs. *J Clin Periodontol*. 2004;31:784–790.
28. Kim SG, Kim WK, Park JC, et al. A comparative study of osseointegration of Avana implants in a demineralized freeze-dried bone alone or with platelet-rich plasma. *J Oral Maxillofacial Surg*. 2002;60:1018–1025.
29. Wolberg AS. Thrombin generation and fibrin clot structure. *Blood Rev*. 2007;21:131–142.
30. Clark RA. Fibrin and wound healing. *Ann N Y Acad Sci*. 2001;936:355–367.
31. Laurens N, Koolwijk P, de Maat MP. Fibrin structure and wound healing. *J Thromb Haemost*. 2006;4:932–939.
32. Weibrich G, Hansen T, Kleis W, et al. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. *Bone*. 2004;34:665–671.