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Use of Un-centrifuged Bone Marrow in Delayed Unions and Non Unions of Diaphyseal Fractures of Long Bones in Adults.

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ABSTRACT

Bone marrow provides osteo-progenitor cells, the main cells in bone formation and fracture healing. The purpose of this study was to ascertain the osteogenic potential of un-centrifuged bone marrow. Twelve patients seven non-unions and five delayed unions of long bone fracture were treated with un-centrifuged bone marrow injection. There were six fracture tibial shaft, four fracture shaft of femur and two fracture shaft of humerus. Average time duration between injury and procedure was 5.9 months (range – 11 wks to 11months 2 wks). The bone marrow was aspirated from anterior superior iliac crest and was injected per-cutaneously at the fracture site under image intensifier guidance. The procedure was carried out as a brief in patient procedure. All but three cases required only one injection of bone marrow. Union was observed radio logically in fifteen cases. The average time of union after injection was 13.18 weeks (range-six week to six months). One case of fracture shaft of femur needed open bone grafting. Percutaneous autologous bone marrow injection is a less invasive and safe alternative to open bone grafting, especially as an early intervention in fracture healing process.

Keywords: Bone marrow injection, delayed union, nonunion.

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INTRODUCTION

Fracture healing is an intricate process about which not everything is known. Reasons for delayed fracture healing can be many including polluted food, multidrug resistant bacteria infection [1]. Prediction of which fracture will go for non union is difficult. The aim of any fracture treatment is re-establishing the connection of the fragments with restoring original mechanical integrity of the bone. For this the bone ends should be opposed, stably fixed or held and should be vascular. There are methods like electrical stimulation, bone grafting, bone-morphogenetic protein injection to stimulate fracture healing. Each has its own disadvantages. For example electrical stimulation is invasive; bone grafting is morbid; bone morphogenetic protein is costly. Also one must not feel that a fracture could have healed even without any augmentation. This is because there is a late healing group classified in Marsh's study [2]. However in contemporary practice if in subsequent radiographs there is no new bone then the fracture can be intervened. Thus regeneration of bridging bone in a fracture is the aim of the fracture. Completion of this is the end point of the fracture treatment. Improved design of fracture fixation devices and their use in bone fracture have tackled the mechanical problems. But still atrophic non-unions due to impaired osteogenic capability need only biological answers [3]. In fracture, a reduction in the blood supply reduces essential raw materials reaching the fracture site and also stops the stem cells reaching the site. This hampers regeneration of the fracture bridging tissue. This further causes tissue death and infection [1].

To increase the chance of union of the problematic fracture, basic process of bone repair and stimulation techniques must be understood .Cell therapy can be effective but needs further research and clinical trials to make it safe and efficacious [3]. The multipurpose nature of the special cells called the stem cells is due to their ability to transform into different cell types. The stem cells in adult are derived from the bone marrow, the adipose tissue and blood [1]. The specialty aptly termed as regenerative medicine, deals with cells with self-renewal capacity and with capacity to become precursor cells for other cell types [1]. Bone marrow cells especially with stroma with osteogenetic progenitor cells called the mesenchymal stem cells MSCs and hematopoietic stem cells or HSCs [1]. MSC harvested from the marrow can be cultured and placed in non union sites with regeneration to achieve union [1].

Still in centres where cell engineering and cell culture is not practiced direct injection of the autologous marrow is the choice. We undertook a study on delayed and non -unions of long bones of limbs to find the effectiveness of direct injection of the autologous marrow.

METHODOLOGY

A prospective study on twelve patients; seven non-unions and five of delayed union of the fracture of long bones were treated with this autologous bone marrow transplant. Of these, six fractures shaft of tibia, four fracture femur, two fracture shaft of humerus. Three femur fractures were already stabilized by interlocking nailing and one by plating. Two tibial fractures were already treated by external fixation as they were open and four were treated by nailing. The two humerus fractures were already treated by nailing. These cases were selected as there was no evidence of new bridging bone in the period after surgery for at least 3 months.

During the procedure, with the patient in supine position, intra operative image intensifier was used to localize the fracture site before the injection of bone marrow.figure 1 and 2. Iliac crest was painted with Povidone Iodine and infiltrated with 2% Xylocaine solution..figure 3. The patient was given intravenous anesthesia. The marrow was opened with a 2.7mm drill bit. The 16 gauge Salas bone marrow aspiration needle with stillete was introduced percutaneously through the above drilled hole in the superior iliac crest the procedures are given in figures 1 to 4. 10 cc and 20 cc syringes were used for aspirating the marrow from superior iliac crest. Another aspiration needle is introduced into the non union site and the aspirated marrow is injected around the fracture site under image intensifier guidance using same syringe that was used to aspirate the marrow. Heparin was not used in the syringes. The quantity of bone marrow injected around various bone fractures varied. The amount of marrow injected for tibia, femur and humerus cases are presented in table 1. The mean time between injury and this procedure was 5.9 months (range – 11 wks to 6 months). All but three cases required only one injection of bone marrow. This procedure was carried out as a brief inpatient procedure. i.e. the patients were kept in ward for overnight and sent home the next day if they had no specific problem . None of the patients had any specific problem. Patients were followed once in 4

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weeks until the union is achieved or up to 6 months whichever is earliest. If at 6 months the fracture did not unite then bone grafting was done. Details of all the cases and the clinico- radiological outcome are presented in table 1

Table 1: Details of all 12 cases that had bone marrow injection

S.NO	NAME	Age /sex	Bone fractured	Presenting time	Diagnosis	Amount of marrow injected	Number of marrow injections	Infection +/_	Bone grafting	Total follw up	United in time
1	RC	30yrs male	Tibia	7 months 10 days	Atrophic nonunion	20 ml	1	-	Not used		6 months
2	RV	50yrs \ male	Tibia	5 months 25 days	Atrophic nonunion	32 ml	1	+	Not used		5 months 25 days
3	RM	32 yrs male	Tibia	6 months	Atrophic nonunion	30 ml	1	-	Not used		6 months
4	VN	20yrs \ male	Femur	6 months	Atrophic nonunion	20 ml	1	-	Not used		6 months
5	NM	30yrs \ male	Femur	1 yr	Atrophic nonunion	50 ml	2	+	Used		10 months
6	**RK**	**30 yrs \ male**	**Humerus**	**3 months 18 days**	Atrophic nonunion	**15 ml**	**1**	**-**	**Not used**		**3 months**
7	RJ	25yrs \ male	Tibia	7 weeks	Delayed union	22 ml	1	-	Not Used		6 weeks
8	SV	29 yrs \ male	Humerus	2 months 20 days	Delayed union	18 ml	1	-	Not used		8 weeks
9	VK	32 yrs \ male	Femur	11 months 2 weeks	Atrophic nonunion	30 ml	1	-	Not used		11 weeks
10	SV	30/m	Distal femur	4 months	Delayed union	30 ml	1	-	Not used		12 weeks
11	DM	50yrs \ male	Tibial	**4 months**	Delayed union	30 ml	2	+	Not used		12 weeks
12	SN	45yrs \ male	Tibia	**3 months 18 days**	Delayed union	30 ml	2	+	Not Used		12 weeks
						Tibia 27.3 Femur 32.5 Humerus 16.5			1/12		13.18 weeks in bone marrow cases

RESULTS

11 fractures of 12 cases united with 91.6% success rate in an average time of 13.18 weeks (range 7 to 20 weeks) after injection. Of these we found upper limb fractures united faster than the lower limb fractures. One of the fractures- a case of femur fracture needed open bone grafting in our series. In another case we had residual prolonged pain at fracture site even after union in one patient.

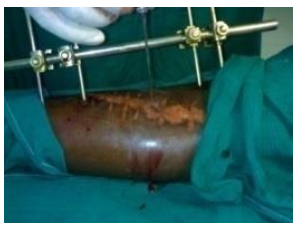

Figure 1: A tibia fracture treated with external fixator

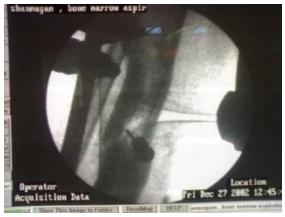

Figure 2: The fracture localized by C-arm image intensifier

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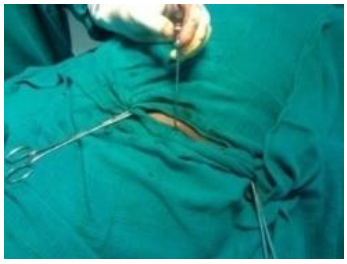

Figure 3: Iliac crest is drilled for bone marrow aspiration

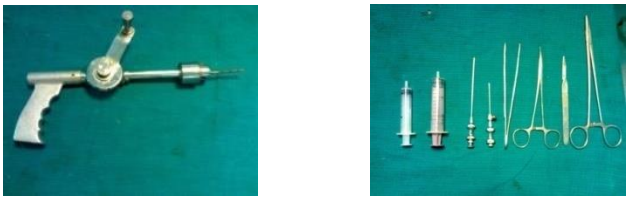

Figure 4: Drill and other instruments required for the procedure

Another patient Mr R. K a 30year old gentleman , on 04\02\07 had a vehicular accident and sustained a closed comminuted middle third of right humerus with brachial artery injury. The patient was otherwise normal with no co -morbid condition like diabetes or immuno-compromised state. On the same day brachial artery exploration and repair was done after open reduction and internal fixation of the humerus facture with a rush nail. Even after three months his radiographs showed no callus figures 5 and 6. Hence a diagnosis of delayed union was made and on 22\05\07 about 15 ml of marrow was aspirated from ipsilateral iliac crest and injected into his humerus fracture site under C-arm control. One month later a diffuse bridging of the anterior cortex which was not seen before, appeared (figures 7 and 8). In further follow ups of two months and three months after the injection, the patient had further abundant bridging callus in radiographs (figures 9-12). The patient had impingement from the rush nail which was removed. The radiograph of the humerus without the implant is seen in the figure.13,14 The final range of movement of the patient is shown in the figures 15-19.

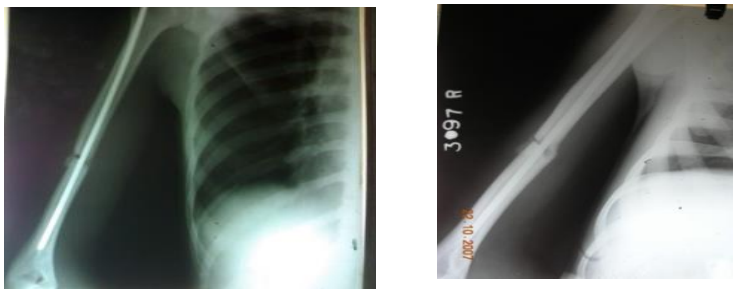

Figure 5 and 6: Radiographs taken 3 ½ months after the open reduction done along with brachial artery repair. This patient later underwent marrow injection

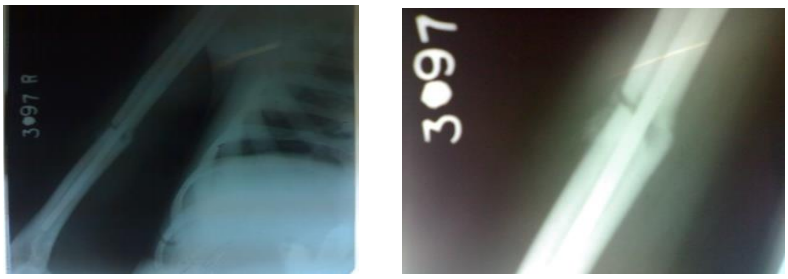

Figure 7 and 8: 14 days radiographs showing an haziness in the anterior aspect which was not before; callus has been forming only posteriorly

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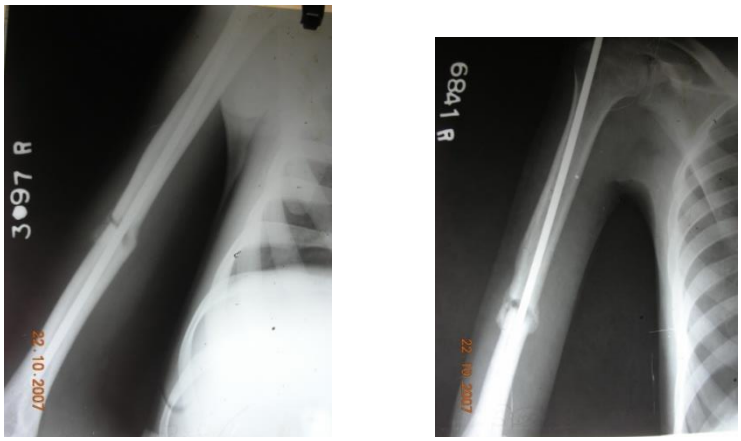


Figure 9 and 10: Radiographs at 2 months showing bridging callus on either side of the fracture both anteriorly and posteriorly

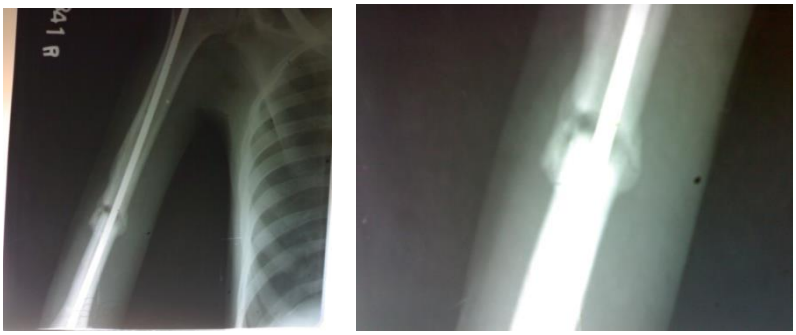


Figure 11 and 12: Radiographs at 3 ½ months showing more bridging callus on either side of the fracture both anteriorly and posteriorly

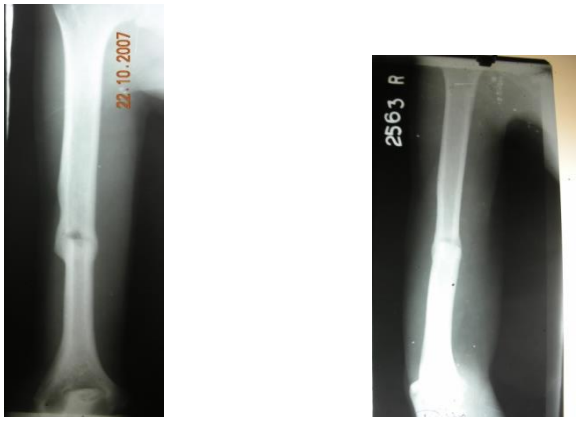
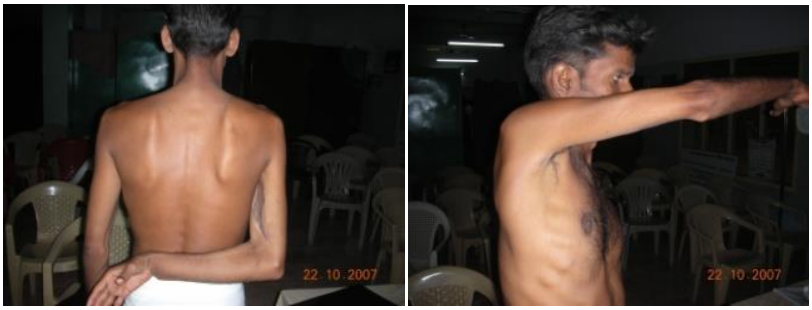


Figure 13 and 14: the radiographs showing union. The implant is removed due to irritation of the rotator cuff



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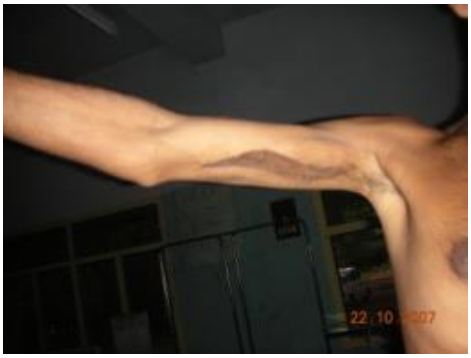


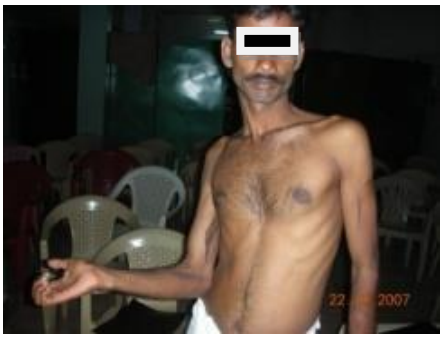
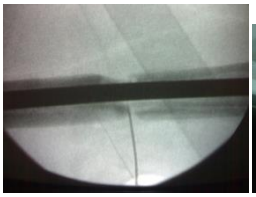
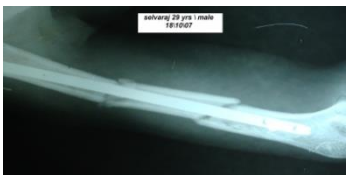
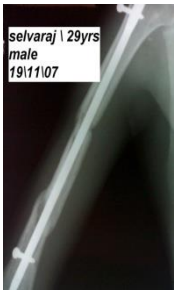


Figure 15,16,17,18 and 19: The range of movements of the patient. The figure 17 show the grafted skin on the medial side of the arm







Figures 20, 21 and 22: Another 29 year old male injured on 16\06\07 Grade 2 open transverse # femur (left), closed segmental # humerus (left) ILN 28\06\07, 09\07\07 delayed union. Time after injury for –1st injection 11 weeks Date of 1st injection 05\09\07.Amount of marrow injected 18 ml. Follow up- 1 month interval –clinical & radiological findings Subsequent visits showed union.

DISCUSSION

Bone grafting provides osteoinduction and osteoconduction and has been performed since the time of Phemister . He found that if a fracture is not united but in satisfactory arrangement, then one or two large bone grafts can be applied to a surface of the cortex roughened and prepared on either side of the fracture site -without removal of the fibrous intermediary callus. No fixation is needed for fracture or graft. Only the soft tissue is sutured over the bone graft and the fracture is supported by plaster. Slowly, the applied graft becomes attached to the fragments, the fibrous callus which was left undisturbed ossifies, and bony union is established in most cases. The graft was not fractured in any case [4].

The iliac crest is a large and easily accessible common donor site for autologous bone graft [5,6]. Lumbar hernias following iliac crest grafting occur sometimes (incidence of 5% to 9%) [6] with at least 15 cases being reported [5]. Iliac crest bone graft site hernia was reported in an obese females [5,7] swelling with repeated abdominal pain and discomfort are the complaints [7].

Sometimes the hernia was initially diagnosed only as a hematoma and when it was about to be evacuated the diagnosis was made. So high degree of suspicion is needed to diagnose such hernia after an iliac

crest bone graft especially in an obese patient [5]. In these cases, bowel loops herniated through the iliac crest bone defect and lie lateral to the iliac crest. This can be confirmed by radiographs and CT [5,7] and CT is diagnostic [6] Surgical repair is advocated due to the risk of strangulation. . Repair of lumbar hernia after iliac crest bone graft harvesting is done with prosthetic mesh [6] or titanium-mesh [7].

Thus bone grafting has advantages of uniting the fracture with a scaffold but also associated with certain complications and certain degree of morbidity attached to it. Problems are painful scars, hematoma formation, subsequent infection, weakening and fracture, sensory loss due to cluneal nerve involvement and gait disorder from the donor site. There is also the need to open the fracture site. This can result in not only infection but also devascularization of the fractured bone.

Bone marrow has determined osteogenic precursor cells (DOPC) with good potential to differentiate and form bone without additional stimuli. These DOPC are stem cells of the bone and belong to the stromal cell line of the bone marrow which is histogenetically independent of hemopoietic cells. Bone marrow stromal cells have osteogenic properties and requirements in growth factors [8].

The osteogenic capacity of the bone marrow was first confirmed in rabbits in 1869, by Goujon. He found and stated that red marrow if put as an auto-graft in an heterotophic site forms new bone [9] . After centrifugation and concentration of the aspirated bone marrow can be injected as progenitor cells. These can also be measured as colony forming units. The bone tissue is permeable to liquid so there can be a risk of fat embolism during intra-osseous infusion of bone marrow. Orlowski et al discovered fat particles in two pulmonary fields in a study in dogs. But these animals did not have a fall in O_2 saturation or problems during the intra-osseous injection. It may be because of small and inadequate amount of fat released to cause respiratory distress or a fall in arterial pressure of oxygen. Till today such problem of fat embolism is not reported in humans. But the repeated demonstration of fat particles in pulmonary fields in animals, suggest avoidance of the bone marrow injection procedures in persons with right to left intracardiac shunts due to the chance of embolism to vital organs like the brain [10].

In a study it was found that there was no specific difference between the centrifuged and un-centrifuged bone marrow. The number of stromal cells in the concentrated bone marrow is low. Stronger concentration of stem cells are needed to make a difference. The main deterrent in using the cell separation and centrifuging is the cost [11]. In the preparation, Connolly et al had tried centrifuged marrow to give more osteogenesis than the gravity sedimentation. This new bone was found even at 5 weeks after the injection of the bone marrow. The effectiveness of aspirated bone marrow can be enhanced by centrifuged or adding the marrow with a demineralised bone matrix or other stimulating factors [12]. When chambers of un-centrifuged and centrifuged marrow is placed inside tissues, the centrifuged marrow had increased percentage of new bone formation [13]. Sheep marrow studies had proven mesenchymal stem cells can as animal models of medical therapy [14,15].

The observations by such authors and the high cost associated with centrifugation and cell separation techniques made us to start the work with un-centrifuged bone marrow injection for our patients. Bone marrow injection is a less invasive percutaneous method which does not need exposure of the fracture site. Thus it avoids almost all complications associated with open bone grafting. More studies are needed to establish a definitive protocol for bone marrow injection in healing of delayed and nonunion of long bones. With about 90% union rate, even in the presence of infection, it can be recommended as a simple, safe, less morbid and reliable alternative to open bone grafting in selected cases. Open bone grafting can be postponed and reserved for cases resisting union.

CONCLUSION

In cases of non-unions and delayed unions of long bones, percutaneous autologous bone marrow injection achieved union even in the presence of infection. It is safer, easier when compared to open bone grafting, especially done early in the fracture healing process.

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