INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

EVALUATION OF BONE MARROW ASPIRATE CONCENTRATE IN NON-UNION OF FRACTURES.

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ARTICLE INFO

Article history

Received 29/04/2020

Available online

03/07/2020

Keywords

Bone Marrow Aspirate Concentrate,

Non-Union,

Ficoll Solution.

ABSTRACT

BACKGROUND: Bone Marrow Aspirate Concentrate (BMAC) is a new class of material known as Orthobiologics. The aspirate is rich in mononuclear cells, of which bone marrow stem cells form an integral part, which develop into osteogenic cell lines and form bone at the site of injection. BMAC gives results similar to surgical methods of treating non-union such as bone grafting. The objective of our study is to investigate the efficacy of the proposed method and to assess the outcome as well as the complications of the procedure. AIM: To evaluate Bone Marrow Aspirate Concentrate in Non union of fractures. MATERIALS AND METHODS: We performed a multi centre study by including twenty one patients who are diagnosed with non-union and delayed union of fractures. This is a non-randomized study. Bone Marrow is aspirated from the iliac crest. Ficoll solution is used for density gradient centrifugation for isolation of mononuclear stem cells. Later the concentrate is injected into the defect site. Bone formation was evaluated by x-rays in two standard planes. Level of significance was set at $P<0.05$. RESULTS:The critical osseous defect reached observable union by a mean of 38 ± 1.38 weeks. A distance of 5 mm or less between the fractures ends resulted in healing. CONCLUSION: Obtained results indicate that the method is feasible and effective in the management of Non union and delayed union of fractures.

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*Please cite this article in press as **Dr. Amarnath Surath MS Ortho** et al. Evaluation of Bone Marrow Aspirate Concentrate in Non-union of Fractures. Indo American Journal of Pharmaceutical Research.2020:10(06).*

INTRODUCTION

BMAC is a class of substances called orthobiologics that are extracted from the human body. They release growth factors, cytokines and are responsible for regeneration of tissues at the site of injury or disease. However, this function is possible when the concentration of these substances or cells is much higher than the physiological normal range. Bone marrow aspirate (BMA) contains mesenchymal stem cells (MSCs), platelets and various growth factors (PDGF, TGF-a, BMP2 and IL-1RA)[1]. MSCs have the capability to differentiate into cell lines like cartilage, bone, tendon, muscle and nerves. Benefits of BMAC are achieved either by proliferation and differentiation into variety of cell lineages or by elution of growth factors and cytokines to hasten the process of healing in tissues with attenuated healing potential.

BMAC helps increase the cell count of mesenchymal stem cells. The concentration of MSC in bone marrow alone is relatively low, hence the aspirate is concentrated by density gradient centrifugation in order to increase the percentage of MSCs. Iliac crest yields the highest concentration of MSCs.

Bone union is the desired end point after any fracture. Three components are needed for any fracture to progress to healing, namely the presence of stem cells, growth factors, and a biologic scaffold. BMA has been utilized as a source of bone marrow derived mesenchymal stem cells (BM-MSC)[2]. BMAC, results in faster healing rates when compared to other conventional methods such as bone grafting. It can be safely assumed that stem cell therapy in impaired fracture healing is a viable option [3].

MATERIALS AND METHODS:

In this prospective, non-randomized study done from 2017-19 twenty one patients [Table 1] suffering from delayed and nonunion were selected for injection of BMAC. The end point was clinical and radiological evidence of union. Informed consent was obtained from the patients and the study was approved by research committee of NRI Medical College.

S.no	Age	Gender	Type of Fracture	Duration of Non union	No. of BMAC injections	Time taken for healing
1	25	M[1]	Rt[2] forearm	10 w[3]	1	24w
2	34	M	Rt humerus	12 w	4	44w
3	24	M	Rt humerus	14 w	1	20w
4	28	M	Rt leg	8 w	1	24w
5	26	M	Lt tibia	20 w	1	28w
6	35	M	Rt femur	16 w	1	12w
7	70	M	Rt femur	16 w	2	20w
8	21	M	Rt femur	12 w	1	40w
9	45	F[4]	Rt femur	14 w	2	16w
10	30	F	Rt forearm	10 w	1	16w
11	25	M	Lt[5] forearm	12 w	1	20w
12	51	M	Lt femur	12 w	1	16w
13	21	F	Prox. Tibia	8 w	1	16 w
14	29	M	Rt femur	6 w	1	12w
15	24	M	Lt humerus	12w	1	40w
16	34	M	Rt humerus	10 w	1	16w
17	31	M	Lt femur osteotomy	16 w	1	12w
18	23	M	Proximal Tibia	10 w	1	16w
19	80	M	Sub Trochanteric Fracture	8 w	1	20w
20	21	M	Distal Femur	6 w	2	24w
21	34	M	Mid shaft Femur	6 w	1	20w

Inclusion criteria and Exclusion criteria

The following were the inclusion criteria: Males and females were included. Age ranged from 21-71 years. Delayed union of fractures. (Gap less than 5mm on x-ray), non union of fractures and stable fracture fixation with persisting gap between the fragments. The exclusion criteria were as follows: Gap between fracture fragments exceeding 5mm, open fractures with unhealthy soft tissue cover, peripheral vascular disease (TAO-Thromboangiitis Obliterans, atherosclerosis) and skeletally immature patients.

[1] Male
[2] right
[3] weeks
[4] female
[5] left

Study design:

Twenty one patients were included in this prospective study based on the gap existing between the fracture fragments. Patients with delayed and non-union of fracture underwent BMAC injection into the fracture site. Demographic data and brief medical history were taken followed by clinical examination (complete blood picture, biochemical investigation and radiographic findings) for all the patients enrolled in the study. Antero-posterior and lateral view x-rays of the fracture site were taken for all the patients. The fractures having a gap exceeding 5mm were excluded from the study. Functional outcome of non- union was assessed evaluating patient satisfaction levels and radiological evidence of union with x-rays in two standard planes (AP and lateral views).

Aspiration of bone marrow

Iliac crest has the highest concentration of mesenchymal stem cells. Bone Marrow is aspirated based on these sector rule in iliac crest, which roughly divides the entire iliac crest into six zones. Of these six zones the posterior crest is preferred site as it contains higher concentration of MSCs (higher by 1.6 times compared to anterior iliac crest). In the present study marrow was aspirated from the anterior iliac crest [4]. Concentrating the bone marrow will eliminate non-nucleated cells like red blood cells and increase the number of mesenchymal cells. Density gradient centrifugation with ficoll was used to concentrate the mono nuclear cells.

Processing of Marrow Aspirate and Density Gradient Centrifugation

Ficoll plaque technique of density gradient centrifugation was employed for the isolation of mono nuclear cells. This technique takes an advantage of density differences between elements found in the blood sample and mononuclear cells. It yields RBC free bone marrow cell suspension. Heparinized marrow was diluted 1:1 in phosphate buffered saline (PBS). A 50ml conical bottom centrifugation tube is taken and 20ml of ficoll added. Thirty ml of diluted marrow was carefully layered over the ficoll taking care to maintain the ficoll marrow interface [5] (Fig 1).
The tubes were placed in centrifuge (REMI R-4C) with a 4x50ml rotor (Fig 2).

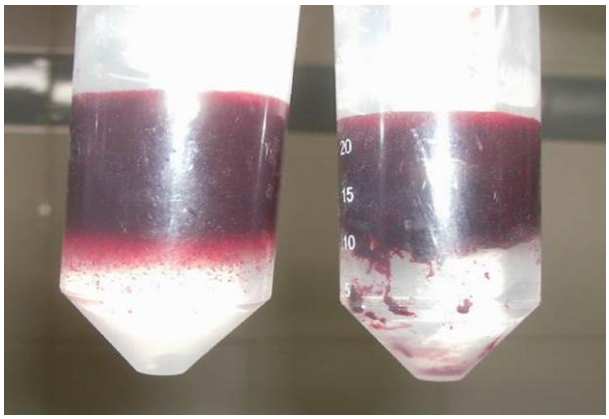


Fig 1.

Fig 2.

The samples were centrifuged at 2500rpm (833g approx.) for 20 minutes without brake. Due to higher density, RBCs and granulocytes sediment at the bottom of the ficoll layer while the platelets and mono nuclear cells form a smoky layer between the ficoll and the plasma (Fig 3). The mononuclear cells were re-suspended in 3-5 times their volume of PBS and centrifuged at 2000rpm (666g) for 10 minutes [6] wash out the ficoll. This step was repeated in order to get a pure cell suspension. At the completion of the procedure a pellet of mononuclear cells (4a)forms at the bottom of the tube (Fig 4b).

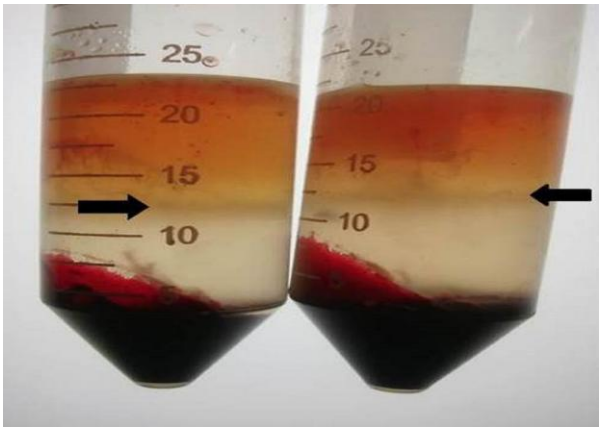


Fig 3

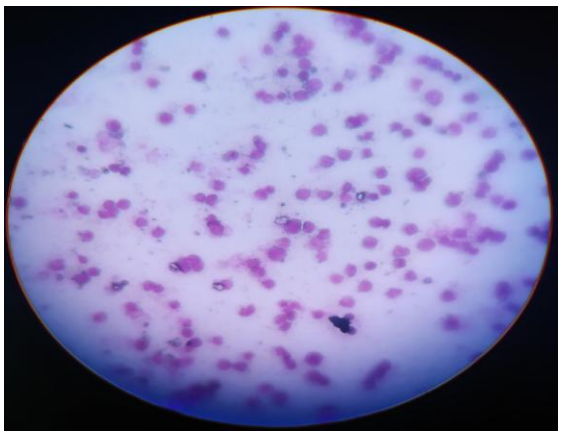


Fig 4a

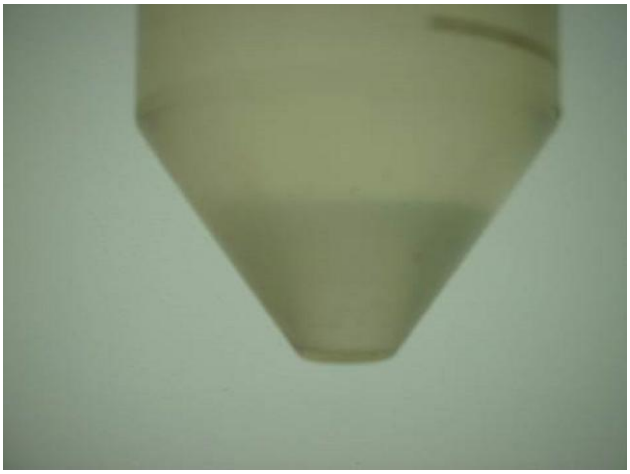


Fig 4b.

The excess PBS is removed and cells re-suspended to yield 2-5ml of BMAC. The cellular components of the reconstituted pellet were assessed by flow cytometry and cell viability evaluated by Trypan blue dye (1:1) stain. Following laboratory evaluation the suspension was injected into the fracture site under radiographic control [7].

Injection of BMAC

Under image intensifier, the fracture site was identified, skin anaesthetized with 1% lignocaine. A slightly oblique path was chosen to reach the gap in order to minimize back flow of the injected cells. A stab incision was made through which a small curette (1-2mm) was passed and the bone ends freshened. The BMAC was then injected into the gap and skin closed with a staple.

Post procedure protocols

Antibiotics were given for 3days and NSAIDs given as needed. Partial weight bearing was allowed after 4to6 weeks followed by full weight bearing depending on clinical and radiological findings. Physiotherapy was routinely advised.

Criteria for evaluation of outcome

Clinical evaluation was based on VAS score to assess pre and post procedure pain. X-rays in two planes were used for measuring size of the osseous defect, cortical continuity and progressive reduction in the fracture lines. Subjective assessment of healing was based on pain relief, satisfaction with procedure and ability to perform daily activities and attend to his occupation.

Illustrative Cases Case 1:

Male, 25 had multiple surgeries for fracture both bones of the forearm with persistent non union of the radius(Fig 5a). He underwent excision of the pseudoarthrosis mass, iliac crest interposition graft and plate fixation (Fig 5b). At 12 weeks, follow up x-rays showed no evidence of union at the proximal graft- bone interface while the distal site consolidated (Fig 5c). BMAC injection into the proximal site after curettage resulted in healing at the end of 20 weeks (Fig 5d).

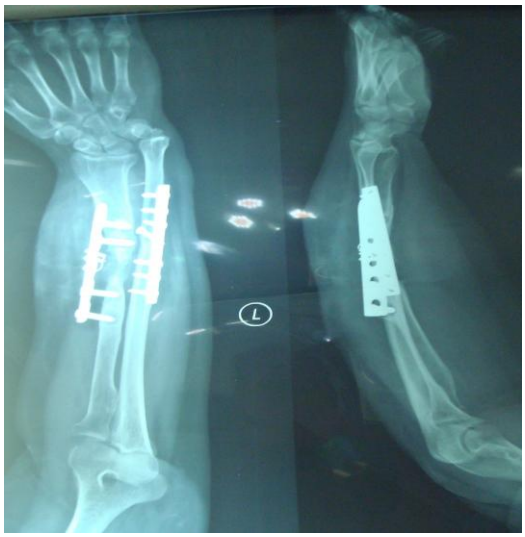


Fig 5a

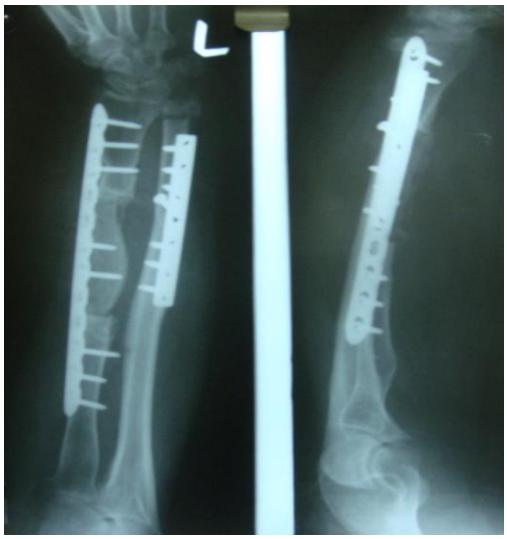


Fig 5b.

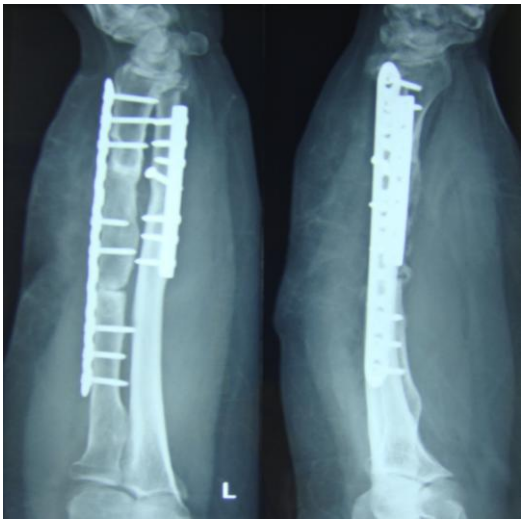


Fig 5c.

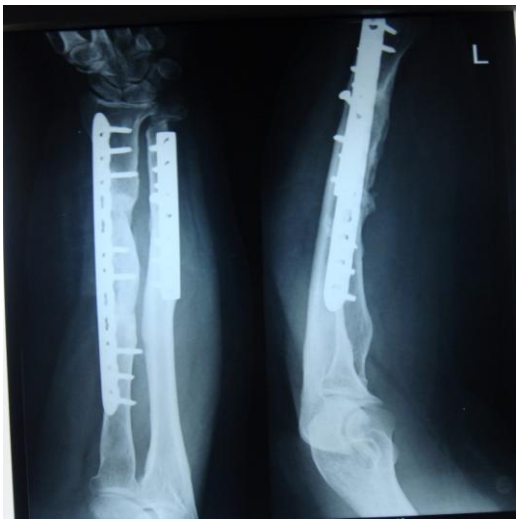


Fig 5d.

Case 2

Male aged 32 presented with basal fracture of left proximal femur (Fig 6a). Osteosynthesis and femoral osteotomy performed, resulting in delayed union at the osteotomy site (Fig 6b). BMAC injection into osteotomy site resulted in union after 12 weeks(Fig 6c).

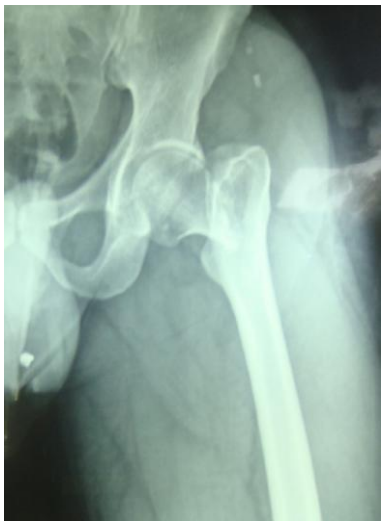


Fig 6a.

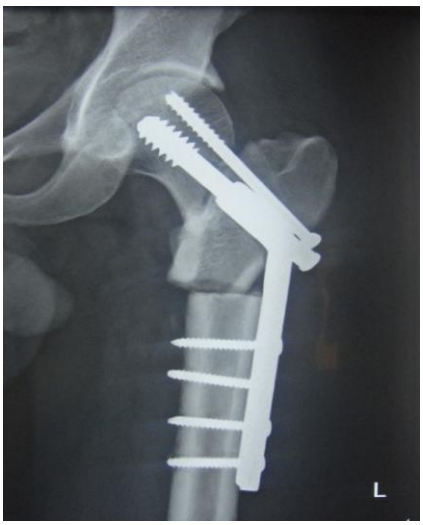


Fig 6b.

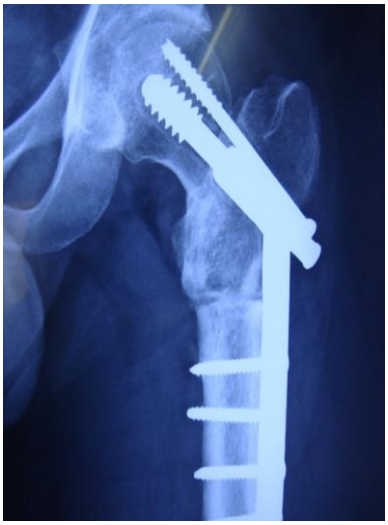
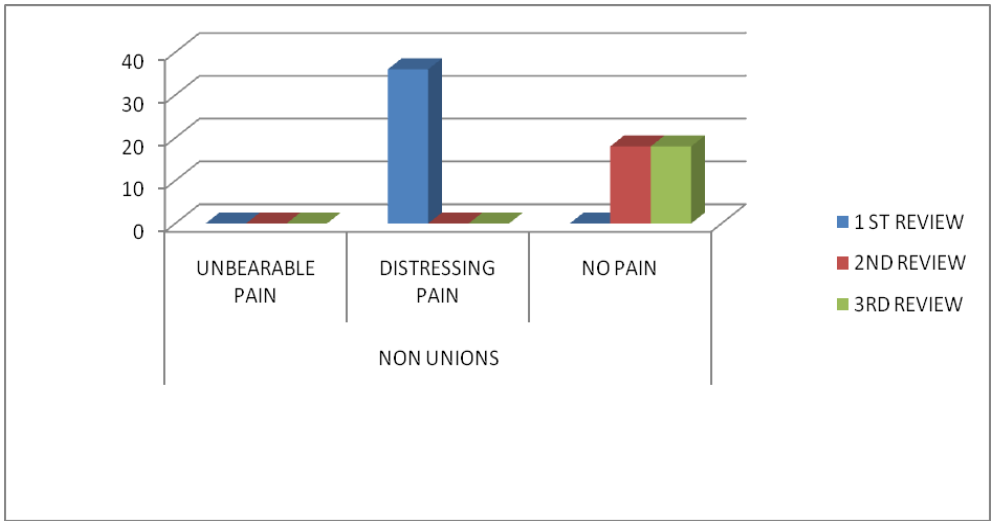


Fig 6c.

RESULTS:

SPSS *ver.*16.0 has been used to perform statistical analysis. Collected data will be analyzed using One sample t-test. A P-value of less than 0.05($P < 0.05$) was regarded as significant.

Outcome was assessed based upon functional activity, pain and radiological evidence of union as seen on two plane radiographs**.** Time from index surgery to BMAC injection was 6-16 weeks. The radiographic response was identified, 6-8weeks after injection. All the patients in our study showed fracture healing and radiological evidence of new bone formation within the follow up and perform their activities well. The follow up x rays of patients are shown in (figure5, 6 and 7). Collected data was analyzed using One sample t-test, we have obtained p-value <0.05 (0.032) which is regarded as significant. VAS score analysis of all patients showed decrease in pain during the follow-up period (graph 1). There was a direct correlation between movements and ability to bear weight and fracture healing as shown in the radiographs(Graph 1).



Graph 1: Analysis of healing using VAS Score at three different reviews.

DISCUSSION

Impaired fracture healing can cause changes in the lifestyle, quality of life, personality of an individual [8]. Different methods are employed to deal with impaired fracture healing, of which bone grafting and bone transplants are the most common. These procedures can produce bone healing but require an additional surgical procedure, with its attendant morbidity and expenditure. MSCs are pluripotent stem cells of bone marrow that have wide proliferative capacity. The procedure of extracting BMAC is low cost when compared to alternative procedures such as bone grafting and can be performed as a day care procedure. Analysis of cell differentiation markers shows that large numbers of MSCs are easily obtainable from bone marrow aspirates of adult patients [9]. Adult mesenchymal stem cells, osteoblasts and fibroblasts respond to bone morphogenetic proteins(BMPs), thus promoting bone formation. Various studies reported that bone marrow-derived mononuclear cells promote angiogenesis and improve vascularity at the fracture site. Delivery of these core components as well as growth factors and cytokines play a vital role in the healing process. The differentiation of osteoprogenitor cells relies on the surrounding microenvironment such as fracture stability and a bridgeable gap. Prior studies reported that normal marrow has a lower proportion of MSCs which are insufficient for stimulating the micro environment. The aspirates contained an average range, 1200-1224 progenitors/cm(3)) before concentration and an average range, 6000-6120 progenitors/cm^3 after concentration. The higher cell counts have a multiplier effect on the healing process.[10] Large volume of aspirate is associated with decreased concentration of progenitor cells because of dilution of the sample with peripheral blood. Peripheral blood admixture can be minimised by aspirating small quantities of marrow (2ml) at a time and withdrawing the syringe by a few mm and rotating it 90 degrees each time. Multiple sites must be chosen on the iliac crest, avoiding marrow aspiration from a single entry point.[11] Most frequent indication is the injection of BMAC into a fracture gap that shows evidence of non union. Stable internal fixation and bridgeable gap (<5mm) are the criteria for advocating the procedure (Fig 7 a,b,c,d). Bone union at the metaphyseal region occurs earlier than diaphyseal region.

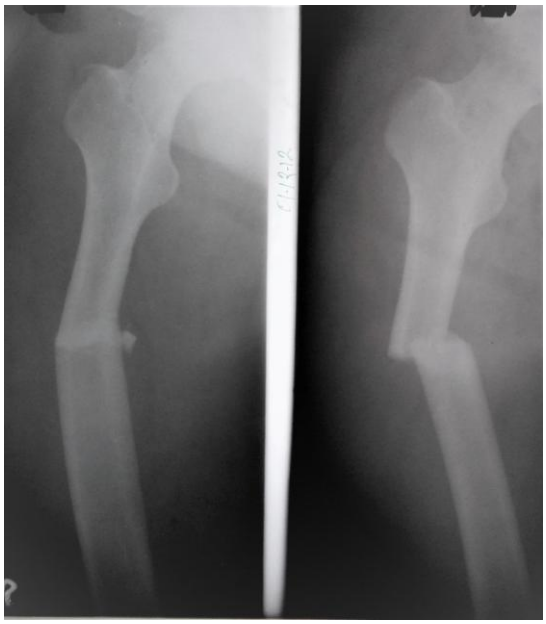


Fig 7a.

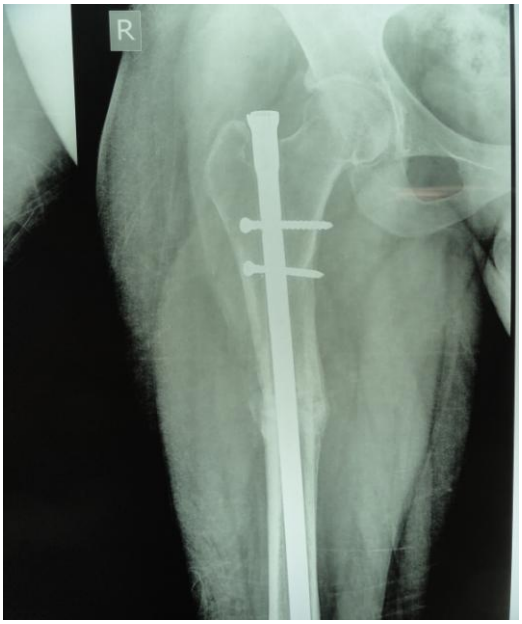


Fig 7b.

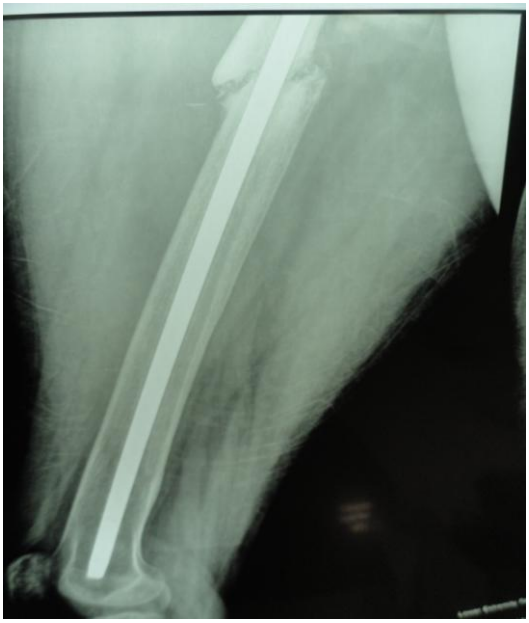


Fig 7c.

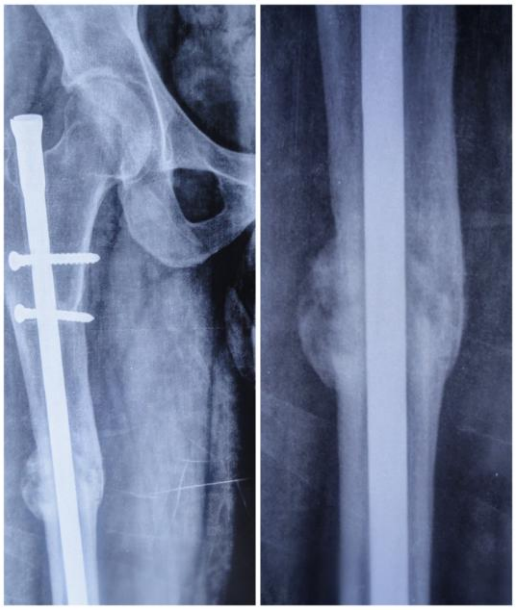


Fig 7d.

CONCLUSION

BMAC is a recent technique that has the potential to heal delayed and non-union of fractures. The underlying mechanism is by proliferation of MNCs into various osteogenic progenitors that generate the formation of osseous tissue required for fracture healing. The procedure is autologous, low cost and can be performed in the operation theatre.

Sources of Support:
Nil

Acknowledgements:
Nil

Conflict of Interest:
Nil

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