Treatment of atrophic non-union fractures by autologous bone marrow aspirate injections: A prospective study

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Abstract

Introduction: Bone healing is a multidimensional process. Fracture treatment can take many forms, usually requiring bone grafting. There is a decrease of progenitor cells at the site of atrophic nonunion and bone healing is limited by the deficiency of these cells. Autologous bone marrow injections containing mesenchymal stem cells (MSC) increases the osteogenic potential of these non-union sites and can obtain healing of these non-unions sites. Aim of our study is to assess the effectiveness of autologous bone marrow aspirate injections for the treatment of atrophic non unions.

Material and method: This was an open label prospective study with consecutive non-probability sampling. The study was conducted from September 2010 to May 2017. Thirty five patients with long bone fractures who were treated with open or closed reduction and internal fixation but subsequently went into non-union and fulfilled the inclusion criteria are included in the study. Approximately 50–60 ml of bone marrow was harvested from the iliac crest and injected at the non-union site under C-Arm guidance. These bone marrow aspirate injections were repeated at intervals of 3 weeks till the radiological evidence of union begins to appear. Regular follow up was done. Results were recorded and statistically analyzed.

Results: Union is achieved in 30 out of 35 cases i.e. in 85.7 % of cases. Thus the success rate of our modality of treatment is 85.7%. While nonunion persisted in 5 out of 35 cases i.e in 14.3% of cases. Average number of bone marrow aspirate injections (time interval between each injection is 3 weeks) at which signs of union i.e. callus formation start appearing: 5 ± 0.788 ( Range: 3-6). Average no. of weeks required for definitive union radiologically after 1st injection: 26.10 ± 5.115 weeks.

Conclusion: Percutaneous injections of bone marrow aspirate injections at the non-union site are a minimally invasive and cost-effective procedure and can be carried on day care basis.

Keywords: Progenitor cells, Bone marrow aspirate, Atrophic non-unions.

Introduction

Bone healing is a multidimensional process consisting of four well established remodeling stages; an initial inflammatory response, soft callus formation, initial bony union and bone remodeling. At the cellular level, inflammatory cells, vascular cells, osteochondral progenitors including mesenchymal stem cells (MSC’s), and osteoclasts are fundamental in the repair process [1]. MSC’s are pluripotent cells found in multiple human adult tissues including bone marrow, synovial tissues, and adipose tissues. Since they are derived from the mesoderm, they have the capacity to differentiate into bone, cartilage, muscle, and adipose tissue[2]. Iwakura et al [3] demonstrated the hematoma at the initial fracture site contains multi-lineage mesenchymal progenitor cells which are critical in the role in bone healing. Animal and early clinical models have shown that MSC’s can be used to regenerate articular cartilage and bone. It has been shown by electron microscopy, that bone marrow cells are responsible for the formation of part of the bony callus[4,5]. The adult skeleton possesses two types of bone marrow, one red and haematopoietically active, the other yellow due to adipose involution. The haematopoietic stem cell in bone marrow has been studied extensively because of its clinical relevance in therapeutic transplantation of bone marrow. Haematopoietic stem cells are pluripotent and capable of producing progeny that can differentiate into any and all of the cells of circulating blood and the immune system through a well-defined series of steps leading to differentiation into mature blood cells. In addition to the haematopoietic element, red marrow also contains a stroma where the osteogenic precursor cells are found. This osteogenic capacity of bone marrow has been exploited by several authors to reinforce the osteogenic properties of allografts [6], xenografts [7] and composite grafts [8] by mixing bone marrow removed during the operation with the bone graft.

The rate of union is influenced by the status of bone marrow of the patient. The relationship between consolidation of
fractures and changes in the bone marrow was observed by Ilizarov [9]. He demonstrated that, following the loss of blood, there was hyperactive haematopoiesis in the bone marrow of the iliac crest [9]. This suggested a link between this haematopoietic activity and the osteogenesis around the osteotomy. This study also showed that if the bone marrow of the iliac crest was more highly populated with blood cells, consolidation of a fracture was more rapid.

Independent of the anatomic location of the fracture, the degree of bone and surrounding soft tissue injury influences the healing potential. The degree of soft tissue injury was recently identified in a survey of surgeons as one of the most important factors that contribute to the development of a nonunion. High-energy fractures cause de-vascularization of the fractured bone in the form of periosteal stripping or disruption of the endosteal blood supply or both. This is clearly evident with open fractures but internal soft tissue stripping can occur equally in closed fractures. In addition, severe high-energy injuries can render the bone ends nonviable either from immediate cell death or via the process of apoptosis [10]. Bone loss, either traumatically associated with open fracture or the result of surgical debridement, is a potential precursor of nonunion. Nonunion is also closely related to the degree of open fracture by virtue of its providing a source of bacterial contamination and creating the potential for infection.

Patients with a history of chemotherapy, alcoholic intoxication and smoking have been shown to have abnormally low levels of progenitors in their iliac crests [11]. Thus, difficulties in consolidation of a fracture may be linked to an overall reduction in the numbers of progenitor cells in the bone marrow as a result of some general physiological problem. Marrow samples taken from some sites of nonunion in the tibia have shown that in adults, after in vitro cloning of the marrow, the number of progenitors is very low compared
with the tibia of normal patients. This suggests that normal tissue repair may be limited by the decreased population of progenitors in local tissues. If the cells in the osteogenic layer of the periosteum and endosteum do not have osteogenic potential, consolidation can only occur by the osteoblasts present at the fracture site. The osteoblasts are not numerous, have a low proliferative capacity and a short half-life of two to three months, which explains the difficulty in achieving bony union.

In the past decade, several clinical studies\cite{10,11} have demonstrated that transplantation of connective tissue progenitors in aspirated bone marrow can provide bone healing in nonunion. Connolly\cite{10} found bone marrow injection to be successful in the treatment of tibial non-unions. The percutaneous technique of autologous bone marrow grafting is a minimally invasive treatment. The effectiveness of this technique for the treatment of atrophic non-unions has been confirmed by several authors \cite{12,13,14}. The aim of our study is to assess the effectiveness of autologous bone marrow aspirate injections for the treatment of atrophic nonunions.

**Material and method**

This was an open label prospective study and the sampling technique was consecutive non-probability sampling. The study was conducted from September 2010 to May 2017. A total of 38 patients were initially included in the study out of which 3 patients were later excluded due to inadequate follow up. We selected 35 patients with long bone fractures who were treated with open or closed reduction and internal fixation but subsequently went into non-union and fulfilled the inclusion criteria. Patients included in this study were based on the following:

- **Inclusion Criteria:**
  1. All patients with age above 18 years and with long bone fractures who were primarily treated with open or closed reduction and internal fixation but subsequently went into non-union (here US-FDA non-union definition was used i.e. no signs for radiographic progression of healing even after 9 months since injury and with failure of progression for 3 consecutive months).
  2. All the existing methods of treating non-union were explained to the patients and only those patients who were willing to take direct percutaneous injections of bone marrow aspirate at the non-union site as the modality of treatment are included in the study.
  3. Well informed verbal and written consent was taken from all these patients.
  4. Patients with normal blood cell counts are enrolled in the study as it is a indirect representation of normal bone marrow function: RBC count (>5.4 X 106/L in males and >4.8 X 106/L in females), TLC (4000–11,000 Cells/L), absolute lymphocyte count (1500-4000 Cells/L), absolute neutrophil count (3000-6000 Cells/L).
  5. Patients who are fit and not suffering from any chronic illness or malignancy were included in the study.
  6. Patients who are willing to come for follow up at regular interval of 3 weeks.
  7. Patients with history of smoking but are willing to quit smoking are enrolled in the study.
  8. Patients with history of alcohol intake but are willing to quit consuming alcohol are enrolled in the study.
  9. Patients with history of diabetes mellitus but are willing to have strict diabetes control by daily exercise or by oral hypoglycemic drugs or by insulin.

- **Exclusion criteria:**
  1. Patients who are not fit and are suffering from any chronic illness or malignancy.
  2. Patients with inadequate blood cells count.
  3. Patients in whom adequate internal fixation was not done primarily or having inadequate reduction of the fracture fragments.
  4. Patients who are on any immunosuppressive agents.
  5. Patients with uncontrolled diabetes mellitus.
  6. Patients with history of smoking but didn't quit smoking.
  7. Patients with history of alcohol intake but didn't quit consuming alcohol.

Of the 35 patients there were 27 males (77.1%) and 8 females (22.9%). Average age in our study 52.03 ± 11.036 years (Range: 34 years -72 years). Of the 35 patients: 9 patients had humerus shaft fracture and all were managed by ORIF by plating primarily; 5 patients had shaft femur fracture and all were managed by closed reduction and IF by femur interlock nailing primarily, 6 patients had shaft tibia fracture and all were managed by closed reduction and IF by tibia interlock nailing primarily, 4 patients had distal femur fracture and all were managed by ORIF by distal end femur lock plating primarily, 4 patients had distal tibia fracture and all were managed by ORIF by distal end tibia lock plating primarily, 7 patients had subtrochanteric femur fracture and all were managed by closed reduction and IF by proximal femoral nailing primarily. All these patients had long bone fractures that subsequently went into atrophic nonunion (as confirmed by radiological examination), even though they were managed soon after injury by adequate open reduction and internal fixation. Average time elapsed since injury : 11.26 months ± 1.975 months (Range: 9-15 months). All these patients were admitted and well informed verbal and written consent was...
taken from all these patients. All these patients were willing to take direct percutaneous injections of bone marrow aspirate at the non-union site as the modality of treatment and were also satisfying other inclusion criteria.

**Technique for Bone Marrow Aspiration:**
Bone marrow aspiration requires the following items: • Scalpel • Mallet • 30 ml syringe(s) • Trocar needle and BMA cannula • ACD-A (anticoagulant). Patient is made to lie supine with a sand bag applied under the right or left hip region. Donor site i.e. anterior and posterior iliac crest is prepared and draped in a standard fashion. The site is usually blocked with 10 ml of local anesthetic of 0.5% buprenorphine and 2% lidocaine hydrochloride. This block also helps to confirm location of the anterior iliac crest bone by direct contact of the needle with bone. A small stab incision is made in the skin, and the trocar needle and cannula assembly are then introduced and advanced into the anterior iliac crest, approximately 4–5 cm posterior to the medial border of the crest. This helps to avoid the lateral femoral cutaneous nerve, which is close to the anterior superior iliac crest. Feel the front and back of the crest with the trocar needle point and choose a midpoint. While holding the trocar assembly in the palm, use gentle but firm pressure to advance the needle, rotating it in an alternating clockwise/counter clockwise motion until it advances between the cortices of the iliac crest. Sharp taps to the handle with the mallet will usually advance the needle easily between the walls of the iliac crest. In general, advance the trocar no more than 5–7 cm.

Once advanced into bone, leave the cannula in place; remove trocar needle, and attach the syringe with ACD-A to the top of the cannula with the syringe having enough space for a 1:5 ratio of ACD-A to bone marrow. Ensure the distal holes of the cannula are buried within the cancellous bone. Pull back on the syringe plunger to aspirate 15–20 ml of bone marrow. Next, detach the syringe from the cannula handle. Shake the syringe gently in an oscillating fashion to ensure the bone marrow and ACD-A are thoroughly mixed. For additional bone marrow, reattach the trocar needle onto the cannula, pull the assembly back to the surface of the iliac crest, and redirect the needle within the iliac crest. Continue to use the same hole initially created or create a new hole within the walls of the iliac crest approximately 10 cm away from the initial hole. For bone marrow aspiration from the posterior iliac crest, probe the medial and lateral edges of the iliac crest and dock the trocar assembly in the middle of the superficial cortex. Aim the trocar 30° lateral from the para-sagittal plane and 20–30° inferior from the transverse plane. Avoid going too far laterally to avoid risk of injury to the cluneal nerves. The exact depth of optimal advancement varies somewhat, but generally, the trocar should not be advanced more than 5–7 cm. As in the anterior approach covered earlier, redirection of the needle and cannula construct allows for additional harvesting of bone marrow.

Approximately 50–60 ml of bone marrow may be harvested from the iliac crest fairly easily by above technique with only one skin incision created above the iliac crest. Apply local pressure to the harvest site and close the incision with one simple suture using a 2-0 absorbable suture. A small bandage may be adequate for dressing. Some patients may experience moderate pain for a couple of days but many find the process relatively pain free.

**Technique For Bone Marrow Injection At The Receipt Non-union Site:**
Patients non-union fracture site is prepared and draped under aseptic precautions. Non-union fracture site is confirmed under C-arm AP and Lateral images and 5-10 ml of 1% xylocaine with adrenaline is injected at the non-union site. Same bone marrow aspiration needle with trocar is placed in the middle of the non-union site and position of the needle is confirmed using C-Arm AP and Lateral images. Once the position of the needle is confirmed, trocar is removed and bone marrow aspirate is injected into the non-union site taking care that the aspirate is retained at the fracture site. Needle is withdrawn slowly and dressing is done at the injection site with the adhesive tapes applied over it.

Patient is discharged on the same day and advised to come for follow up in intervals of 3 weeks. At each follow up, AP and lateral views x-rays of the fracture sites were done and these x-rays were looked for appearance of the radiological evidence of beginning of union. Till the point such an evidence begins to appear, at each follow up that is at the interval of 3 weeks, bone marrow aspirate is injected into the fracture site using above mentioned technique. Once the radiological evidence of union begins to appear, we stop repeating the bone marrow aspirate injection at that vary follow up but we ask the patient to keep on coming for follow up at intervals of 3 weeks. Thereafter at each follow up we keep on taking AP and lateral views x-rays of the fracture sites until there is callus bridging the fracture sites in both these views. (Refer to Case 1 and Case 2 mentioned below)

If there is no radiological evidence of beginning of union at 8th follow up i.e at 24th week, we consider it as a failure of our technique i.e non-union fracture site is not showing any healing response with our modality of treatment.

**Results**
Union is achieved in 30 out of 35 cases i.e. in 85.7 % of cases. Thus the success rate of our modality of treatment is 85.7%.
While nonunion persisted in 5 out of 35 cases i.e in 14.3% of cases.

Average number of bone marrow aspirate injections (time interval between each injection is 3 weeks) at which signs of union i.e. callus formation start appearing: 5 ± 0.788 (Range: 3-6)

Average number of weeks required for definitive union radiologically after 1st injection: 26.10 ± 5.115 weeks.

In our study there is a significant difference in the time taken for union in patients with no comorbidities and patient who has history of smoking alone. There is highly significant difference in the time taken for union in patients with no comorbidities and patient who has history of other comorbidities along with smoking.

In our study there is a significant difference in the number of bone marrow injections required for union in patients with no comorbidities and patient who has history of smoking along with other comorbidities.

In our study there is a significant difference in the time taken for union in patients with no comorbidities and patient who has history of diabetes mellitus alone. There is highly significant difference in the time taken for union in patients with no comorbidities and patient who has history of other comorbidities along with diabetes mellitus.

In our study there is a significant difference in the number of bone marrow injections required for union in patients with no comorbidities and patient who has history of diabetes mellitus along with smoking.

In our study there is a significant difference in the time taken for union in patients with no comorbidities and patient who has history of alcohol intake along with other comorbidities.

In our study there is a significant difference in the number of bone marrow injections required for union in patients with no comorbidities and patient who has history of alcohol intake alone. There is highly significant difference in the time taken for union in patients with no comorbidities and patient who has history of other comorbidities along with alcohol intake.

In our study there is a significant difference in the time taken for union in patients with no comorbidities and patient who has history of alcohol intake along with infection.

In our study there is a significant difference in the number of bone marrow injections required for union in patients with no comorbidities and patient who has history of other comorbidities along with INFECTION.

**Discussion**

Bone marrow concentrate isolated contains progenitor cells
such as Hematopoietic Stem Cells (HSCs), Mesenchymal Stem Cells (MSCs) and Endothelial Progenitor Cells (EPCs) along with abundant cytokines and growth factors. As bone marrow contains osteogenic progenitor cells, its implantation was proposed in order to potentially lead to efficient bone regeneration.

Hernigou et al. [15] demonstrated in their study that the number of progenitor cells injected play an important role in determining the volume of callus formed and thereby, determine the healing of atrophic non-union fractures. Their group measured the concentration of progenitor cells harvested from bone marrow using the ‘Fibroblast- Colony Forming Unit’ (CFU-F) assay, and found a direct relationship with the amount of healing and indirect relation with the time of healing versus the concentration of CFU-F.

The success rate of our modality of treatment is 85.7%. These results are comparable to the results of the study of PonemoneV et al [16] with success rate of 82%.

Specific conditions that are most notably considered to affect fracture healing are smoking, diabetes, alcohol intake and infection. Several studies have shown that consolidation of a fracture is often delayed in heavy smokers and drinkers. Experiments in animals showed that nicotine had an adverse effect on consolidation[17]. Studies on bone marrow haven shown that there is a significant adipose involution of the bone marrow in heavy smokers and drinkers and therefore a potential decrease in the number of progenitor cells [18].

In our study, number of bone marrow aspirate injections and time taken for union are significantly increased with these co-morbidities.

Researchers are now beginning to understand the differences among the progenitor cells harvested from various individuals. These differences depend on many variables, such as age, gender, and local and systemic diseases and the variability in the osteogenic potential from patient to patient represents a limitation of this autologous bone marrow grafting technique.

**Conclusion**

Grafting with autologous bone marrow containing mesenchymal stem cells increases the osteogenic potential of these non-union sites and can obtain healing of these non-unions sites. However, correct placement of the needle at the center of the fracture non-union site is of tremendous importance. Moreover, retention of the bone marrow aspirate at these non-union sites should be emphasized. Percutaneous injections of bone marrow aspirate injections at the non-union site is a minimally invasive and cost-effective procedure and can be carried on day care basis.

**References**

Conflit of Interest: Nil.
Source of Support: None

