

**“STUDY OF EFFICACY OF BONE MARROW INJECTION IN
DELAYED UNION OF FRACTURES OF LOWER LIMB”**

By

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SDUAHER

**DISSERTATION SUBMITTED TO SRI DEVARAJ URS ACADEMY
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In partial fulfillment of the requirements for the degree of

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IN

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Under the Guidance of

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APRIL-MAY 2019

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LIST OF ABBREVIATIONS

BMP	Bone Morphogenic Protein
FGF	Fibroblast Growth Factor
IGF	Insulin like Growth Factor
IMIL	Intramedullary Interlocking Nail
IL	Interleukin
LCP	Locking Compression Plate
PDGF	Platelet Derived Growth Factor
PFN	Proximal Femoral Nailing
DM	Diabetes Mellitus
HTN	Hypertension
RTA	Road Traffic Accident
NSAIDS	Non Steroidal Anti Inflammatory Drugs
US-FDA	United States Food and Drug Administration

ABSTRACT

STUDY OF EFFICACY OF BONE MARROW INJECTION IN DELAYED UNION OF FRACTURES OF LOWER LIMB

BACKGROUND: Fracture healing is a complex series of events involving the array of biological as well as biomechanical processes. Some, in the setting of an unfavourable cellular microenvironment and mechanical instability, some fractures unpredictably end up in delayed union or non-union despite interventions with internal or external fixation, or cast application.

Various variables including the patient and fracture have been attributed for the delay in the process of fracture union.

In view of this, open autologous bone grafting, where bone is harvested and then implanted at the site of fracture has been the gold standard method for the treatment of delayed union and non-union.

However, post-operative complications have render this method less favourable. In contrast, stem cell therapy have been the choice of treatment in many conditions because of their regeneration potential into different cells and tissues. Bone marrow is one of the richest sources of these mesenchymal stem cells which act as osteoprogenitor cells. It was in 1955 Herzog first demonstrated the use the bone marrow injection for healing of fractures.

Injecting bone marrow rich in osteo-progenitor cells is a simpler procedure having lower post-operative complications, lower cost, as well as a shorter hospital stay.

This study aims to present the functional outcome of only autologous bone marrow injection in the treatment of delayed union of lower limb fractures which were previously treated by internal or external fixation, or cast application.

MATERIALS AND METHODS:

The study is conducted in 30 patients presented with delayed union of long bones to Department of Orthopaedic surgery at R.L. JALAPPA HOSPITAL attached to SRI DEVARAJ URS medical college, Tamaka, Kolar, during the period of December 2017 to June 2018.

Patients with Lower limb long bone fractures with no signs of clinical and radiological union after 3 months and age above 18 years were included in the study. Patients with bone marrow disease, pathological fractures, and active infection were excluded.

RESULTS:

In this study the average age of the subject is 40 years, and male: female are 9:1. 53.3% of the fractures involve tibia, 46.7% of the fractures involve femur. The initial mode of treatment provided to the patients are IMIL nailing in 76.7%, Plate application in 6.7% and external fixation in 16.7% patients. The volume of bone marrow injection was 40-60 ml in 60% patients and 60-80 ml in 40% of total patients. The average union time is around 18 weeks. 16.7% of the patients had union at 2 months, 36.7% at 4 months, 40% at 6 months and 6.7% had absent union. The results are satisfactory in 28 patients and poor in 2 patients.

INTERPRETATION AND CONCLUSION: After our follow up of 30 patients for a period of 6 months post bone marrow aspirate injection, we observe that there is accelerated bone healing, even in patients with associated co-morbidities.

We also observed that even single dose of unconcentrated bone marrow aspirate injection is enough to achieve union. The procedure is simple, cost effective, easily reproducible with nil or minimal complications. Even though all of our subjects underwent the procedure under spinal anaesthesia, many studies have concluded that this procedure can be done under local anaesthesia or short general anaesthesia.

We finally conclude that, the earlier the intervention faster the union and less chances of progression to non-union.

Key words: bone marrow aspirate, delayed union, tibia, femur

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INTRODUCTION

Fracture healing includes a complex series of events involving the array of biological as well as biomechanical processes. Some, in the setting of an unfavourable cellular microenvironment and mechanical instability, some fractures unpredictably end up in delayed union or non-union despite interventions with internal / external fixation or cast application.¹

Various variables including the patient and fracture have been attributed for the delay in the process of fracture union. Different modalities of treatment are available for the management of the same. The biological environment has been shown to be affected by osteogenic, osteo-conductive and osteo-inductive substances which are collectively present in osteo-progenitor cells.²

Goujon followed by McGaw and Harbin, first demonstrated the osteogenic potential of these osteoprogenitor cells in bone marrow and thereby, hold the key to the formation as well as healing of bone.^{3,4}In view of this, open autologous bone grafting, where bone is harvested and then implanted at the site of fracture has been the gold standard method in the management of delayed union and non-union.

However, post-operative complications such as pain, hematoma formation, surgical site infection and scarring, meralgia paresthetica and gait changes have render this method less favourable, not to mention the high cost and also the need for prolonged hospital stay. Opening the non-union site where healing is already hampered also contributes to devascularisation of fracture fragments.

In contrast, stem cell therapy, have been the choice of treatment in many conditions because of their regeneration potential into different cells and tissues.⁵ Bone marrow is one among the richest sources of these mesenchymal stem cells which act as osteoprogenitor cells.⁶ It was in 1955 Herzog first demonstrated the use of bone marrow injection for healing of fractures.⁷

Injecting bone marrow, rich in osteo-progenitor cells is a simpler procedure having, lower post-operative complications, cost effective, as well as a shorter hospital stay.

This study is done in an aim to present the functional outcome of only autologous bone marrow injection in the treatment of delayed union of fractures of lower limb, which were previously treated by internal / external fixation, or cast application.

OBJECTIVES

To evaluate the efficacy of bone marrow aspirate injection in delayed union of fractures using union scale score with reference to

- a. Rate of healing
- b. Functional and Radiological outcome

TABLE 1: UNION SCALE SCORE⁸

Score	Mobility	Tenderness	Radiological features
0	Frank mobility in both planes	Present	No callus at all
1	Restricted mobility in both planes	Absent	Minimum ensheathing callus
2	Minimum mobility in one plane	Absent	Good ensheathing callus and internal callus bridging at least two cortices
3	No mobility at all	Absent	Good ensheathing callus bridging all 4 cortices

Union scale score includes-

A. Clinical scoring (tenderness and abnormal mobility)

B. Radiological scoring (callus formation)

HISTORICAL REVIEW

Sporadic use of bone grafts has been described in the oldest medical records, referred even in myths and legends, and depicted in centuries old religious arts. Since the first recorded bone graft attempt by the Dutch surgeon Job Van Meekeren, bone grafting has become one of the most frequently performed orthopaedic procedures.⁹

Despite advances in operative fixation techniques and the consideration of many substances for use as implant or transplant materials, fresh autologous cancellous bone remains as the most effective graft material for most clinical situations, and the method of open harvesting from iliac crest of pelvis and operative implantation at fracture site remains largely unchanged since the classic work of Phipps.¹⁰

McGraw and Harbin were among the first to demonstrate the osteogenic activity of bone marrow.^{3,4} The osteogenic potential of bone marrow is because of stromal and endosteal cells of the marrow. As bone marrow is the only tissue that contains an abundance of both determined and inducible osteoprogenitor, it is logical graft of choice.

REVIEW OF LITERATURE

A study conducted on 8 patients in the year 1990, with primary sarcomas of bone who developed delayed unions or non- unions after being treated by resections and reconstruction were treated by injection of autologous bone marrow aspirate. Formation of new bone was noted in 7 patients, whereas union was achieved in 5 patients.¹¹

A retrospective study done during 1991 in Singapore, reported bone marrow injection stimulates early callus formation, sufficient to unite fractures with median time of 10 weeks clinically and radiologically by 17 weeks.³

Connolly et al, used marrow injections to treat 20 tibial delayed and nonunions with a union rate around 80%. The results showed that the most effective application of bone marrow injection, is in the treatment of delayed union so as to prevent the progression to non-union.¹²

A study between 1990 and 2000 which included 60 patients with established delayed and nonunion of tibial shaft, concluded that the efficacy is related to high concentration of stem cells that will enhance the rate and amount of bone formation.¹³

After a long- term followup, a study in 2001, which included 72 patients, results showed only 4 failures (5.5%) which are encouraging and suggest that percutaneous autologous bone marrow injection is found to be a simple solution for complex problem of delayed or non-union.¹⁴

A prospective study conducted around 2003 reported that percutaneous bone marrow injection along with demineralised bone matrix helps in acceleration of bone healing on an outpatient basis treatment for the delayed and non-union.¹⁵

A study conducted over a period from 2005 to 2006 in fractures with delayed union, 37 out of 38 patients had successful union to bone marrow injection with mean union time of 14.6 weeks.¹⁶

Another study conducted in 2007 which included 28 patients, bone marrow was aspirated from the iliac crest and injected into the fracture site, Union was observed in 23 cases with an average healing time of 12 weeks.⁸

In a study done in 2009, 50 cases of post-traumatic delayed and non-union administered with bone marrow aspirate injection were examined clinically and radiographically. It was observed that 90% of the cases had union. This technique was found to be minimally invasive and is a biological method of bone grafting because it will not disturb the vascularity at the fracture site.¹⁷

The mesenchymal stem cells in bone marrow aspirate described by a study in 2012, as the main key factor in bone healing as they are the progenitor cells to osteoblasts and adipocytes which are the main bone cells.¹⁸

A retrospective study of the radiographs of 50 patients with delayed and non-union published in 2013 showed that percutaneous bone marrow injection accelerates bone healing and is a useful procedure in patients who are unfit for general anaesthesia. It was also described as a patient- and surgeon-friendly procedure.¹⁹

In the same year, a study comparing different modalities of management of delayed union concluded that multiple bone marrow injections achieved similar results and are equally efficacious in fracture healing as bone grafting and platelet rich plasma but at a lower cost.²

Similarly, another study done during the same period showed that autogenous bone marrow injection significantly accelerates bone healing in 19 out of the 20 patients with non-union due to biological causes.²⁰

A study done in 2014 showed several factors which decrease the efficacy of autologous bone marrow injection namely cigarette smoking, elderly age and open fracture.²¹

In diabetics, as described by a clinical study done in 2015, proved that those with delayed and non-union who were treated with autologous bone marrow concentrate had a higher rate of successful union and less complications compared with patients receiving autologous bone grafting.²²

After a long 8 years of study involving 45 patients with delayed and non-union published in the same year showed that early injection of the bone marrow concentrate helps in accelerating the bone healing especially in patients with poor tissue coverage and other comorbidities.²³

Bone marrow was found to possess high osteogenic potential in a study of 30 patients done around the same time and can be an easy substitution for complex problem such as delayed union as it can be done on outpatient basis under local anaesthesia with minimal morbidity at recipient and donor sites.²⁴

After studying 80 patients in 2016, the authors came to a conclusion that a trial of percutaneous bone marrow injection should be done in cases of delayed union as bone marrow grafting can be done secondarily if the former fails.²⁵

In another study done in the same year, the author reported a study which showed the osteoconduction and osteoinduction properties of bone marrow aspirate at the delayed union and non-union site.²⁶

A result of a prospective study published in 2017 showed that out of 34 patients, a satisfactory response was seen in 78% of delayed union whereas only 40% of non-union cases showed good response. Therefore, autologous bone marrow injection was recommended before delayed union progresses to non-union.²⁷

Around same time of the year, a study reported that 16 out of 20 hypertrophic non-union cases and 6 out of 8 atrophic non-union cases were united after bone marrow injection, thereby concluding that as compared to bone grafting, bone marrow injection has the same osteo-induction and osteo-conduction properties but with less morbidity.²⁸

In the same year, 50 surgically unfit patients with long bone fractures were divided into 2 groups, one treated with cast application alone and the other with cast application and percutaneous bone marrow injection. It was found that the group with percutaneous bone marrow injection showed accelerated bone healing thereby decreasing the duration of immobilisation hence improved rehabilitation.²⁹

At around same time, another clinical study has documented the use of mesenchymal stem cells of bone marrow in playing a pivotal role not only in non-union and delayed union, but also in the management of acute fracture healing with bone loss.⁶

In a study done in 2018, the author evaluated 93 patients and concluded that by using multiple hole tip needle in bone marrow aspiration, the repeated entry of the donor site can be prevented thereby reducing morbidity.³⁰

Recently this year, after a follow up of 15 patients for a period of 1 year post-bone marrow injection, the authors reported that it is cost effective, easily reproducible with good functional outcome and less complications.³¹

In the recent most study published this year, after a long term follow up period of 2-6 years in patients younger than 18 years, percutaneous autologous bone marrow transplantation was concluded to be a simple, effective and safe strategy for the treatment of delayed union in long bones of children. Early bone marrow transplantation was shown to reduce the need for bone grafting later on, thereby shortening the course of treatment.³²

RELEVANT ANATOMY

DEVELOPMENT OF BONE³³

Formation of bone is essentially the replacement of connective tissue derived from mesenchymal cells, which occur in 2 ways:

1. Intramembranous bone formation
2. Endochondral bone formation

Intramembranous bone formation

- Bones forming from layers of connective tissue are called intramembranous bones.
- Osteoprogenitor cells will directly differentiate into osteoblasts.
- Bone matrix is deposited over the surface by the osteoblasts and bone grows in width (appositional growth).

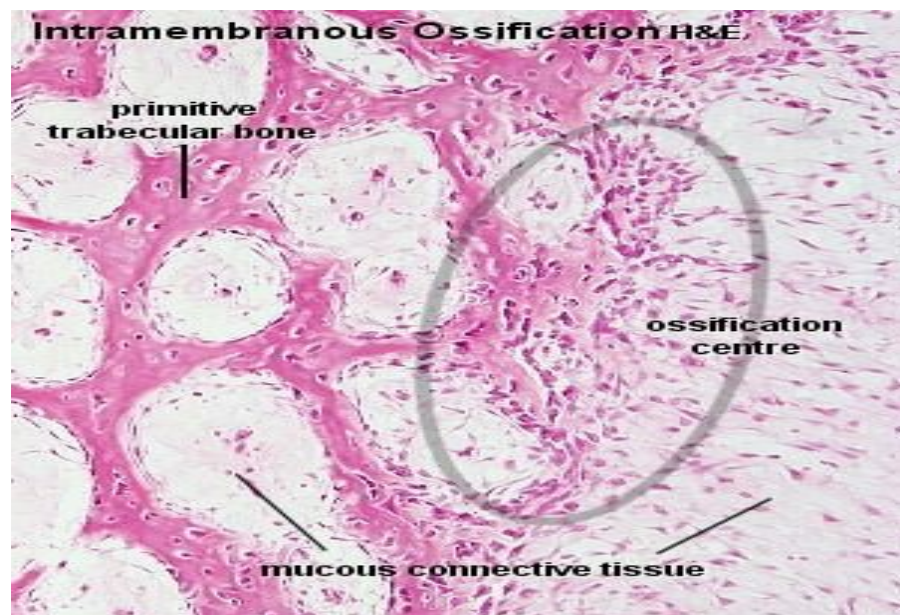


Figure 1: Intramembranous ossification³³

Endochondral bone formation

- Bones forming by replacing the hyaline cartilage are called endochondral bones.
- Most of the bones in the body are formed by chondral ossification.
- In the areas of low oxygen tension chondrocytes hypertrophy, degenerate and calcify.
- Oxygen tension increases when cartilage is invaded by the proliferation of capillaries which is followed by ossification.
- Chondral ossification in the growth plate helps in the longitudinal growth of the long bone.

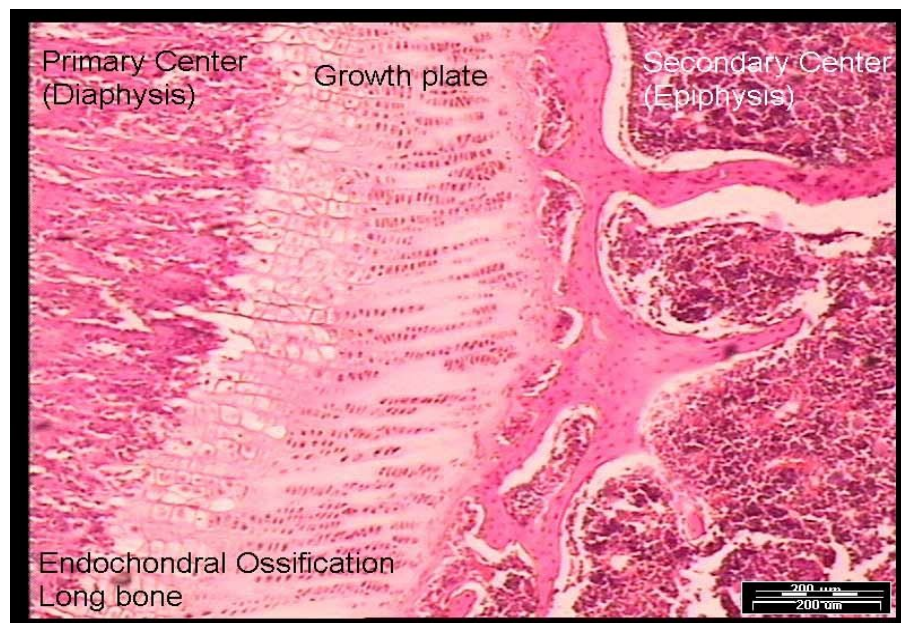


Figure 2: Endochondral ossification³³

BONE

- Bone is a strong and rigid connective tissue providing support and protection for the body.
- The rigidity of the bone helps in adapting to resist stress, supporting the body and providing leverage for the movement.
- Bone is a highly organized, vascularised, and dense mineralised with matrix of organic and inorganic materials.

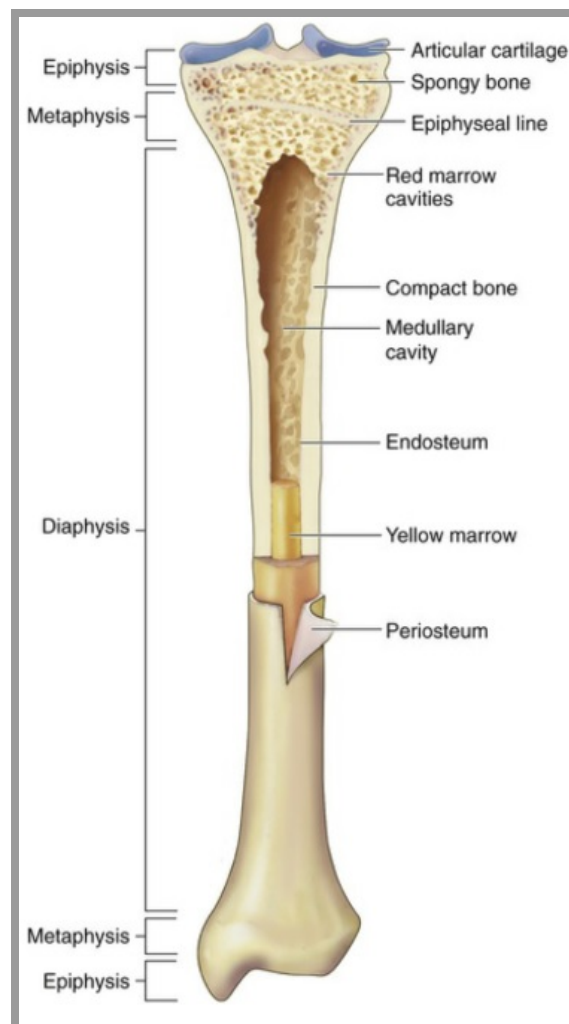


Figure 3: Long bone³⁴

MACROSCOPIC ANATOMY OF THE BONE³⁴

The living bone is white in colour.

Depending upon the texture, it can be either of the two components:

- a) Ivory (compact bone)
- b) Honey-combed (trabecular or cancellous or spongy bone)

Compact bone

- Generally limited to outer shell or cortex of the mature bone for providing strength and rigid articular surfaces
- Commonly seen in the diaphysis of long bones

Cancellous bone

- Also formed in the cortex, consisting of bony trabeculae, providing strength to the cortex while minimizing the weight of the bone
- It provides strength in compression, so found abundantly in the epiphysis of the long bones
- The trabecular arrangement follows the pressure and tension stress adding to its mechanical strength

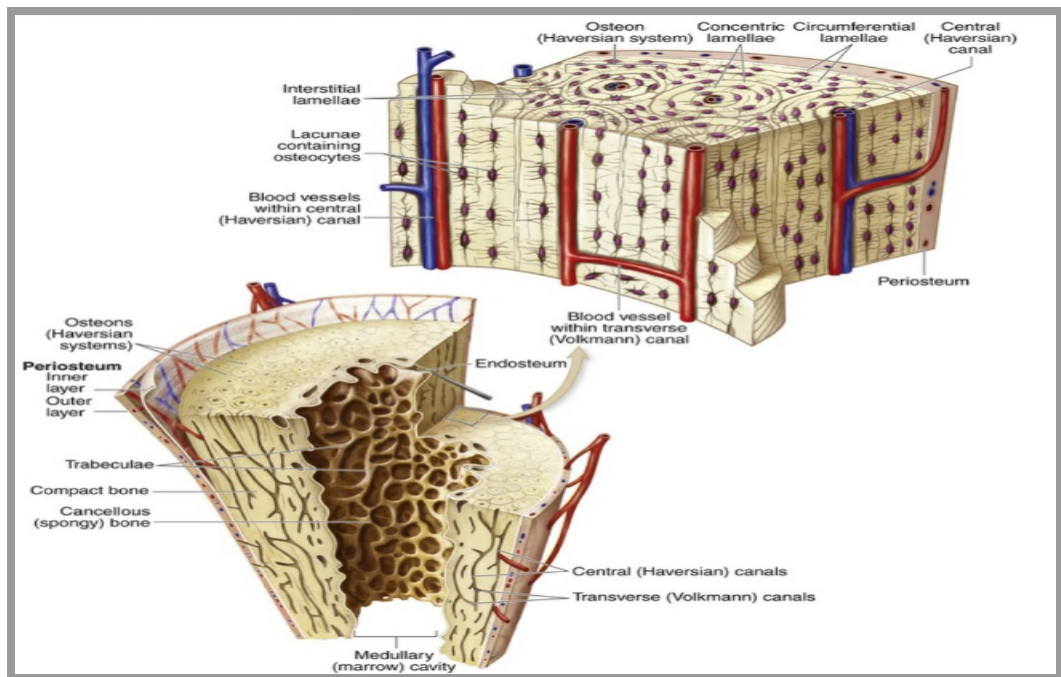
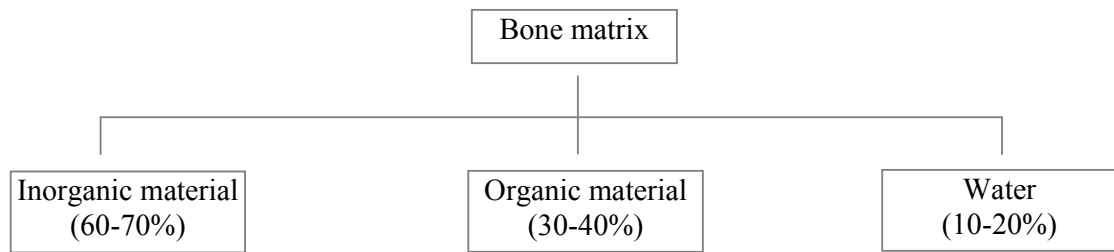


Figure 4: Illustration of Bone Structure³⁴

MICROSCOPIC ANATOMY OF THE BONE³⁵

It constitutes of:

- 1) Extracellular bone matrix
- 2) Specialized cells
 - Osteoprogenitor cells
 - Osteoblasts
 - Osteocytes
 - Osteoclasts
 - And other cells
- 3) Periosteum, endosteum and marrow



Inorganic material

- Formed by a process initiated by osteoblasts called mineralization.
- Mineralisation provides the required compression strength to bone.
- It is made up of inorganic mineral salts – calcium hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ in the microcrystalline form.
- Bone must be mineralised at least 50% to be visible on the radiograph.

Organic material

- Most of the organic material is formed by type I collagen
- The remainder includes non-collagenous proteins, glycoproteins and carbohydrates.
- Depending on the laminar arrays of the collagen and the cells, bone can be 2 types:

a) Woven/Immature bone

- Random arrangement of collagen and cells.
- Ex: Initial stages of bone healing.

b) Lamellar bone

- Orderly distribution of collagen and cells.
- Lamellar bone can be structured either in osteons (compact/cortical bone) or in the form of trabeculae which are seen in cancellous/spongy bones.

Compact bone

- The specialised and basic architectural unit is provided by the osteons (Haversian systems) which are parallel to the long axis of bone.
- The haversian system consists of central haversian canal surrounded by concentric bone lamellae which are intervened by numerous lacunae.
- The oblique channels that is Volkmann's canal which consists of neurovascular bundle connect the haversian canals to the medullary cavity and with surface of the bone.
- The surface of compact bone is covered by periosteum and the inner surface is lined by endosteum except at the articular surfaces and at apophysis.

Cancellous bone

- The space between the trabeculae are filled with bone marrow and blood vessels.

Bone cells

1) Osteoprogenitor cells

- Also called as osteogenic cells or pro-osteoblasts.
- These are from mesenchymal precursor cells which can differentiate into osteoblasts, chondroblasts and fibroblasts under appropriate conditions.
- They divide by mitosis and differentiate into osteoblasts at the fracture site.
- They are found in the endosteum and cambium layer of periosteum or directly from mesenchymal stem cells during intramembranous ossification.

- They are of two types:
 - Committed osteoprogenitor cells – committed to form bone tissues
 - Inducible osteoprogenitor cells – depending on the inducer and the inducing effect
these cells differentiate into either osteoblasts / chondroblasts or fibroblasts

2) Osteoblasts

- These are basophilic roughly cuboidal mononuclear cells derived from the osteoprogenitor cells when stimulated by bone morphogenic protein (BMP).
- Osteoblasts form contiguous monolayer over the surface of growing bone.
- Functions:

These cells are responsible for synthesizing proteins of the bone:

1. Collagen (mainly type 1)
 2. Non-collagenous proteins or glycoproteins
 3. Osteocalcin and alkaline phosphate – required for bone mineralization
 4. Osteonectin – crystallization of minerals
 5. Osteoprotegerin – restricts osteoclasts differentiation
- Hormonal regulation of bone resorption via receptors for parathyroid hormone (PTH) and calcitriol.
 - As the lifespan of osteoblasts in humans is only 2-3 months, the mediator mechanisms will replace the exhausted osteoblasts with new ones during fracture healing.

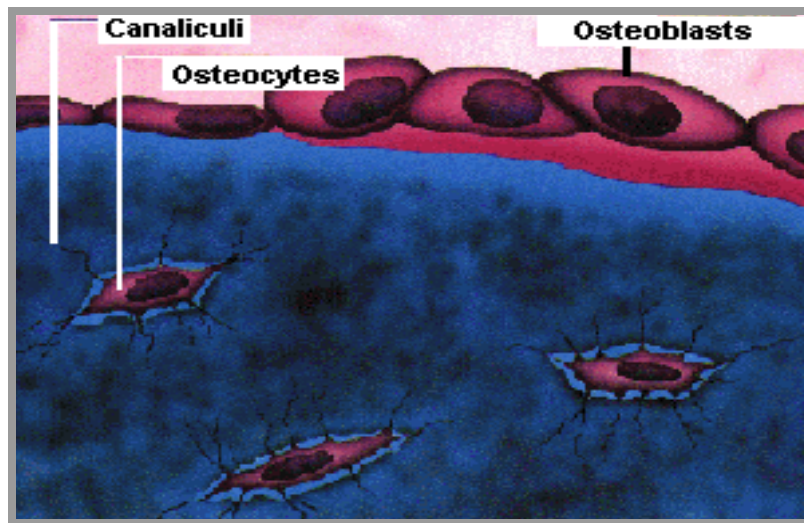


Figure 5: Illustration of Osteoblasts³⁵

3) Osteocytes

- They form the major cell type in mature bones.
- Osteocytes are derived from osteoblasts which lost the ability to divide/secrete new matrix after enclosed in the matrix.
- Lacunae are spaces in which osteocytes rest.
- Canaliculi are the interconnecting channels with other cells.
- Function:
 - Act as sensors for mechanical changes in the microenvironment of bone.
 - Helps in inducing bone remodeling.

4) Osteoclasts

- Formed by fusion of monocytes.
- They are multinucleated giant cells with numerous mitochondria and lysosomes.
- Active osteoclasts have ruffled membrane.
- Functions:
 - Only cells which can degrade and resorb mineralised bone tissue.
 - Important cells in calcium metabolism.

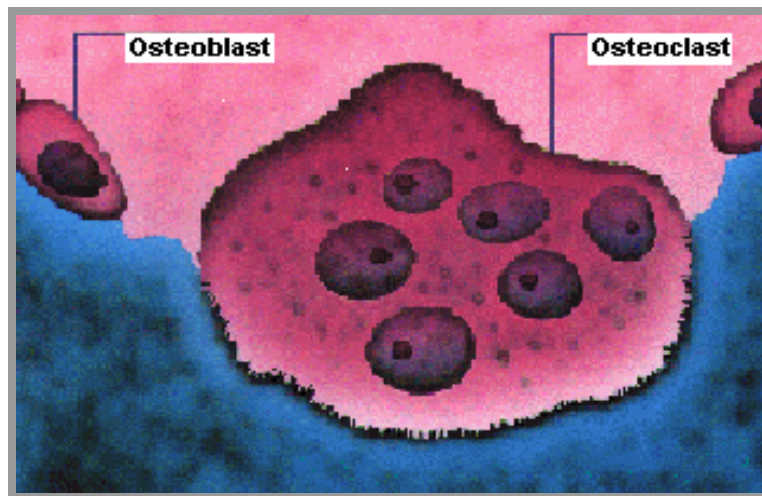


Figure 6: Illustration of Osteoclast³⁵



Figure 7: H&E stain showing Osteoclast³⁵

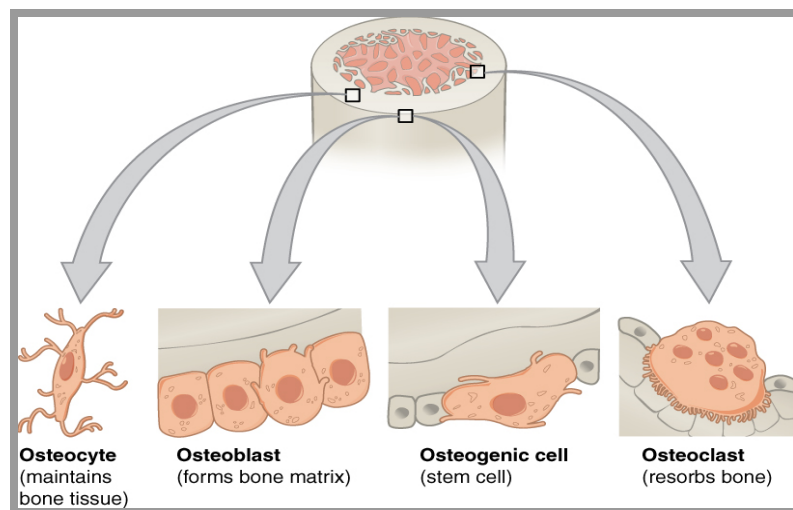


Figure 8: Differentiation of Osteoprogenitor cells³⁵

5) **Reticular cells**

6) **Endothelial cells**

7) **Fibroblasts**

PERIOSTEUM

- Covers the outer surface of bone
- It has 2 layers:
 1. External fibrous layer
 2. Internal osteogenic layer (cambium)

Cambium layer activation leads to appositional growth

ENDOSTEUM

- It lines the inner surface and cavities of the bone
- It has osteogenic potential

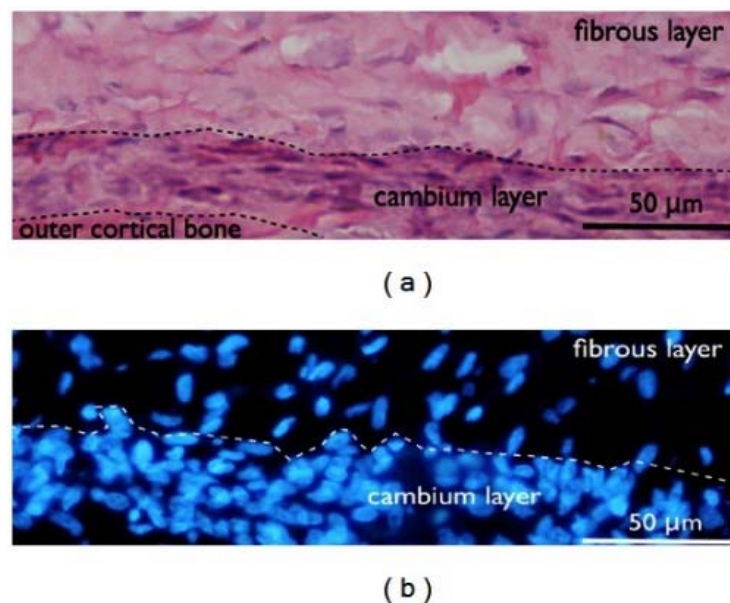


Figure 9: Microscopy of periosteum³⁵

NEUROVASCULAR SUPPLY

Bone derives its blood supply mainly from

- Nutrient artery
- Epiphyseal/metaphyseal arteries
- Periosteal arteries
- Muscular, ligamentous and capsular arteries

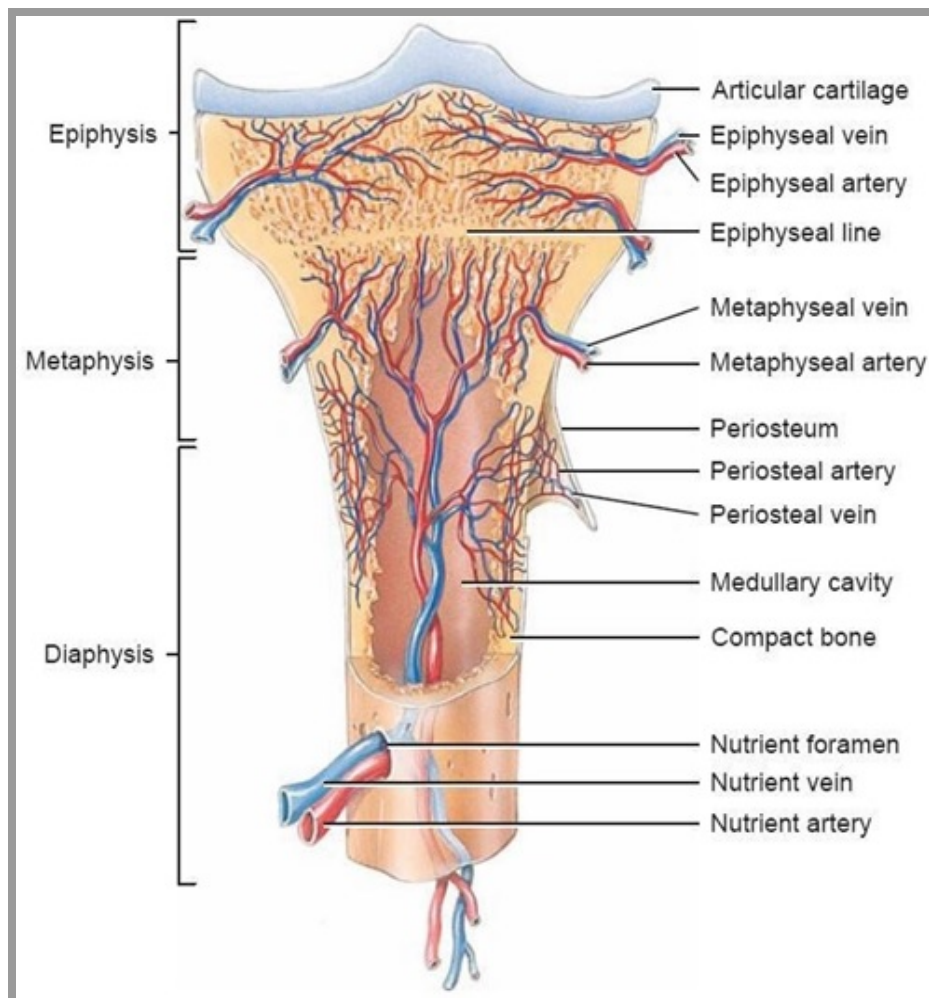


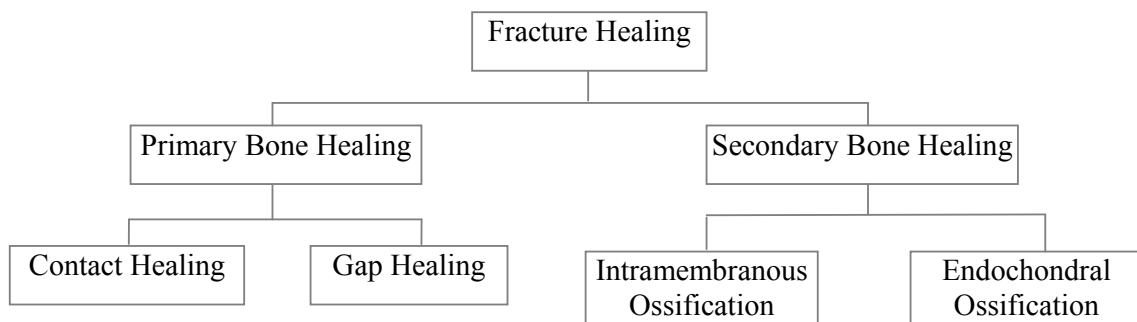
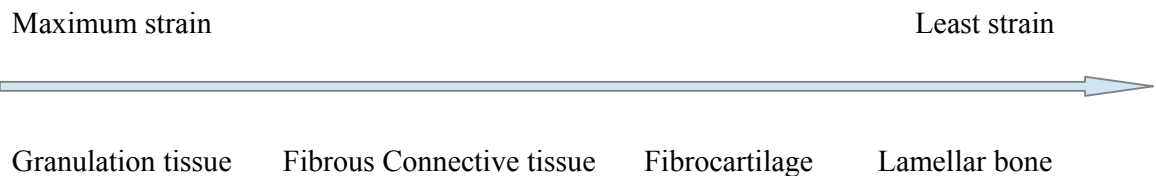
Figure 10: Vascular supply of a long bone³⁵

REGENERATION OF BONE (FRACTURE HEALING)³⁶

- The process of fracture healing requires appropriate cells and the genes at appropriate location and time.
- Fracture healing is similar to soft tissue healing except its ability to heal like pre-fracture state without formation of scar.
- There are different theories about fracture healing but the most widely accepted was proposed by Perren.
- He described the stages of fracture healing depending on the degree of strain/movement.

$$\text{Fracture gap strain} = \frac{\text{Relative change in the fracture gap}}{\text{Original fracture gap}}$$

- Depending on the inter-fragmentary strain the cellular response changes and leads to forming different tissues in between the fracture fragments.



Primary bone healing

(also known as Direct cortical / Osteonal / Haversian bone healing)

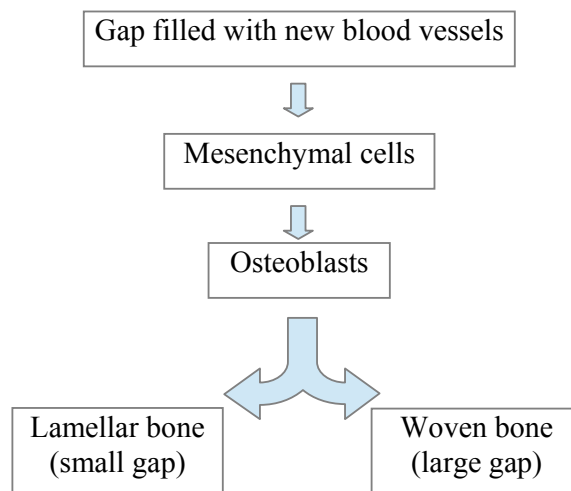
- Pre-requisites: Absolute stability which is achieved by
 - Anatomical reduction.
 - Inter fragmentary compression.
 - Cutting cones formation by osteoclasts.

1. Contact healing

- At the site of the contact and compression.
- Simultaneous bone union and remodeling.
- Formation of lamellar bone by osteons which directly extends between the fracture ends.

2. Gap healing

- At the tension site.
- Bone union and remodelling is sequential.



Secondary bone healing³⁷

1. Intramembranous ossification
 - Bony callus at the periosteum
2. Endochondral ossification
 - Bridging callus of fibrocartilagenous

Stages of secondary bone healing

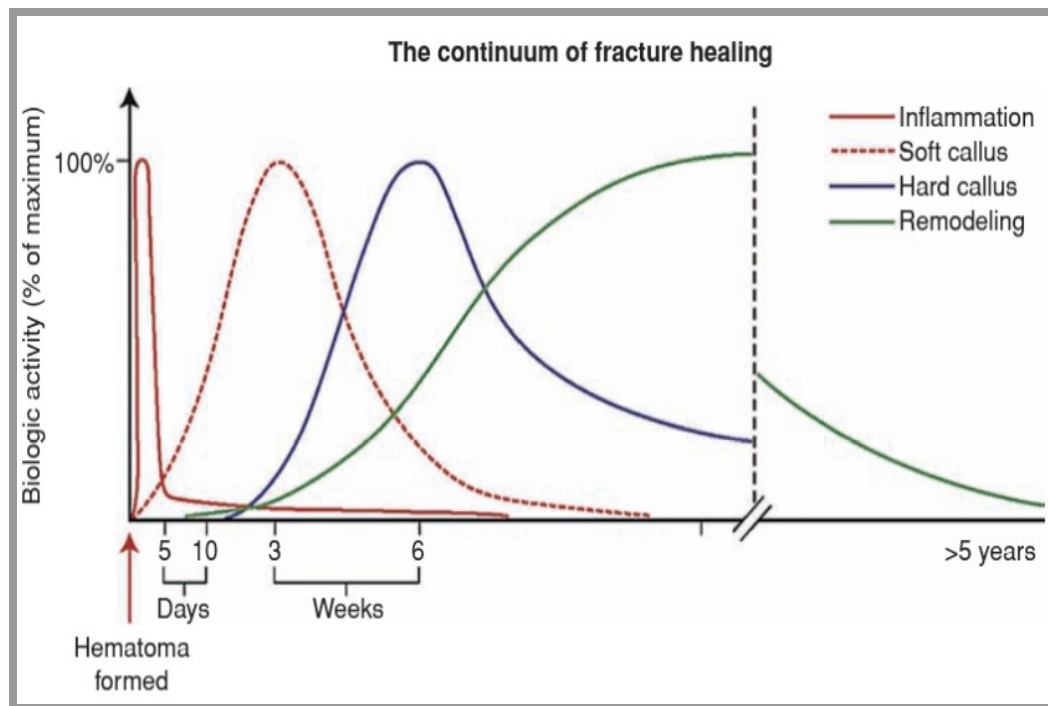
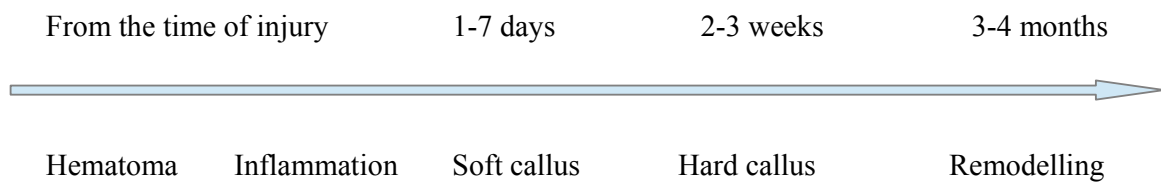


Figure 11: Continuum of fracture healing³⁹

Hematoma formation

- Starts immediately after injury.

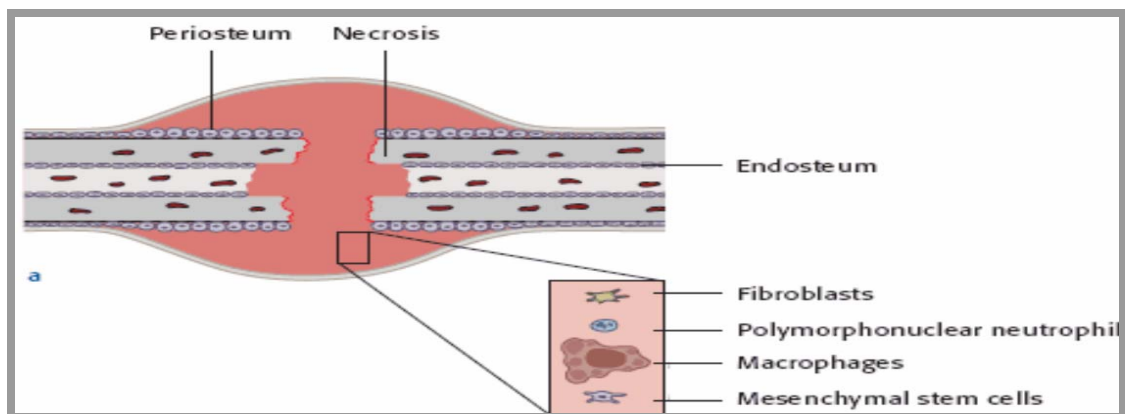
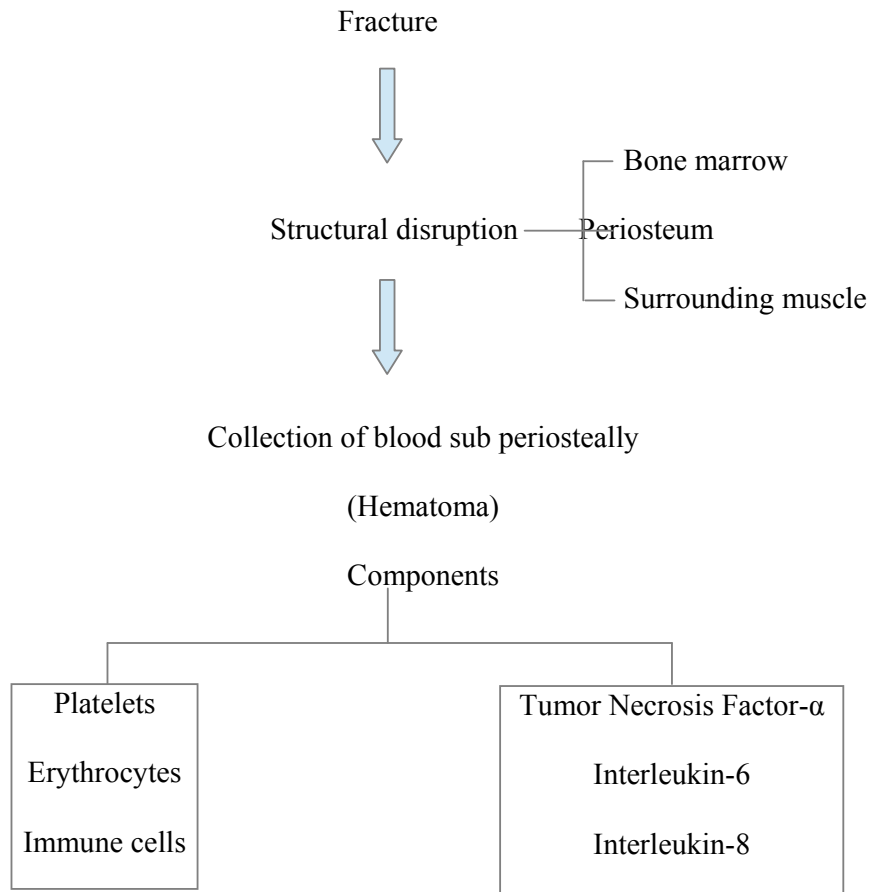


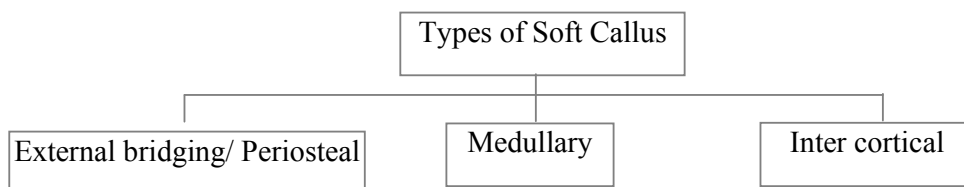
Figure 12: Hematoma formation³⁸

Stage of Inflammation

- Damage to the bone and soft tissue after fracture leads to the disruption of blood vessels.
- The site adjacent to fracture site will be relatively hypoxic leading to local tissue necrosis and debris formation.
- Neutrophils will arrive first and persist for a minimum of 3 hours, followed by other cells which help in phagocytosis.
- Inflammatory stage and fracture healing go side by side rather than as distant steps.

Stage of Soft Callus Formation

- Before beginning of this stage, there is differentiation of osteoprogenitor cells into either chondrocytes or osteoblasts.
- It generally begins by 3 weeks.
- Depending on various factors, fibrous tissue and hematoma is replaced by either cartilage or osteoid in the callus.
- Type I and Type II collagen formation help to restore matrix and its stability.



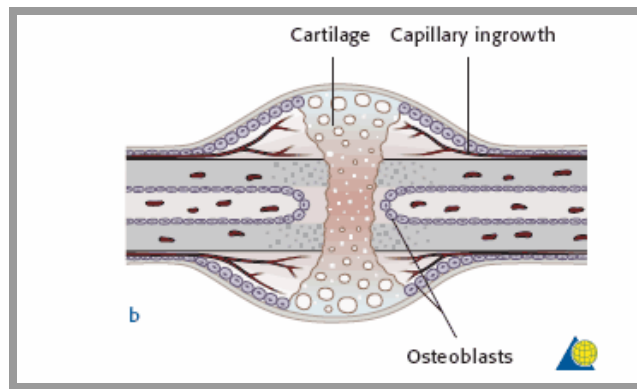


Figure 13: Stage of Callus³⁸

Stage of Hard Callus

- In this stage, cartilage becomes calcified and blood vessels invade the callus.
- The main cells involved in this are osteoblasts and osteoclasts.
- Woven bone is deposited by osteoblasts which strengthens the callus.
- Hard callus mainly consists of type I collagen.

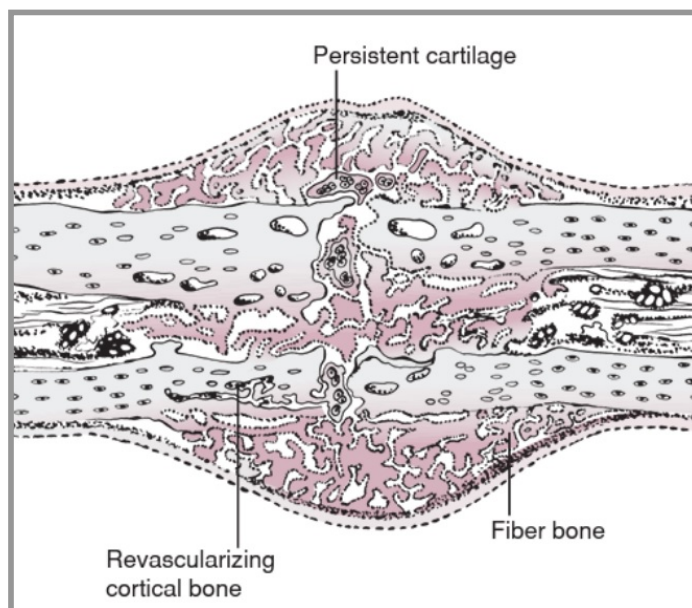


Figure 14: Stage of Hard Callus³⁹

Stage of Remodelling

- In this stage, bone returns to its pre-injury state.
- Both canalicular architecture and haversian system is restored.
- This process of remodelling continues for months to years till solid osseous union is achieved.
- Lamellar bone is formed because of communication channels between the osteoblasts and osteoclasts along the lines of mechanical stress which follows Wolff's law.
- The disorganized woven bone is removed by cutting cones formed by osteoclasts followed by organized lamellar bone formation by osteoblasts around central blood vessels.

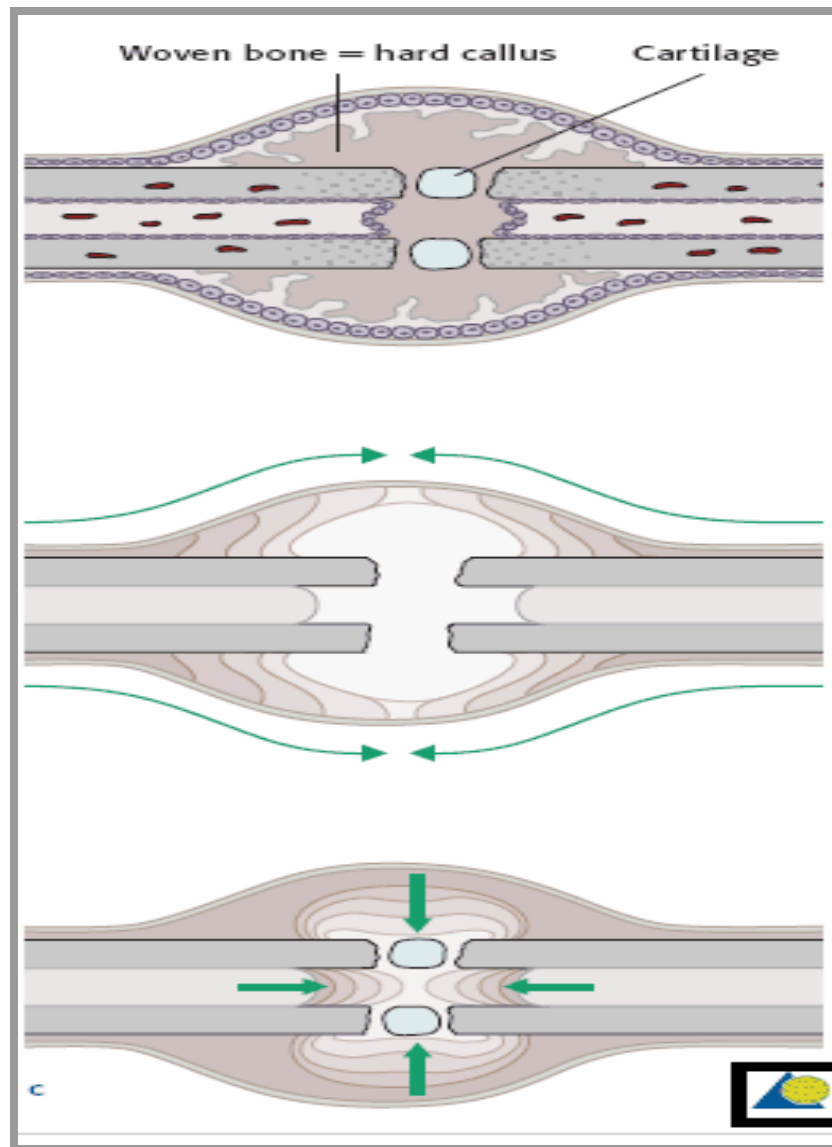


Figure 15: Different stages of callus³⁸

THE BIOLOGY OF FRACTURE HEALING^{40,41}

As mentioned above, fracture healing is a remarkable process as it is very similar to soft tissue healing except its ability to heal like pre-fracture state without the formation of scar. Instead of fibrous proliferation, granulation tissue grows in bone healing which differentiates into various bone cells, ultimately forming bone.

Traditionally, osteoblasts were assumed to play the main role in healing of fracture. However, recent advances have elucidated this process and concluded that there are more factors involved. Fracture healing is a series of interrelated and interdependent events which involve multiple cells performing specific functions in each stage, playing various roles in neovascularisation, fibroproliferation along with bone and cartilage formation.

It is interesting to note, however, that healing can occur without fracture. In pathological conditions such as Paget's disease, myositis ossificans, myelofibrosis or in physiological growth plates of bones, growth and proliferation similar to fracture healing occur without fracture being the primary initiator.

Osteoinduction, Osteoconduction and Osteogenesis:

Osteoinduction, the first step in fracture healing, as the name suggests, is the stage where mesenchymal cells are induced to differentiate into a variety of cells which then proliferate and secrete chemical substances which forms a cycle by further stimulating the undifferentiated mesenchymal cells to participate in differentiation. This continues throughout fracture healing.

The osteoinducing factors are various growth factors such as transforming growth factor, bone morphogenetic proteins (BMP), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF). Cytokines, prostaglandins and multiple hormones similarly play contributing roles in osteoinduction.

The transforming growth factor is a part of a superfamily of growth factors, consisting a total of 34 members. It acts on serine/threonine kinase cell wall receptors which functions to promote the proliferation and differentiation of the mesenchymal precursors into bone cells such as osteoblasts, osteoclasts, and chondrocytes. It also stimulates endochondral and intramembranous bone formation and induces osteoblasts to synthesize cartilage-specific proteoglycans and type II collagen.

BMP is a group of osteoinductive proteins which were first isolated from demineralised bone matrix. It was found to induce mesenchymal cell differentiation in bones. Of worth mentioning is the BMP-3, also known as osteogenin, is a potent inducer of mesenchymal tissue differentiation into bone cells and promoter of endochondral ossification. BMP-2 and BMP-7 on the other hand induce endochondral bone formation especially in segmental defects, whereas BMP-1 functionally cleaves the carboxyl terminals of pro-collages I, II and III. These BMPs regulate extracellular matrix production and follow a dose: response ratio.

FGFs are divided based on chemical forms into FGF-1 (acidic) and FGF-2 (basic). Their functions are to amplify the proliferation of bone cells such as osteoblasts and chondrocytes and the formation of callus. FGF-2 also promotes neovascularisation.

PDGF is formed as dimer of PDGF-A and PDGF-B which increases bone cell proliferation (Eg: osteoblasts) and ultimately increases the synthesis of type I collagen. They also serve as a mitogen for mesenchymal cells. A subcategory of PDGF-B, known as PDGF-BB, has been found to stimulate the proliferation of osteoclasts, thereby increasing bone resorption.

IGFs are synthesised in various tissues. IGF-I is synthesised by hepatocytes, stimulated by growth hormone. Collectively, IGFs function to increase bone matrix and collagen synthesis and inhibit collagen degradation in bones. They also stimulate the proliferation of osteoblasts.

Cytokines, which include Interleukins (IL) 1, 4, 6 and 11, granulocyte colony stimulating factors and tumour necrosis factor, of which IL-1 is the most potent, mainly functions to stimulate bone resorption. Notably, the production of IL-1 and IL-6 is decreased in the presence of estrogen and it was proved that reduced estrogen is the main cause of post-menopausal bone resorption.

On the other hand, the effect of prostaglandins and leukotrienes, were found to be species-dependent and their overall effects in humans remain unknown. Some studies suggest that prostaglandins especially the class E series stimulate the formation of bone by osteoblasts and inhibit the activity of osteoclasts. Leukotrienes also stimulate formation of bone by osteoblasts, however they also stimulate the formation of resorption pits by osteoclasts.

The various hormones implicated in fracture healing are estrogen which amplifies fracture healing, thyroid hormones which induce bone resorption by osteoclasts, glucocorticoids which raise parathyroid hormone levels hinder the calcium absorption and thereby stimulating resorption of bone by osteoclasts. Finally, growth hormone stimulates and accelerates callus formation via IGF-1.

Various vascular factors play a role in the regulation of new blood vessels formation at the fracture site. Metalloproteinase's firstly degrade bones and cartilage to allow the invasion of blood vessels near the fracture site, followed by the growth of new blood vessels in the site mediated by vascular endothelial growth factors and angiogenic factors.

Osteoconduction is the process where scaffold of the matrix is formed, which serves as base, on which bone cells synthesize callus and then bone. This is important for the formation of bridging callus between fracture fragments followed by its mineralisation in a systematic way.

Osteogenicpotential (osteogenesis) is the ability to provide osteoprogenitor cells which can differentiate into osteoblasts and osteocytes during bone formation.

Autografts have osteoinductive, osteoconductive and osteogenetic potential, in contrast, allograft have only osteoinductive and osteoconductive properties.

DEFINITIONS⁴²

Delayed Union

It is when fracture healing has not progressed at the expected rate for the specific fracture type and fracture site for the particular bone (usually between 3 to 6 months).

Non- Union

US - FDA defines non-union as “established when a minimum of 9 months has elapsed since fracture, with no progressive radiological signs of healing for 3 consecutive months”.

CAUSES OF DELAYED UNION AND NON-UNION

The causes of delayed and non-union were described by Cruess and Buck-Walters in 4 groups of variables.

INJURY VARIABLES

- Open fractures
- Severity (high velocity) of injury
- Intra-articular fracture
- Segmental fracture
- Soft tissue interposition

TISSUE VARIABLES

- Form of bone (cortical)
- Bone necrosis
- Bone diseases
- Infection

PATIENT VARIABLES

- Age (elderly)
- Nutrition (poor)
- Systemic hormones (PTH, estrogen)
- Nicotine usage

TREATMENT VARIABLES

- Improper apposition of fracture fragments
- Loading and increased micromotion
- Unstable fracture fixation

BONE MARROW³⁵

The skeletally mature adults have two types of bone marrow:

- Yellow marrow filled with adipose tissue
- Red bone marrow which is hematopoietically active

The stem cells in the active bone marrow are pluripotent in nature and are capable to differentiate into any cell lineage. The colony forming units of fibroblasts in bone marrow were proved to have the pluripotency, thereby differentiating into osteoprogenitor cells.

The ability of the mesenchymal stem cells to differentiate into bone producing cells has been utilized therapeutically to accelerate fracture healing. The concentration of these osteoblastic progenitor cells is higher in iliac crest compared to tibia and calcaneum.

Bone marrow aspirate has osteoinductive property where it augments the differentiation of osteoprogenitor cells, as well as osteoconductive property by which a scaffold is laid down for bone formation by the differentiated osteoprogenitor cells.

MATERIAL AND METHODS

Source of data:

The study is conducted in 30 cases presented with delayed union of long bones of lower limb to Department of Orthopaedic surgery in R.L.JALAPPA HOSPITAL, attached to SRI DEVARAJ URS MEDICAL COLLEGE, Tamaka, Kolar, during the period of December 2017 to June 2018.

Method of collection of data:

Inclusion criteria:

Patients with

1. Lower limb long bone fractures with no signs of clinical and radiological union after 3 months
2. Age above 18 years

Exclusion criteria:

1. Patients with
 - a. Bone marrow disease
 - b. Pathological fractures
 - c. Active infection
2. Patients refusal

Materials:

Mentioned below are the materials utilized for harvesting of bone marrow and its instillation.

Instruments:

- Salah bone marrow aspiration needles with stiletto of sizes 14-16G
- 16-18G wide bore needles
- Disposable syringes of capacities 10mL and 20mL



Figure 16: INSTRUMENTS

Pre-operative preparation:

Both donor and recipient sites are prepared aseptically.

Pre-medication:

A stat dose of Inj. Cefotaxime 1gm after test dose is given.

Operative details:

a)Position of the patient:

Patient is in supine position. In order to stabilize the back, a sand bag is placed under the gluteal region. In this position, the donor site is made prominent which provides ease for the entry of needle through iliac crest. Risk of damaging pelvic organs would also be minimized. In the same way, a sand bag is also placed under the recipient site for stabilization.

b)Procedure in detail

Under Spinal anaesthesia, both donor and recipient sites are draped following the aseptic principles. Under C-arm guidance, the fracture site is localized. 2 needles (16G or 18G) are inserted into the fracture site, being visualized anteroposteriorly and mediolaterally.

The bone marrow needle is inserted about 3cm posterior to the anterior superior iliac spine at the centre of the broadest portion of iliac crest. The needle is inserted up to the guard, followed by readjustment to a higher point and further insertion with rotatory thrust is done. This is repeated until 2-3cm of the needle has been inserted. The depth of the needle ensures sufficient bone marrow that is being harvested. Finally, the stylet is removed and a 20cc non-heparinised syringe is attached to the needle.

Now that the needle is in position, aspiration of bone marrow is done by simultaneous retraction of syringe plunger and needle rotation to prevent back filling of venous

blood. Bone marrow of about 40-80mL depending on need at recipient site is aspirated and injected. Following aspiration and injection, sterile dressings are applied at both the donor and recipient sites.

Post-procedure, a single dose of Inj. Cefotaxime 1gm is administered. Patient's vitals and general well being are monitored until recovery from anesthesia.

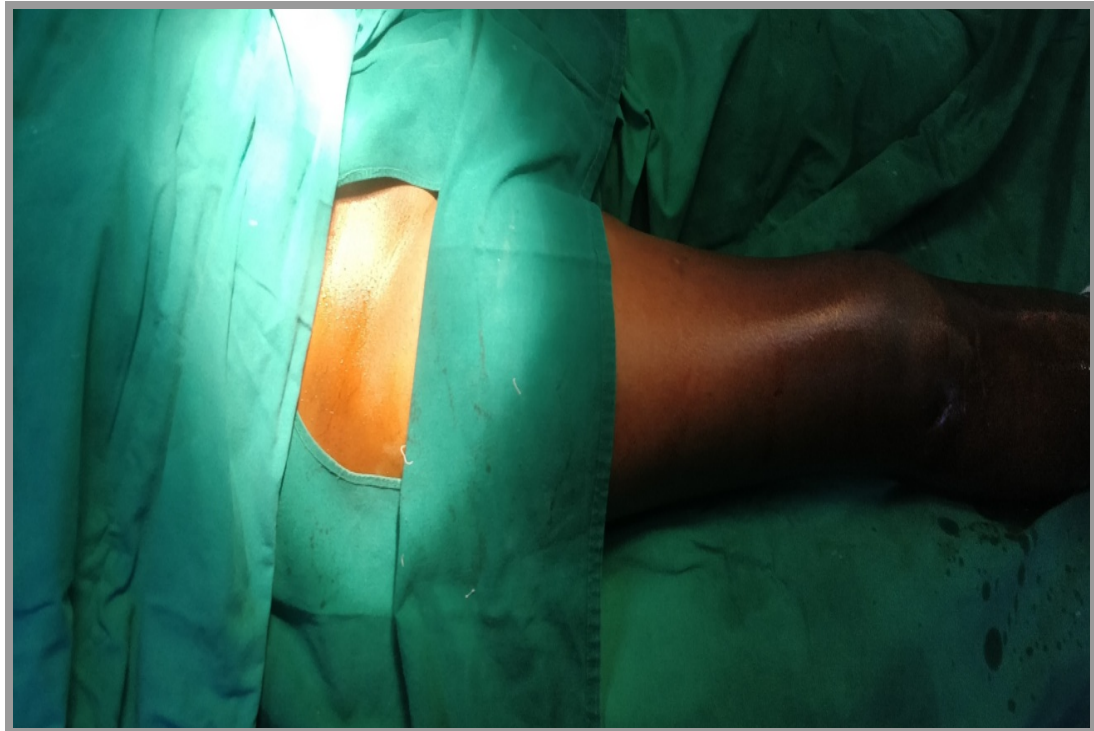


Figure 17: POSITION OF PATIENT

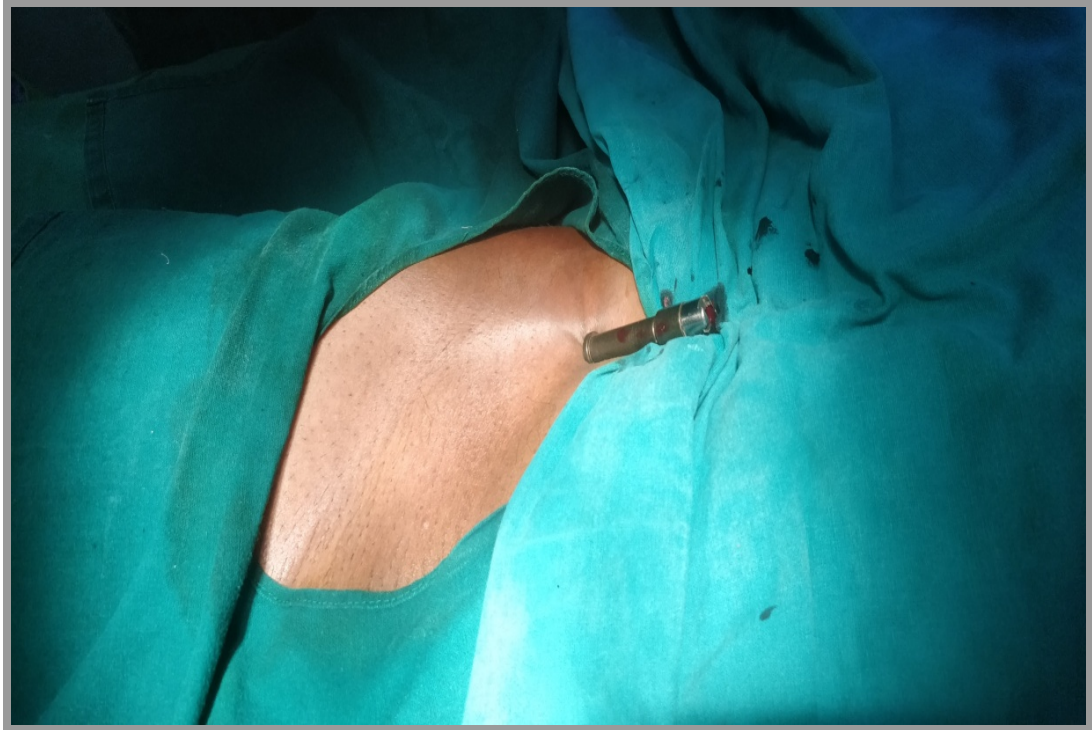


Figure 18: BONE MARROW ASPIRATION SITE (ANTERIOR ILLIAC CREST)

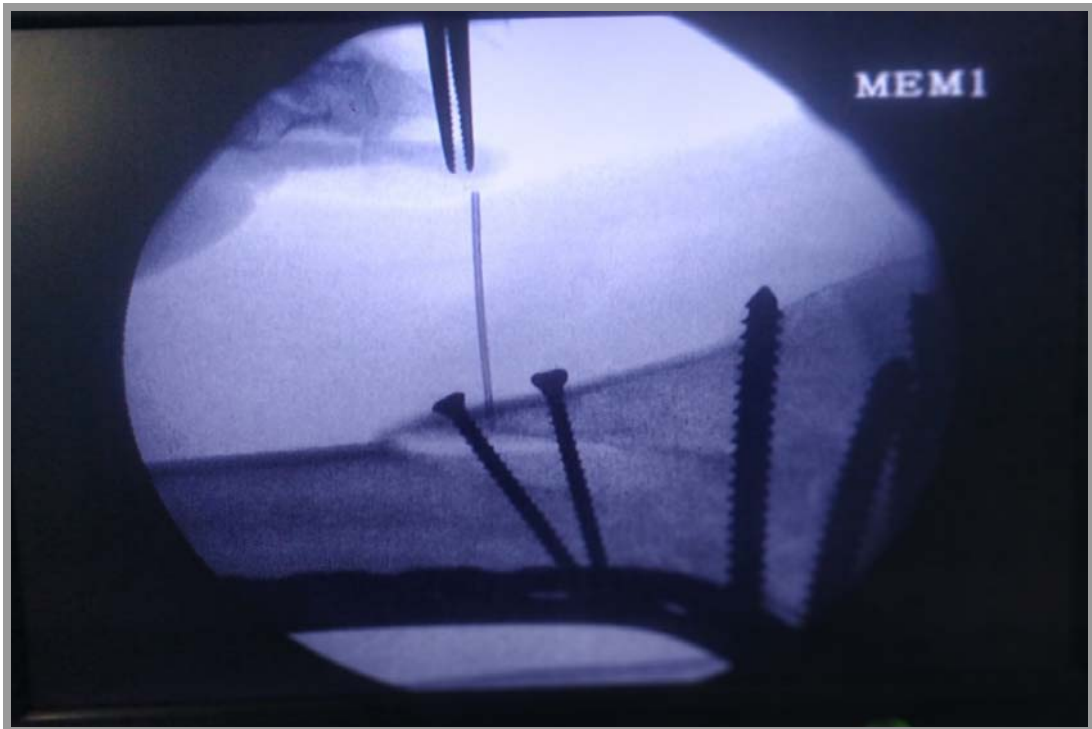


Figure 19: C-ARM IMAGE

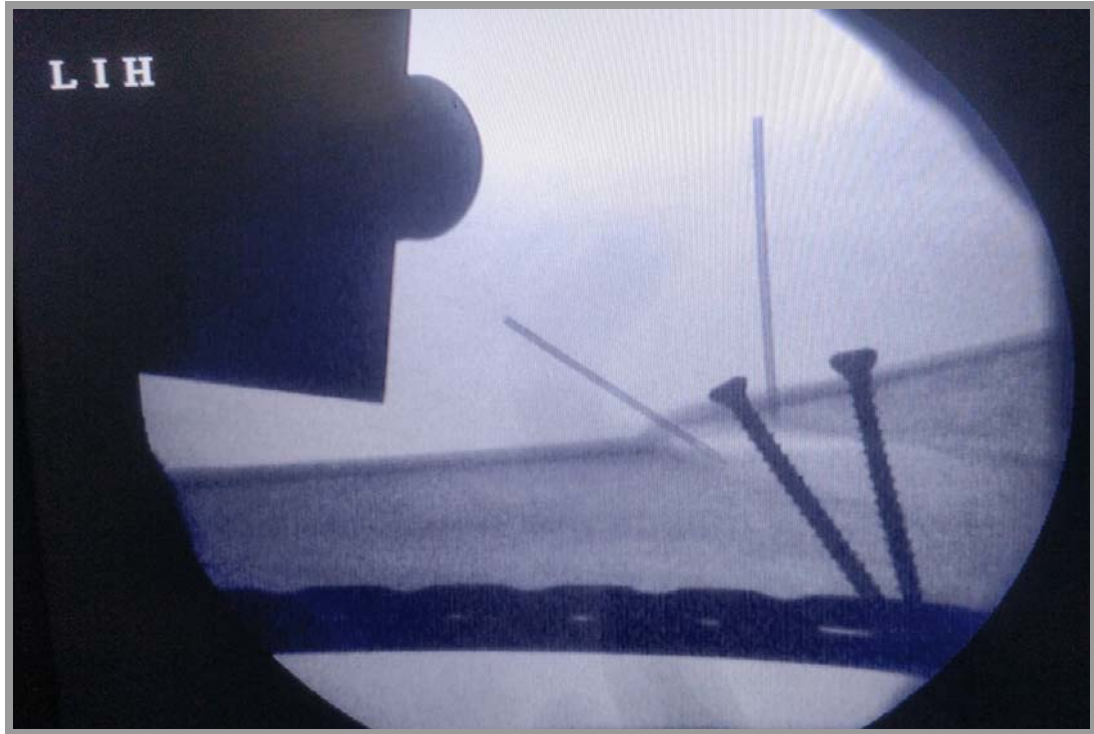


Figure 20: C-ARM IMAGE

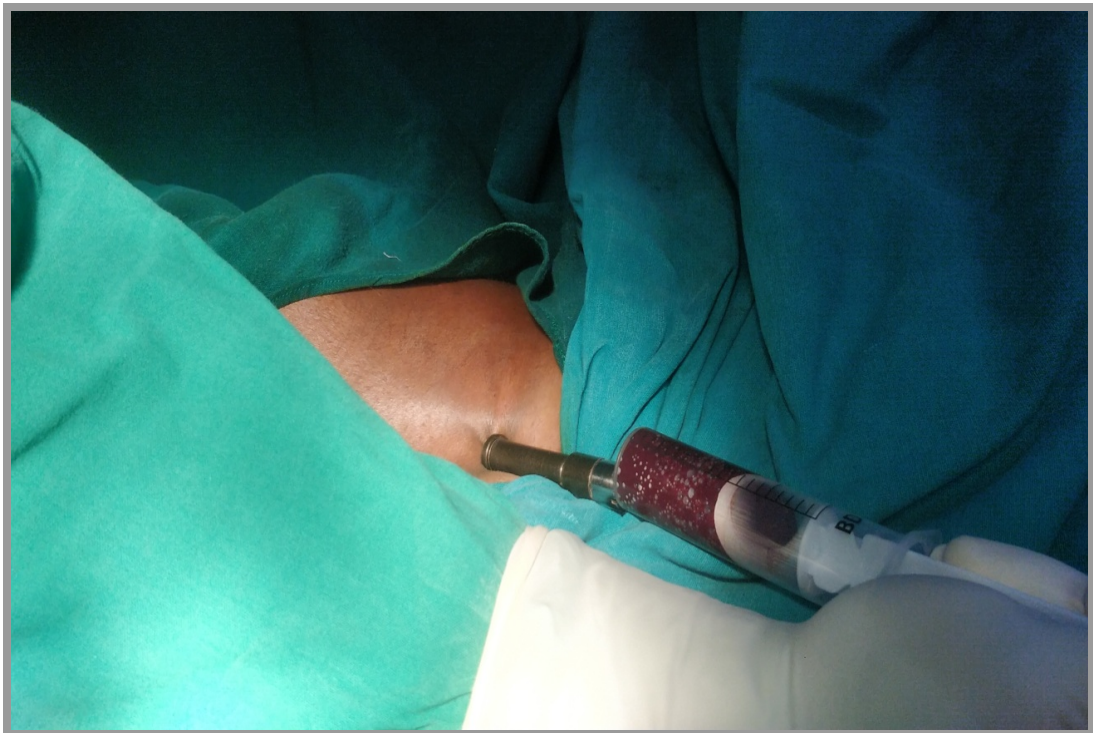


Figure 21: INTRA OPERATIVE (A)



Figure 22: INTRA OPERATIVE (B)



Figure 23:INTRAOPERATIVE (C)



Figure 24: INTRAOPERATIVE (2)

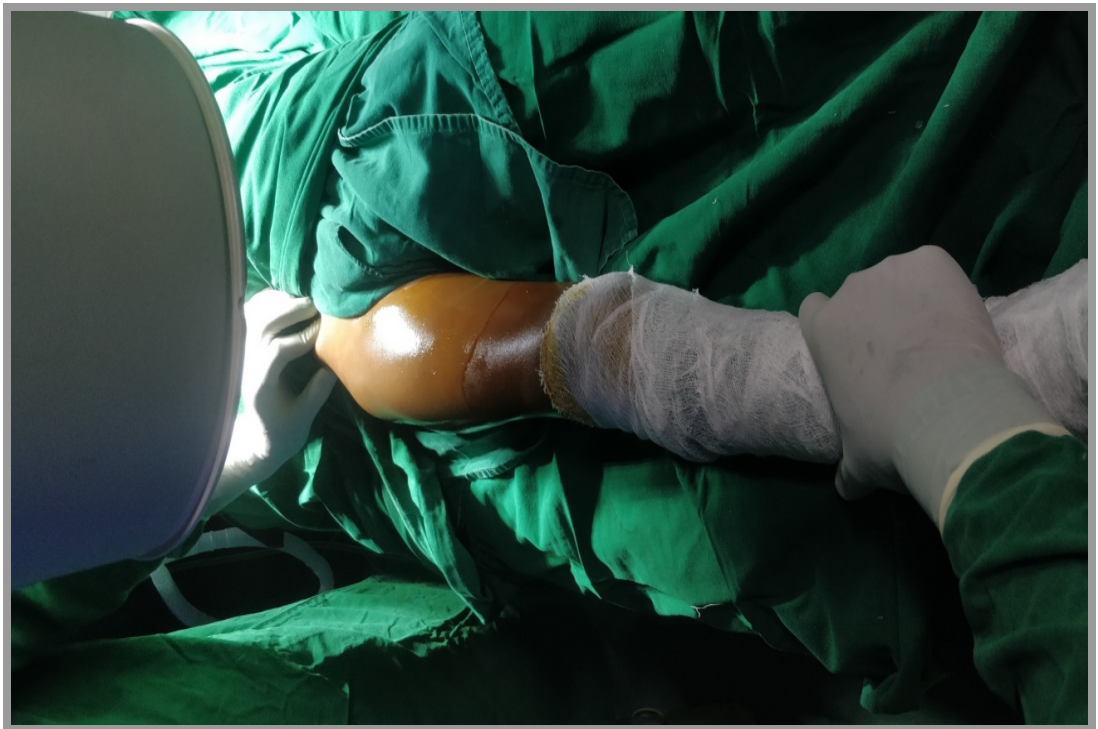


Figure 25: INTRAOPERATIVE (2A)



Figure 26: INTRAOPERATIVE (2B)



Figure 27: INTRAOPERATIVE (2C)



FIGURE 28: INTAOPERATIVE (2D)

c)Follow up

Patients are followed up at 2nd, 4th, and 6th month both clinically and radiologically using union grade score. Patients are advised to avoid medications like NSAIDs that may hinder bone healing.

SAMPLING PROCEDURE

All patients with delayed union fractures of lower limb who satisfied the inclusion criteria were taken up for the study.

A thorough history and clinical examination was done after taking informed consent.

Ethical committee approval was taken for the study.

The following investigations were done pre operatively for pre-anaesthetic evaluation

- Complete haemogram.
- Bleeding time and clotting time.
- Random blood sugar.
- Blood urea and serum creatinine.
- X-ray of the bone involved (antero-posterior and lateral views).

STATISTICAL METHOD

Descriptive statistics

The descriptive statistical procedure is useful to describe basic and simple features with graphic analysis of the data. Univariate analysis, a type of descriptive study in which histograms are used to describe the distribution of central tendency and the dispersion of a single variable.

The present study is purely a descriptive observational study and the observations are tabulated and presented in the form of bar diagrams. Hence, statistical analysis is not necessary.

OBSERVATIONS

In this series, 30 cases with delayed union of fractures of the lower limb admitted at R.L.JALAPPA hospital attached to Sri Devaraj Urs Medical College between December 2016 and June 2018 were injected with autologous bone marrow aspirate at delayed union sites and the following observations are done.

AGE DISTRIBUTION

TABLE: 2

Age	Total no. of patients	Percentage
<30	10	33.3%
30-39	7	23.3%
40-49	7	23.3%
>50	6	20%

The average age of the subject is 40 years. The youngest being 23years and oldest being 80years.

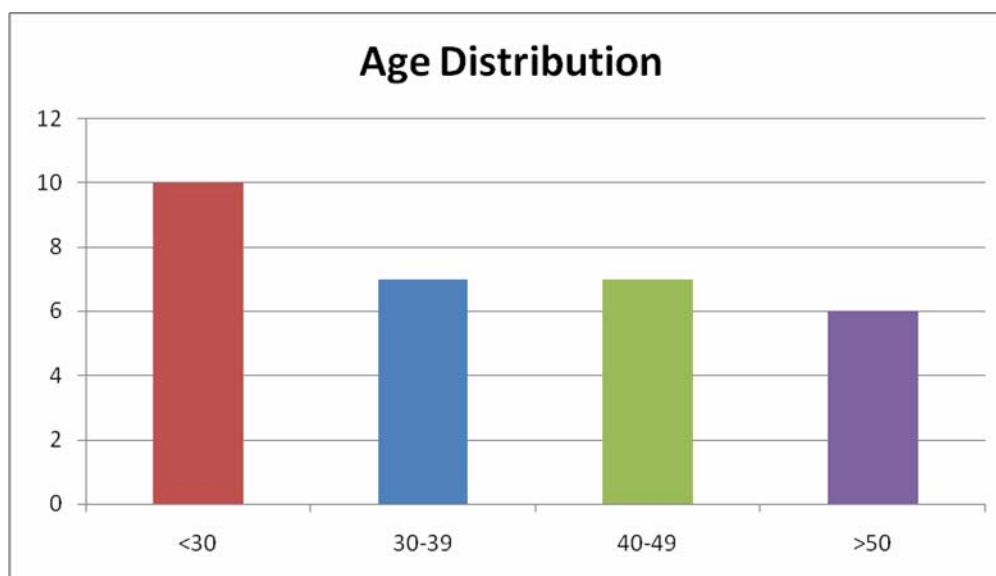


CHART 1:BAR DIAGRAM SHOWING AGE DISTRIBUTION

GENDER DISTRIBUTION

TABLE:3

Sex	Total no. of patients	Percentage
Male	27	90%
Female	3	10%

Most of the subjects in the study are males with male: female ratio at 9:1

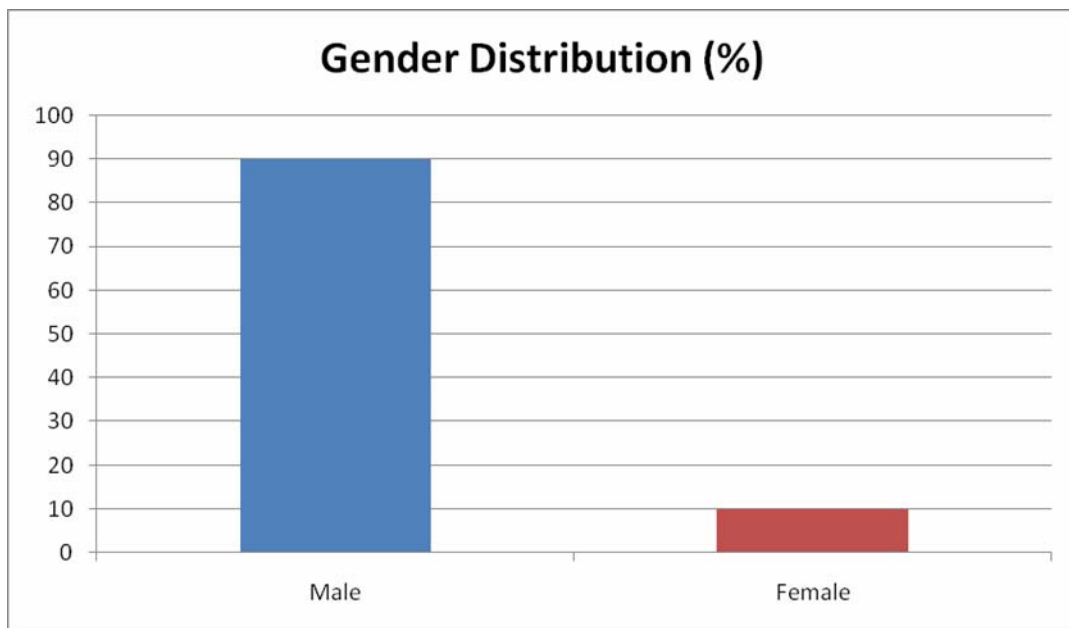


CHART 2: BAR DIAGRAM SHOWING GENDER DISTRIBUTION

BONE INVOLVED AND SITE OF FRACTURE

TABLE:4

Site		Total no. of Cases	Percentage
Tibia	Proximal 1/3rd	1	3.3%
	Middle 1/3rd	7	23.3%
	Distal 1/3rd	8	26.7%
Femur	Proximal 1/3rd	2	6.7%
	Middle 1/3rd	8	26.7%
	Distal 1/3rd	4	13.3%

In this study, 53.3% of the fractures involve tibia, out of which 3.3% over proximal 1/3rd, 23.3% over middle 1/3rd and 26.7% over distal 1/3rd. 46.7% of the fractures involve femur, out of which 6.7% over proximal 1/3rd, 26.7% over middle 1/3rd and 13.3% over distal 1/3rd.



CHART 3: BAR DIAGRAM SHOWING BONE INVOLVED AND SITE OF FRACTURE

TYPE OF FRACTURE

TABLE: 5

Type	Total no. of patients	Percentage
Simple	23	76.7%
Compound	7	23.3%

In the study, 76.7% of fractures were of simple type and 23.3% of compound type.

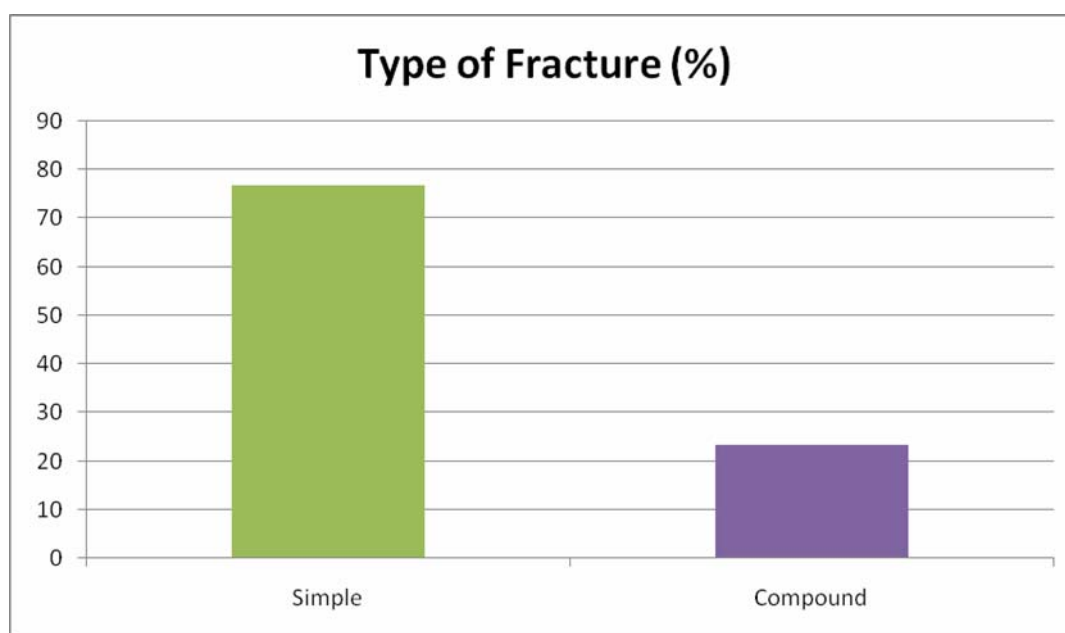


CHART 4: BAR DIAGRAM SHOWING DISTRIBUTION OF TYPE OF FRACTURE

PATTERN OF FRACTURE

TABLE: 6

Pattern	Total no. of patients	Percentage
Transverse	4	13.3%
Oblique	3	10%
Spiral	1	3.3%
Comminuted	20	66.7%
Segmental	2	6.7%

In the study, the patterns of fracture are as follows: 13.3% transverse, 10% oblique, 3.3% spiral, 66.7% comminuted and 6.7% segmental.

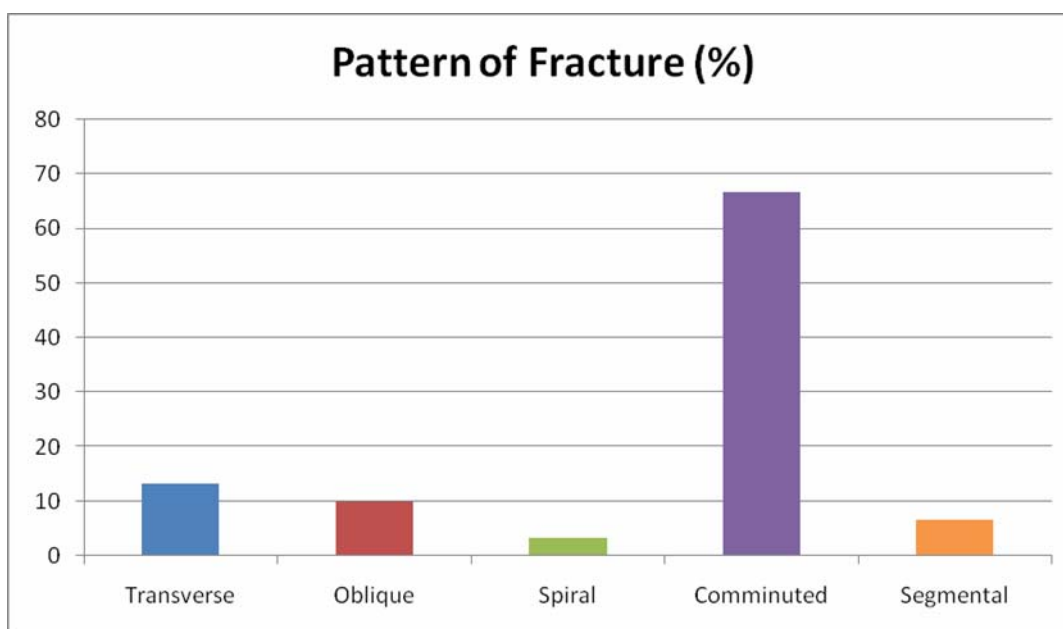


CHART 5: BAR DIAGRAM SHOWING DISTRIBUTION OF PATTERN OF FRACTURE

TIME OF VISIT AFTER FRACTURE

TABLE: 7

Time	Total no. of patients	Percentage
Less than 4 months	15	50%
4-5 months	14	46.7%
5 months or more	1	3.3%

Among the subjects, 50% came for first visit at less than 4 months following fracture, 46.7% between 4 to 5 months and 3.3% at 5 months or more.

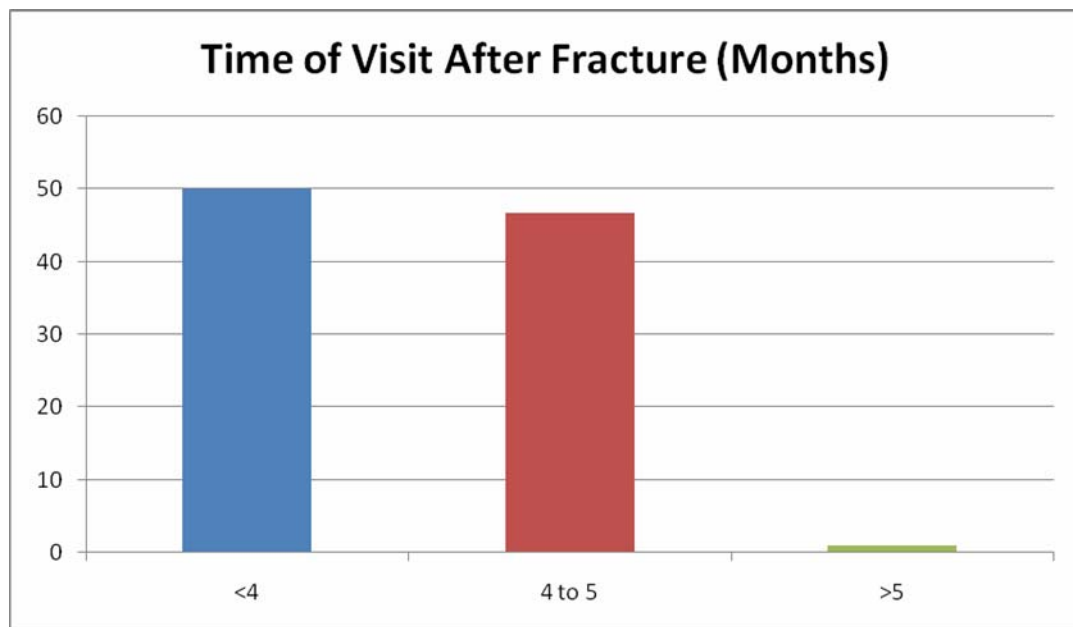


CHART 6: BAR DIAGRAM SHOWING TIME OF VISIT AFTER FRACTURE

INITIAL MODE OF TREATMENT

TABLE: 8

Mode		Total no. of patients	Percentage
Internal	IMIL Nailing	23	76.7%
	Plate	2	6.7%
External		5	16.7%

The initial mode of treatment provided to the patients are IMIL nailing in 76.7%, Plate application in 6.7% and external fixation in 16.7% patients

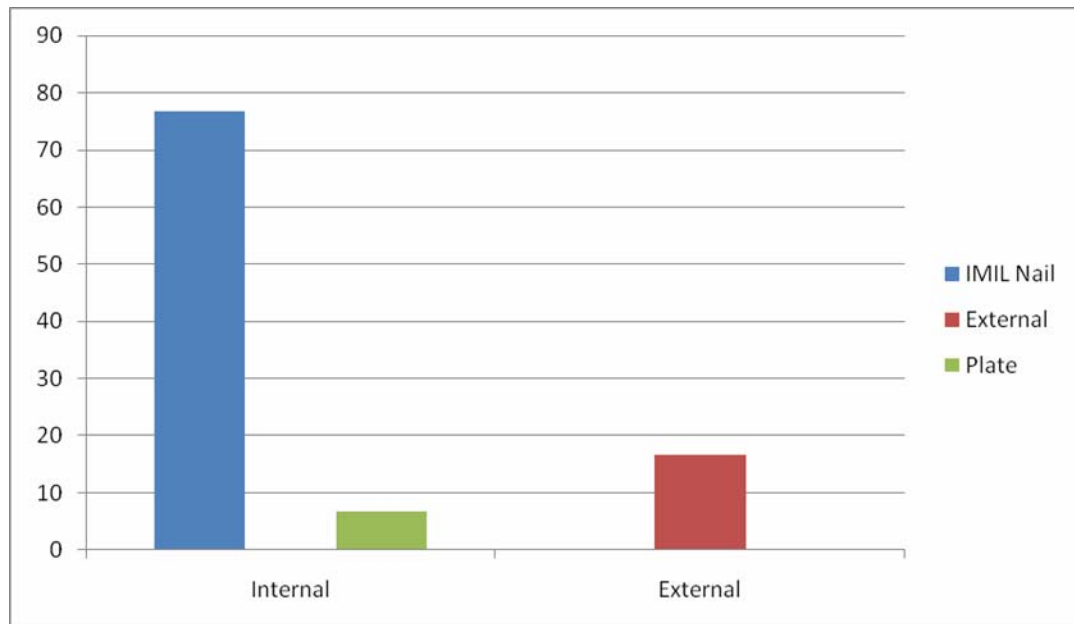


CHART 7: BAR DIAGRAM SHOWING DISTRIBUTION OF INITIAL MODE OF TREATMENT OF FRACTURE

VOLUME OF BONE MARROW ASPIRATE INJECTED

TABLE: 9

Volume(ml)	Total no. of patients	Percentage
40-60	18	60%
61-80	12	40%

Among the volume of bone marrow aspirate injected, 60% of the patients were 40-60mL and 40% were 60-80mL.

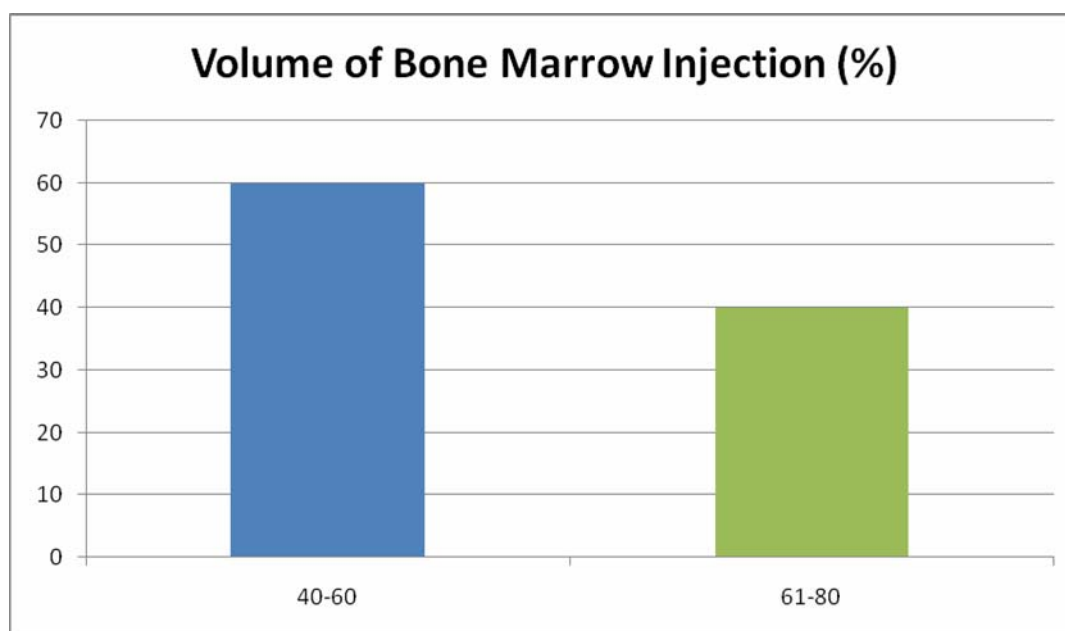


CHART 8: BAR DIAGRAM SHOWING VOLUME OF BONE MARROW ASPIRATE INJECTION

TIME OF CALLUS APPEARANCE ON X-RAY

TABLE: 10

Time	Total no. of patients	Percentage
2 months	18	60%
4 months	10	33.3%
6 months	2	6.7%

60% of the subjects have developed callus which was detected on X-ray at 2 months, 33.3% at 4 months and 6.7% at 6 months after bone marrow injection.

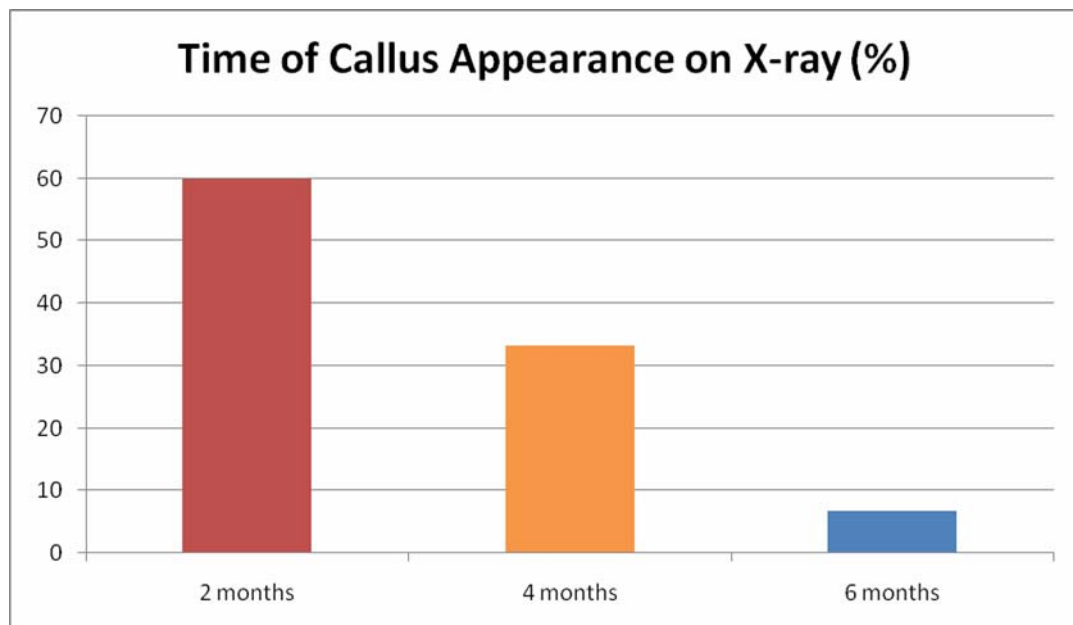


CHART 9: BAR DIAGRAM SHOWING TIME OF CALLUS APPEARANCE ON X-RAY

CLINICAL SCORING (OF UNION SCALE)

TABLE: 11

Score	Total no. of patients	Percentage
6-7	27	90%
4-5	1	3.3%
Less than 4	2	6.7%

A total of 90% of the subjects were clinically scored as 6 to 7, 3.3% were scored between 4 to 5 and 6.7% were scored less than 4.

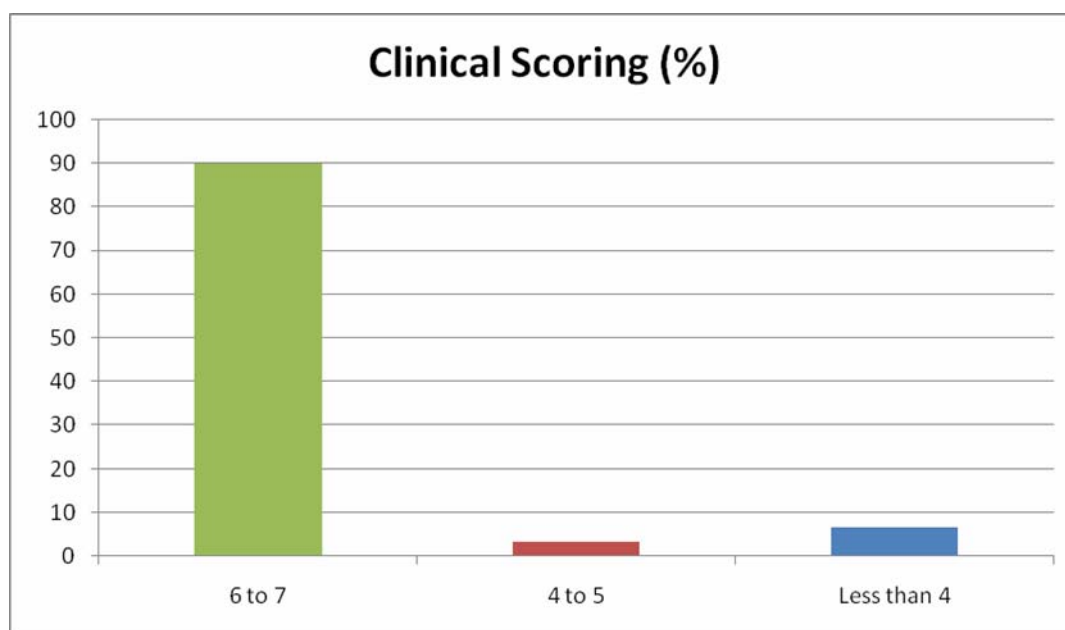


CHART 10: BAR DIAGRAM SHOWING DISTRIBUTION OF CLINICAL SCORING

RADIOLOGICAL SCORING (OF UNION SCALE)

TABLE: 12

Score	Total no. of patients	Percentage
3	27	90%
2	1	3.3%
1	2	6.7%

In the study, 90% of the patients were given radiological score 3, 3.3% were given score 2 and 6.7% were given score 1.

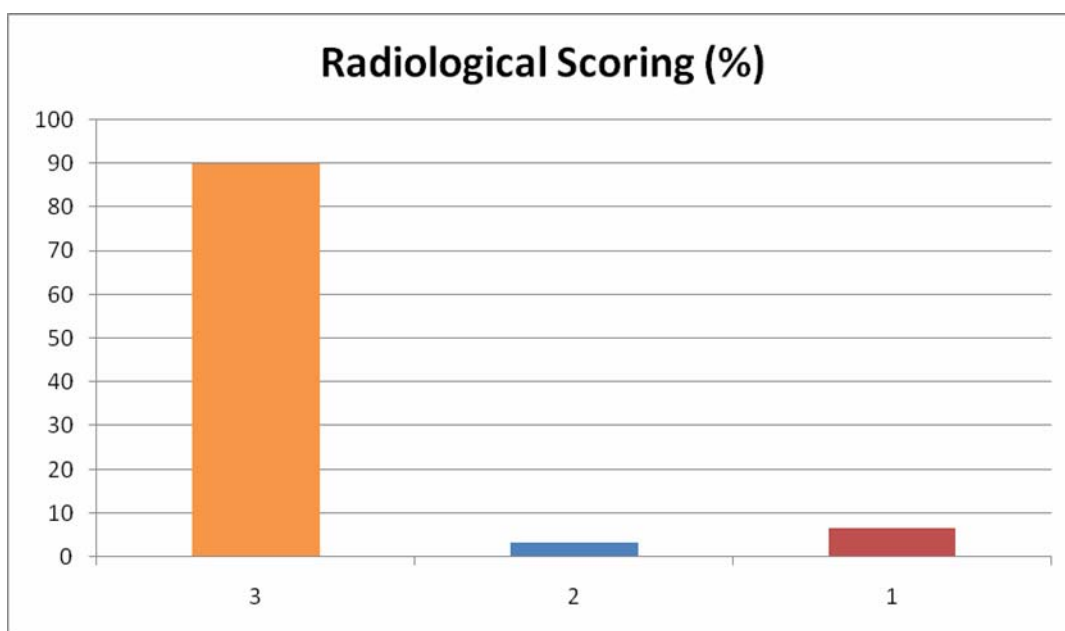


CHART 11: BAR DIAGRAM SHOWING DISTRIBUTION OF RADIOLOGICAL SCORING

TIME OF FRACTURE UNION

TABLE: 13

Time (months)	Total no. of patients	Percentage
2	5	16.7%
4	11	36.7%
6	12	40%
Absent	2	6.7%

16.7% of the patients had union at 2 months, 36.7% at 4 months, 40% at 6 months and 6.7% had absent union.

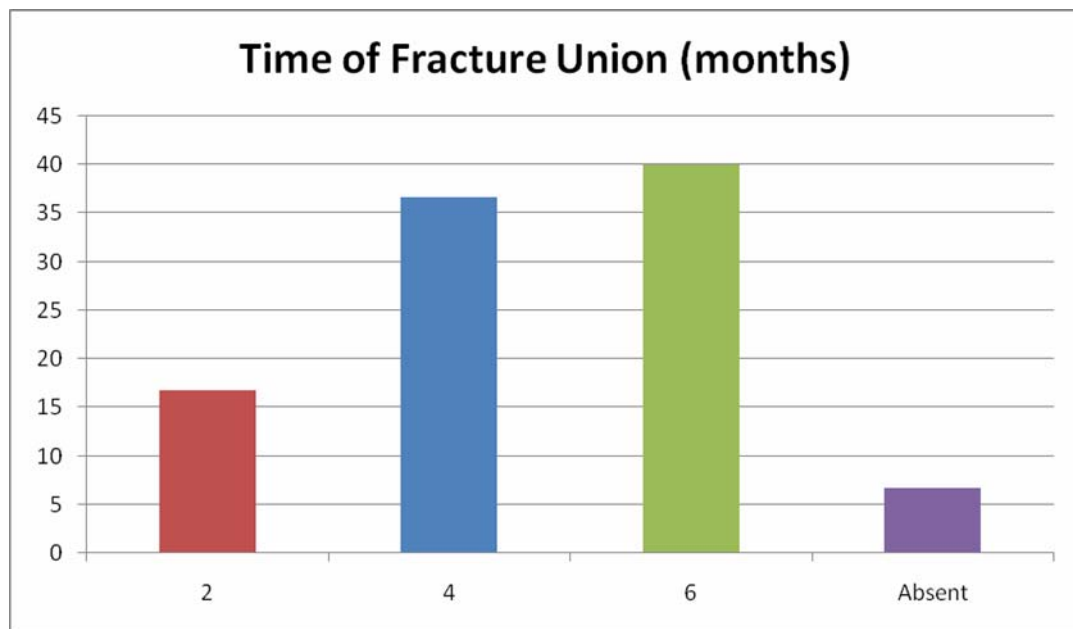


CHART 12: BAR DIAGRAM SHOWING DISTRIBUTION OF TIME OF FRACTURE UNION

UNION SCALE GRADING

TABLE: 14

Grade	Total no. of patients	Percentage
7	27	90%
5-6	1	3.3%
Less than 5	2	6.7%

In this study, 90% of the patients had union of grade 7, 3.3% between 5 to 6 and 6.7% were grade less than 5.

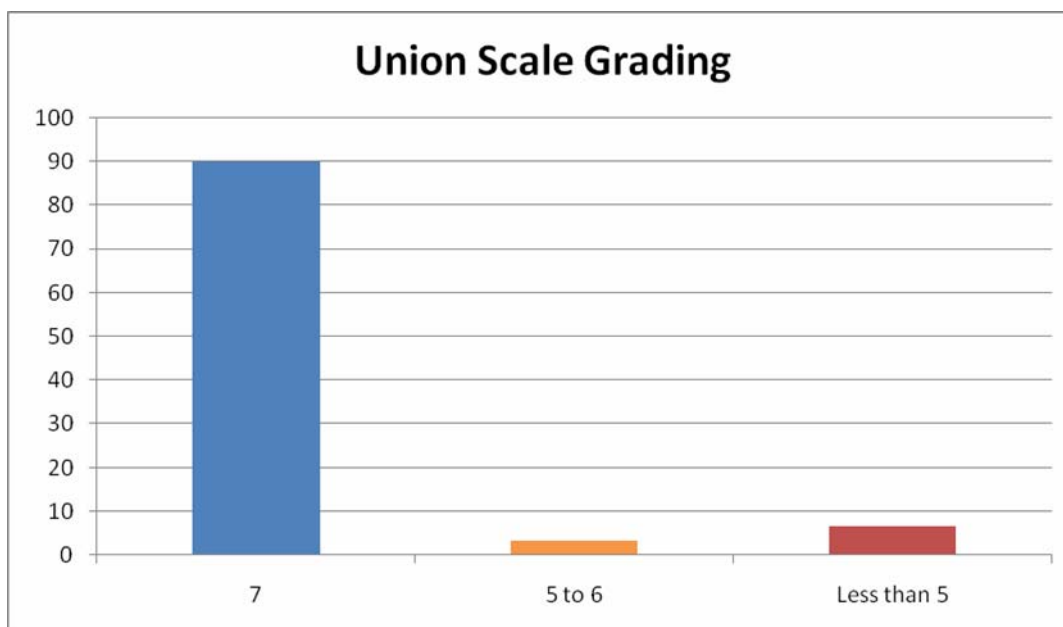


CHART 13: BAR DIAGRAM SHOWING DISTRIBUTION OF UNION SCALE GRADING

OUTCOME

TABLE: 15

Outcome	Total no. of patients	Percentage
Good	27	90%
Fair	1	3.3%
Poor	2	6.7%

In this study, 90% of the patients had good outcome, 3.3% had fair outcome and 6.7% had poor outcome.

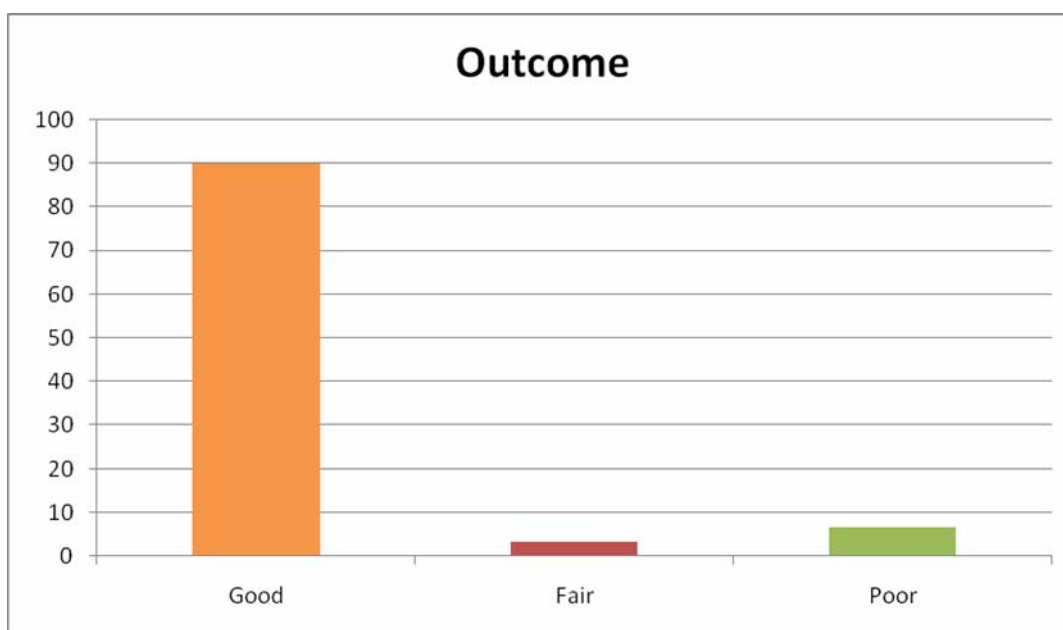


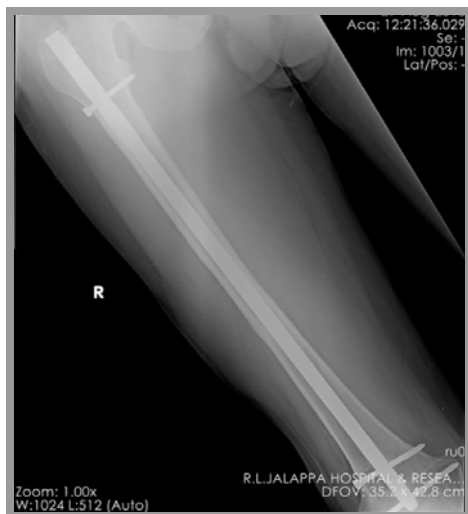
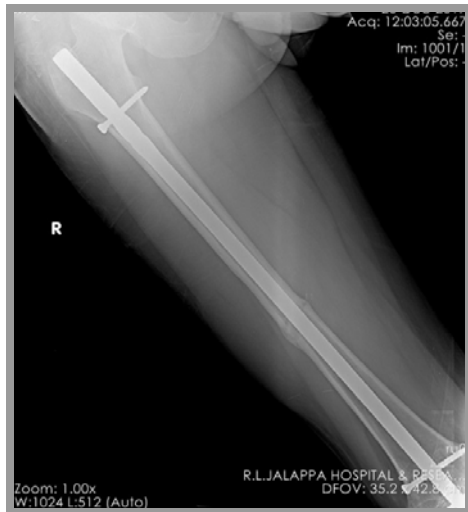
CHART 14: BAR DIAGRAM SHOWING THE DISTRIBUTION OF OUTCOME

X RAYS

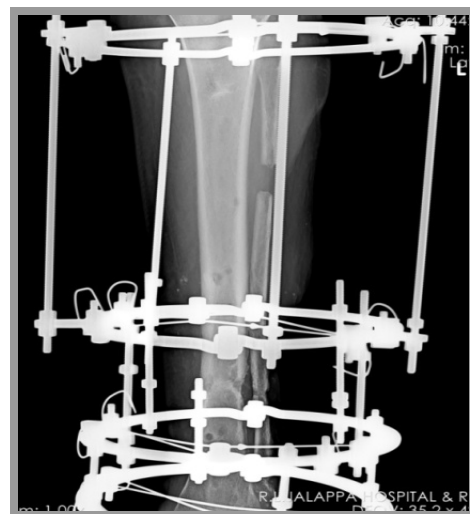
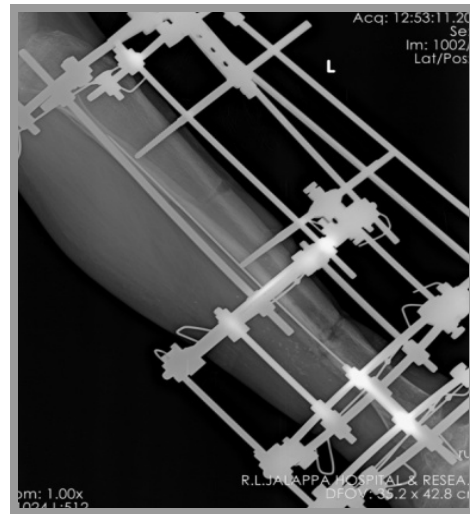
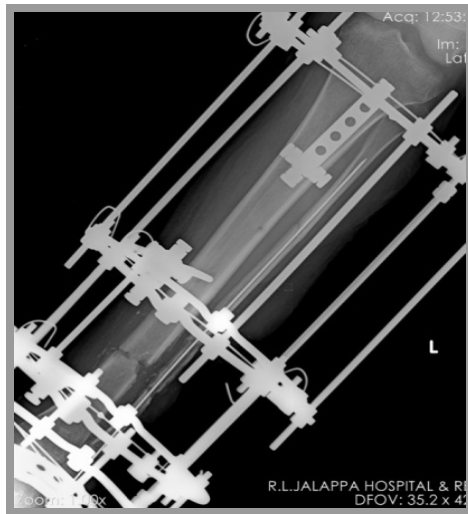
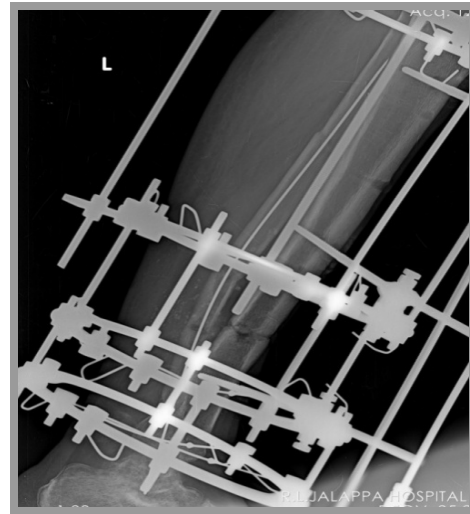
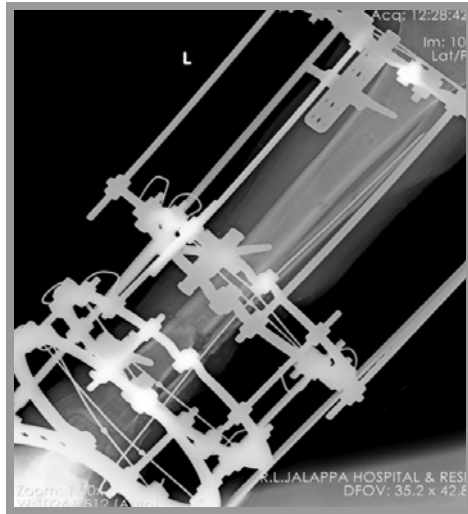
CASE 1



CASE 2



CASE 3



DISCUSSION

Most of the delayed unions in the long bones are treated by various ways like bone stimulators i.e. ultrasonic or pulsed electromagnetic waves, Platelet rich plasma (PRP) injections, bone marrow injection, bone grafts, and bone graft substitutes. This study is performed to know the effectiveness of the bone marrow injection and the outcome in the delayed unions.⁴⁰

For proper and adequate bone healing, re-establishment of the biology at the fracture site is necessary. It is proposed as diamond concept by Giannoudis, which includes “cell recruiting molecules, bone matrix, osteogenic cells and vascularity.”⁴³

AGE DISTRIBUTION

In the present study the mean age is 40 years which is similar to other studies.

TABLE: 16

Authors	Year	Mean age (years)
Sim et al⁴⁴	1992	38
Connolly et al¹²	1999	34
Siwach et al¹⁴	2001	41.2
Helio¹⁵	2003	42
Subash Y³¹	2018	44.2
Elsattar et al⁴⁵	2014	37.6
Brady H L et al⁴⁶	2013	40.1
Willkins et al⁴⁷	2003	42
Upadhyay S et al⁴⁸	2016	39.5
Present study	2018	40

GENDER DISTRIBUTION

In our study, the male to female ratio is 9:1 similar to study done by Sim et al.

TABLE: 17

Authors	Year	Male	Female
Sahu ³⁰	2018	67 (70.5%)	28 (28.5%)
Braly H L et al ⁴⁶	2013	7 (63.6%)	4 (36.3%)
Helio ¹⁵	2003	37 (56.1%)	29 (43.9%)
Sim et al ⁴⁴	1992	9 (90%)	1 (10%)
Elsattar et al ⁴⁵	2014	16 (80%)	4 (20%)
UpadhyayS et al ⁴⁸	2016	6 (75%)	2 (25%)
Present study	2018	27 (90%)	3 (10%)

BONE INVOLVED

In this study, 53.3% of the cases involved tibia, while 46.7% of the cases involved femur which is comparable to other studies.

TABLE: 18

Authors	Year	Femur	Tibia
Denver¹⁵	2003	16 (23.2%)	36 (52.2%)
Elsattar et al⁴⁵	2014	6 (30%)	14 (70%)
Bhutia²⁴	2015	4 (13.3%)	23 (76.7%)
Nazar²⁵	2016	18 (22.5%)	42 (52.5%)
Abdelghany⁴⁹	2007	4 (30.8%)	9 (69.2%)
Konde et al²⁸	2017	5 (18%)	20 (72%)
Peter R F⁵⁰	2018	21 (34.4%)	40 (65.6%)
Present study	2018	14 (46.7%)	16 (53.3%)

SITE OF FRACTURE

In this study, 10% of the fractures involved proximal 1/3rd, 50% involved middle 1/3rd and 40% involved distal 1/3rd which is comparable to other studies.

TABLE: 19

Authors	Year	Proximal 1/3 rd	Middle 1/3 rd	Distal 1/3 rd
Upadhyay S et al ⁴⁸	2016	2 (25%)	3 (37.5%)	3 (37.5%)
Present study	2018	3 (10%)	15(50%)	12(40%)

TYPE OF FRACTURE

In our study, 76.7% of the fractures are of simple type while 23.3% are of compound type which is comparable to other studies.

TABLE: 20

Authors	Year	Simple	Compound
Brady H L et al ⁴⁶	2013	7 (63.6%)	4 (36.3%)
Bhutia ²⁴	2015	14 (46.7%)	16 (53.3%)
Sahu ³⁰	2018	80 (86%)	13 (14%)
Helio ¹⁵	2003	30 (52.6%)	27 (47.4%)
Sim et al ⁴⁴	1992	4 (36.3%)	7 (63.6%)
Elsattar et al ⁴⁵	2014	12 (60%)	8 (40%)
Present study	2018	23 (76.7%)	7 (23.3%)

PATTERN OF FRACTURE

In our study we observed, transverse pattern constitutes 13.3%, oblique 10%, spiral 3.3%, comminuted 66.7% and segmental 6.7% which is different than the study done by Sahu as the subjects in this present study mostly consists of younger patients involved in road traffic accidents.

TABLE: 21

Authors	Year	Linear	Comminuted	Segmental
Sahu ³⁰	2018	67 (73%)	23 (26%)	2 (1%)
Present study	2018	8 (26.6%)	20 (66.7%)	2 (6.7%)

INITIAL MODE OF TREATMENT

We have noticed in our study, initial modalities of management are Intra medullary interlocking (IMIL) nailing (76.7%), plate (6.7%) and external (16.7%) which is comparable to other studies.

TABLE: 22

Authors	Year	Internal fixation	External fixation
Konde et al ²⁸	2017	IMIL Nailing - 18 (72%) Plate - 4 (16%)	3 (12%)
Peter R F ⁵⁰	2018	IMIL Nailing - 35(63.6%) Plate - 12 (21.8%)	8 (14.5%)
Present study	2018	IMIL Nailing - 23 (76.7%) Plate - 2 (6.7%)	Supracutaneous LCP - 4 (13.4%) Illizarov - 1 (3.3%)

TIME FOR UNION

In our study, average time of fracture union is 18 weeks which is comparable to other studies.

TABLE: 23

Authors	Year	Average Time of Union
Braly H L et al⁴⁶	2013	17 weeks
Singh et al²	2013	16 weeks
Subash Y³¹	2018	13.4 weeks
Sim et al⁴⁴	1992	17 weeks
Hernigou et al⁵¹	2005	12 weeks
Bhargava et al⁸	2007	12 weeks
Nazar²⁵	2016	16 weeks
Rahimnia et al²⁶	2016	12 weeks
Konde et al²⁸	2017	20 weeks
Present study	2018	18 weeks

DURATION OF INITIAL VISIT

In this study, the average time of visit after initial fracture management is about 4 months, which is comparable to other studies.

TABLE: 24

Authors	Year	Average time of first visit
Sim et al⁴⁴	1992	4 months (17 weeks)
Denver¹⁵	2003	21 months
Bhargava et al⁸	2007	14-30 weeks
Present study	2018	4 months

AMOUNT OF ASPIRATE INJECTED

In the present study, the amount of bone marrow aspirate injected is between 40-80ml which is comparable to other studies.

TABLE: 25

Authors	Year	Volume of BM aspirate(ml)
Brady H L et al⁴⁶	2013	40-80
Sharma D et al²⁹	2017	50-60
Sim et al⁴⁴	1992	50-200
Bhargava et al⁸	2007	50-90
Present study	2018	40-80

TIME OF APPEARANCE OF CALLUS

In the present study, the average time of callus seen on X-ray is 12 weeks which is similar to a study by Upadhyay S et al.

TABLE: 26

Authors	Year	Time of Callus seen on X-ray
Upadhyay Set al ⁴⁸	2016	12 week
Wani H ¹⁹	2013	21 weeks
Present study	2018	12 weeks

UNION SCALE GRADING

In the present study, the clinical scoring at the end of 6 months follow up are 90% with 6-7 score, 3.3% with 4-5 score and 6.7% with less than 4 score. The radiological scoring is 90% with score 3, 3.3% with score 2 and 6.7% with score 1. Based on clinical and radiological scoring, the union scale grading is derived, which is comparable to other study.

TABLE: 26

Author	Year	Union Scale Grading
Bhargava R ⁸	2007	6-7: 23 (82.1%) Less than 6: 5 (27.9%)
Present study	2018	6-7: 27 (90%) Less than 6: 3 (10%)

FINAL OUTCOME

In the present study, the final outcome is 93.3% which is comparable to other studies.

TABLE: 28

Authors	Year	Outcome
Sim et al⁴⁴	1992	9/11 (81.8%)
Bhargava et al⁸	2007	25/28 (89.3%)
Elsattar et al⁴⁵	2014	16/20 (80%)
Connolly et al¹²	1991	18/20 (90%)
Sahu³⁰	2018	82/93 (88%)
Singh et al²	2013	10/12 (83.3%)
Thua et al⁵²	2015	10/10 (100%)
Hernigou et al⁵¹	2006	88.3%
Sugaya et al⁵³	2014	76%
Present study	2018	28/30 (93.3%)

ANAESTHESIA AND DURATION OF PROCEDURE

All the patients underwent this procedure under spinal anaesthesia and the mean duration of the procedure is around 30 minutes which is comparable to other studies.

COMPLICATIONS

No complications were noted during our study, even though 2 patients complained pain at donor site, which subsided with oral analgesics within 48 hours. Published literature also concluded that there are almost nil complications associated with this procedure.

CONCLUSION

Fracture healing is a diverse process which needs to be addressed depending on various variables related to patient, injury, tissue and treatment. Any imbalance in these variables will disturb the biology of bone healing leading to delayed union.

With increased high velocity and open injuries especially in the younger population, there is an increase in incidence of comminuted fractures which often go for delayed or non-union even after aggressive management. Even though the gold standard for the treatment of delayed or non-union is bone grafting, there is always an associated morbidity with the procedure.

After our short term follow up of 30 cases for a period of 6 months post bone marrow aspirate injection, we could observe that there is accelerated bone healing, even in patients with associated co-morbidities.

We also observed that even single dose of unconcentrated bone marrow aspirate injection is enough to achieve union. The procedure is simple, cost effective, easily reproducible with no or minimal complications. Even though all of our subjects underwent the procedure under spinal anaesthesia, many studies have concluded that this procedure can be done under local anaesthesia or short general anaesthesia on OPD basis. We preferred non heparinized syringes as the time interval between aspiration and injecting at the delayed union site is very short.

We finally conclude that, the earlier the intervention in delayed union faster the union and less chances of progression to non-union.

Although bone marrow aspirate injection at the delayed union of fractures of lower limb is found to be effective in our study, there are limitations from our observations, i.e. small sample size, inclusion of only lower limb fracture delayed unions which needs to be addressed.

SUMMARY

In our study, 30 cases of delayed unions of long bones of lower limb after a follow up of 6 months by clinical and radiological observations are tabulated and evaluated the outcome.

The mean age of subjects is about 40 years with predominance of males as indicated by the mode of injury as high velocity road traffic accidents. Tibia is the most commonly involved bone and internal fixation is the preferred initial fixation in many subjects.

All the cases are done under spinal anaesthesia and an average of 66.6 ml of bone marrow aspirate was injected. The average rate of union is around 18 weeks after bone marrow aspirate injection. No complications were observed during our period of study indicating the safety of this procedure. The post-operative stay is just for 24 hours and the success rate in our study is 93% which indicates the effectiveness.

BIBLIOGRAPHY

1. Charles M, James D, Margaret M, William M, Paul T, Michael D. Rockwood and Green's Fractures in Adults. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2014. p 127.
2. Singh AK, Shetty S, Saraswathy JJ, Sinha A. Percutaneous autologous bone marrow injections for delayed or non-union of bones. J Orthop Surg 2013;21:60-4.
3. Goujon E. Recherches experimentales sur les proprietes physiologiques de la moelle des os. J Anat Physiol. 1869;6:399-412.
4. McGaw WH, Harbin M. The role of bone marrow and endosteum in bone regeneration. An experimental study of bone marrow and endosteal transplants. J Bone Joint Surg 1934;16:816-21.
5. Jensen GS, Drapeau C. The use of in situ bone marrow stem cells for the treatment of various degenerative diseases. Med Hypotheses 2002;59:422–8.
6. Schotell PC, Warner SJ. Role of bone marrow aspirate in orthopedic trauma. OrthopClin North Am 2017;48:311-21.
7. Herzog K. Verlängerung osteotomie unter Verriegelung des percutan gezeit Verriegelten Markkangels. Unfallheilkunde 1951;42:226-30.
8. Bhargava R, Sankhla S, Gupta A, Changani R, Gagal K. Percutaneous autologous bone marrow injection in the treatment of delayed or nonunion. Indian J Orthop 2007;41:67–71.
9. Meekren J Van. Observationes medicochirurgicae. Amsterdam: Henrici and Bloom;1682.
10. Phemister DB. Treatment of ununited fractures by onlay bone grafts without screw or tie fixation and without breaking down of the fibrous union. J Bone Joint Surg Am 1947;29:946-60.

11. Healey JH, Zimmerman PA, McDonnell JM, Lane JM. Percutaneous bone marrow grafting of delayed union and nonunion in cancer patients. Clin Orthop Relat Res 1990;256:280–5.
12. Connolly JF, Guse R, Tiedeman J, Dehne R. Autologous marrow injection as a substitute for operative grafting of tibial nonunions. Clin Orthop Relat Res 1991;266:259-70.
13. Hernigou P, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. J Bone Joint Surg Am 2005;87:1430-7. Siwach RC, Sangwan SS, Singh R, Goel A. Role of percutaneous bone marrow grafting in delayed unions, nonunions and poor regenerates. Indian J Med Sci 2001;55:326-36.
14. Wilkins RM, Chimenti BT, Rifkin RM. Percutaneous treatment of long bone nonunions: the use of autologous bone marrow and allograft bone matrix. Orthopedics 2003;26:549-54.
15. Hernigou P, Mathieu G, Poignard A, Manicom O, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Surgical technique. J Bone Joint Surg Am 2006;88:322-7.
16. Padha V, Mahajan N, Kalsotra N, Salaria A, Sharma S. Role Of Percutaneous Bone Marrow Injection In Delayed Union And Non Union. The Internet Journal of Orthopedic Surgery 2009;18:1-8.
17. Raulo BC, Dash C, Rath S, Chakrabarty S, Rautray P, Sahoo J et al. Use of bone marrow derived stem cells in a fracture non-union. Journal of Acute Disease 2012;6189:60082-7.

18. Wani IH, Padha V, Jan M, SalariaAQ. Percutaneous bone marrow grafting in delayed union and non-union. *International Journal of Medicine and Medical Sciences*. 2013;5:110-5.
19. Kassem MS. Percutaneous autogenous bone marrow injection for delayed union or non-union of fractures after internal fixation. *Acta Orthop Belg* 2013;79:711-7.
20. Barrena EG, Rosset P, Lozano D, Stanovici J, Ermthaller C, Gerbhard F. Bone fracture healing: Cell therapy in delayed unions and nonunions. *Bone* 2015;70:93-101.
21. Hernigou P, Guissou I, Homma Y, Poignard A, Chevallier N, Rouard H et al. Percutaneous injection of bone marrow mesenchymal stem cells for ankle non-unions decreases complications in patients with diabetes. *Int Orthop* 2015;39:1639-43.
22. Gross JB, Diligent J, BensoussanD, Galois L, Stoltz JF, Mainard D. Percutaneous autologous bone marrow injection for treatment of delayed and non-union of long bone: A retrospective study of 45 cases. *Biomed Mater Eng* 2015;25:187-97.
23. Bhutia KU, Bary AA, Singh AK, Singh AM, Raghuvanshi R, Hmar C. Role of Percutaneous Autologous Bone Marrow Injection in Treatment of Delayed Union and Non Union of Long Bones. *IOSR-JDMS* 2015;14:7-13.
24. Nazar M, Shafiq M, Ahmed A, Ahmad S, Javed S, Aziz A. Autologous percutaneous bone marrow injection in long bone fractures with delayed and nonunion. *J Surg Pakistan* 2016;21:23-6.
25. Rahimnia A, Dorostgan A, Kazemian GH, Rahimnia A. Injection of Autologous Bone Marrow versus Hanging Cast in Treatment of Humeral Fracture. *Thrita* 2016;5:e35643.

26. Guttarlapalli PG, Challa S. Outcome of percutaneous bone marrow injection in delayed union and nonunion of long bone fracture. *MedPulse International Journal of Orthopedics* 2017;3:16-9.
27. Konde SS, Borkar SS, Thosar SR, Allamwar AR, Kamath PS. Role of Percutaneous Bone Marrow Injection in Non Union of Fractures. *National journal of Medical Research* 2017;7:135-7.
28. Sharma D, Kumar K. Role of prophylactic autologous bone marrow aspirate injection in accelerating union and rehabilitation in long bone fractures in surgically unfit patients. *Int. J. Orthop. Sci* 2017;3:278-81.
29. Sahu RL. Percutaneous autogenous bone marrow injection for delayed union or non-union of long bone fractures after internal fixation. *Rev Bras Ortop* 2017;53:668-73.
30. Subash Y. Our experience with percutaneous autologous bone marrow injection in the management of delayed and nonunion of long bone fractures. *Int J Res Orthop* 2018;4:291-5.
31. Wu J, Guo H, Liu x, Li M, Cao Y, Qu X et al. Percutaneous autologous bone marrow transplantation for the treatment of delayed union of limb bone in children. *Ther Clin Risk Manag* 2018;14:219–24.
32. Charles M, James D, Margaret M, William M, Paul T, Michael D. Rockwood and Green's Fractures in Adults. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2014. p 128.
33. Patton K T, Thibodeau G A. The Human Body in Health & Disease. 7th ed. St. Louis: Mosby; 2018.
34. Standring S. Gray's Anatomy the Anatomical Basis of Clinical Practice. 41st edition. Philadelphia: Elsevier; 2015.

35. Ramachandran M. Basic Orthopaedic Sciences: The Stanmore guide. 2nd edition. Boca Raton: Chapman and Hall; 2018. p 125.
36. Charles M, James D, Margaret M, William M, Paul T, Michael D. Rockwood and Green's Fractures in Adults. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2014. p 115.
37. Ruedi T P, Buckley R E, Moran C G. AO Principles of Fracture Management. 3rd ed. Thieme; 2007.
38. Charles M, James D, Margaret M, William M, Paul T, Michael D. Rockwood and Green's Fractures in Adults. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2014. p116-7.
39. Frederick M A, Canale S T, James H Beaty, Willis C Campbell. Campbell's Operative Orthopaedics. 13th ed. Philadelphia: Elsevier; 2016. p 2675.
40. Charles M, James D, Margaret M, William M, Paul T, Michael D. Rockwood and Green's Fractures in Adults. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2014. p 113-4.
41. Nandra R, Grover L, Porter K. Fracture non-union epidemiology and treatment. Trauma 2015;0:1-9.
42. Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. Injury 2007;38:3-6.
43. Sim R, Liang TS, Tay BK. Autologous marrow injection in the treatment of delayed and non-union in long bones. Singapore Med J 1993;34:412-7.
44. Elsattar TA, Alseedy AI, Khalil AAE. Bone marrow injection in treatment of long bone nonunion. Menoufia Med J 2014;27:632-5.

45. Braly HL, O'Connor DP, Brinker MR. Percutaneous Autologous Bone Marrow Injection in the Treatment of Distal Meta-diaphyseal Tibial Nonunions and Delayed Unions. *J Orthop Trauma* 2013;27:527–33.
46. Wilkins RM, Chimenti BT, Rifkin RM. Percutaneous treatment of long bone nonunions: the use of autologous bone marrow and allograft bone matrix. *Orthopedics* 2003;26:549-54.
47. Upadhyay S, Varma HS, Yadav V. Percutaneous autologous stem cell enriched marrow concentrate injection for treatment of cases of impaired fracture healing with implant in situ: A cost-effective approach in present Indian scenario. *J Orthop Allied Sci* 2016;4:18-29.
48. Abdel-Ghany M. Bone marrow injection for treatment of infected non-union. Conference paper 2007.
49. Reynders-FP, Reynders-FC, Reynders-FN, Jose SD, Illes T. Intramedullary Injection of Processed Autologous Bone Marrow in the Treatment of Cases with Impaired Bone Healing Long Bones. *Nov Tech Arthritis Bone Res* 2018;2:555-94.
50. Hernigou P, Pognard A, Manicom O, Mathieu G, Rouard H. The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone. *J Bone Joint Surg Br* 2005;87:896-902.
51. Thua THL, Nguyen DT, Pham DN, Le KL, Le QNB, Nguyen PH et al. Mini-invasive treatment for delayed or non-union: the use of percutaneous autologous bone marrow injection. *Biomed Res Ther* 2015;2:389-95.
52. Sugaya H, Mishima H, Aoto K, Li M, Shimizu Y, Yoshioka T et al. Percutaneous autologous concentrated bone marrow grafting in the treatment for nonunion. *Eur J Orthop Surg Traumatol* 2014;24:671-8.

ANNEXURES

PROFORMA

NAME:

IP NUMBER:

AGE:

DOA:

SEX:

DOP:

ADDRESS:

DOD:

OCCUPATION:

INFORMANT:

HISTORY

- **PRESENTING COMPLAINTS**

1.PAIN

2.SWELLING

3.DEFORMITY

4. RESTRICTION OF MOVEMENTS

- **ASSOCIATED INJURIES**

- **PAST HISTORY**

MODE OF FRACTURE FIXATION PREVIOUSLY

- **INTERNAL FIXATION**

- **EXTERNAL FIXATION**

- **CAST APPLICATION**

- **FAMILY HISTORY**

- **PERSONAL HISTORY**

SOCIOECONOMIC STATUS

DIET

APPETITE &SLEEP

BOWEL/BLADDER

EXAMINATION

- **GENERAL PHYSICAL EXAMINATION**

1. BUILT:

2. NOURISHMENT: WELL MODERATE POOR

3. WEIGHT:

4. PALLOR EDEMA CYANOSIS ICTERUS LYMPHADENOPATHY

5. VITALS

PR:

BP:

RR:

TEMP:

- **SYSTEMIC EXAMINATION**

CVS:

RS:

PA:

CNS:

- **LOCAL EXAMINATION**

SIDE AFFECTED: **R** **L**

SITE OF OLD FRACTURE**PROXIMAL 1/3rd****MIDDLE 1/3rd****DISTAL 1/3rd****THIGH****LEG****DEFORMITY/SINUS****THIGH****LEG****TENDERNESS****THIGH****LEG****ABNORMAL MOBILITY AT FRACTURE SITE****RESTRICTION OF MOVEMENTS****HIP****KNEE****ANKLE****SHORTENING****THIGH****LEG****DISTAL NEUROLOGICAL DEFICITS****YES****NO****DISTAL VASCULAR DEFICITS****YES****NO****INVESTIGATIONS****BLOOD: Haemoglobin****TC****ESR****DC**

Blood urea

HIV

Sodium

- GERY AND

- ATE INJ**

- # AVENUE
- ## G

ANNEXURE-II

PATIENT/ SUBSTITUTE DECISION MAKER INFORMATION SHEET

Title of the study: “EFFICACY OF THE BONE MARROW INJECTION FOR DELAYED UNION OF FRACTURES OF LOWER LIMB”.

This study involves the administration of autologous bone marrow aspirate at the site of delayed union to accelerate bone healing. The objective of this study is to assess the Efficacy of the bone marrow injection for delayed union of fractures of lower limb.

Purpose of the research:

Before opting for the invasive procedure like bone grafting, simple, cost effective and

Type of Research Intervention: This study will involve injection of bone marrow aspirate at delayed union fracture site

Procedures and Protocol: After obtaining the consent, under spinal anaesthesia, bone marrow aspiration is done from the anterior iliac crest and injected at delayed union site under C-arm guidance. One dose of antibiotic pre operatively and one dose post operatively will be given. post procedure follow up will be done at 2, 4 and 6 months both clinically and radiologically using Union scale grading.

Risk and benefit: Bone marrow injection for the delayed union is a proven procedure for delayed union.

Reimbursements: You will not be given money or gifts to take part in this research.

Confidentiality: We will not be sharing the identity of the participant. The information we collect from you will be kept confidential and only researchers involved in this project will have access to it.

Right to Refuse or Withdraw: You do not have to take part in this research if you do not wish to do so and you can refuse to participate.

Whom to Contact: If you have any questions you may ask us now or later, even after the study has started, you may contact the following persons:

For more information:

- | | |
|----------------------------------|----------------------------------|
| 1. Dr Madamanchi harsha | 2. Dr Nazeer B S |
| Post Graduate Student | Professor |
| Department of Orthopaedics | Department of Orthopaedics |
| Sri Devaraj Urs Medical College, | Sri Devaraj Urs Medical College, |
| Tamaka, Kolar. | Tamaka, Kolar. |

Mobile – 9494107272

Email – harshavignan1@gmail.com

ANNEXURE-III

INFORMED CONSENT FORM

Name of the Institution: Sri Devaraj Urs Academy of Higher Education and Research

Title of the study: “EFFICACY OF BONE MARROW INJECTION IN DELAYED UNION OF FRACTURES OF LOWER LIMB”.

Name of the Investigators: **Dr. Madamanchi Harsha and Dr. Nazeer B S**

Name of Participant / Patients substitute decision maker:

The following has been explained to me:

1. Patient will be evaluated prior to surgery.
2. The study requires routine investigations like complete haemogram, bleeding time, clotting time, random blood sugar, serum electrolytes, renal function tests, chest radiograph and ECG. No special investigations are needed.
3. After consent, under spinal anaesthesia every patient will receive bone marrow aspirate from the anterior iliac crest as per the requirement at the delayed union site.
4. Patient will be given stat dose of antibiotic pre post operatively
5. Every patient will be followed up at every 2,4,6 months both clinically and radiologically using Union scale grading score.

I give my consent to participate in the foresaid study and authorize the collection and disclosure of my personal information as outlined in this consent form.

I understand that I remain free to withdraw from this study at any time and this will not change my future care. I have read and received a copy of this consent form.

I understand the information provided in this document and I have had the opportunity to ask questions.

Participant/ substitute decision maker's signature:

Date:

Witness name and signature:

1. Date:

2. Date:

Investigator obtaining consent form & his/her signature:

Date:

For any clarification you are free to contact the Investigator:

Principal Investigators

Dr. Madamanchi Harsha (Mobile 9494107272)

Dr. Nazeer B S

A copy of this informed consent form has been provided to the participant.

KEY TO MASTER CHART

M	Male
F	Female
A	Proximal third fracture
B	Middle third fracture
C	Distal third fracture
IMIL	Intramedullary interlocking nail
LCP	Locking compression plate
PFN	Proximal femoral nail
S.S WIRE	Stainless steel wire
T2DM	Type 2 diabetes mellitus
HTN	Hypertension

MASTER CHART

SL NO	Age	Sex	UHID No	Bone	Site	Type	Pattern	Initial Treatment	Time (months)	Quantity (mL)	Callus	Clinical scoring		Radiological Scoring	Total Score	Union (months)	Comorbid conditions	Complications	Outcome
												Mobility	Tenderness						
1	35	M	447914	Femur	C	Closed	Comminuted	IMIL	3	80	2	3	1	3	7	4	Nil	Nil	Good
2	35	M	447914	Tibia	B	Closed	Segmental	IMIL	3	60	2	3	1	3	7	4	Nil	Nil	Good
3	45	F	274556	Femur	A	Closed	Comminuted	PFN	4	80	4	3	1	3	7	6	Nil	Nil	Good
4	45	M	425974	Femur	C	Closed	Comminuted	IMIL	3.5	80	2	3	1	3	7	4	Nil	Nil	Good
5	55	M	336587	Femur	B	Closed	Transverse	IMIL	4.5	80	4	3	1	3	7	6	T2DM	Nil	Good
6	25	M	223307	Femur	B	Closed	Transverse	IMIL	4	60	2	3	1	3	7	4	Nil	Nil	Good
7	37	M	374249	Femur	B	Closed	Oblique	IMIL	3.5	80	2	3	1	3	7	2	Nil	Nil	Good
8	50	M	578258	Tibia	C	Open	Comminuted	Supracutaneous LCP	3	60	4	3	1	3	7	6	Nil	Nil	Good
9	37	F	291941	Femur	B	Closed	Comminuted	IMIL + S.S. wiring	4	80	2	3	1	3	7	4	Nil	Nil	Good
10	45	M	454430	Tibia	C	Closed	Transverse	IMIL	3.5	60	2	3	1	3	7	2	Nil	Nil	Good
11	33	M	322525	Tibia	B	Closed	Comminuted	LCP	4	60	2	3	1	3	7	4	Nil	Nil	Good
12	45	M	360779	Femur	B	Closed	Transverse	IMIL	4	80	4	3	0	2	5	6	Nil	Nil	Fair
13	25	M	384663	Femur	C	Closed	Comminuted	IMIL	3.5	60	2	3	1	3	7	2	Nil	Nil	Good
14	27	M	519498	Both bone leg	C	Open	Comminuted	Supracutaneous LCP	3	60	4	3	1	3	7	6	Nil	Nil	Good
15	44	M	472281	Femur	A	Closed	Comminuted	PFN	4.5	80	6	3	0	1	4	Absent	Nil	Nil	Poor

MASTER CHART

16	25	M	463412	Femur	B	Closed	Comminuted	IMIL	3	80	2	3	1	3	7	4	Nil	Nil	Good
17	70	M	294175	Both bone leg	C	Open	Comminuted	Supracutaneous LCP	4	80	4	3	1	3	7	6	T2DM	Nil	Good
																	HTN		
18	28	M	523363	Tibia	B	Closed	Segmental	IMIL	3	60	2	3	1	3	7	4	Nil	Nil	Good
19	28	M	523363	Tibia	B	Closed	Oblique	IMIL	3	60	2	3	1	3	7	2	Nil	Nil	Good
20	45	M	266026	Tibia	C	Open	Comminuted	IMIL	5	60	6	3	0	1	4	Absent	Smoker	Nil	Poor
21	24	M	566496	Femur	B	Closed	Spiral	IMIL	4.5	80	2	3	1	3	7	6	Nil	Nil	Good
22	63	F	333935	Tibia	A	Closed	Comminuted	LCP	3.5	60	4	3	1	3	7	6	T2DM	Nil	Good
																	HTN		
23	80	M	428950	Femur IT and	B	Closed	Comminuted IT	Long PFN	3.5	80	2	3	1	3	7	4	HTN	Nil	Good
24	48	M	416584	Both bones leg	B	Closed	Comminuted	IMIL	3.5	40	2	3	1	3	7	2	T2DM	Nil	Good
25	60	M	292442	Tibia	B	Closed	Comminuted	IMIL	4	60	2	3	1	3	7	4	Nil	Nil	Good
26	30	M	422645	Tibia	B	Closed	Oblique	IMIL	3	60	2	3	1	3	7	4	Nil	Nil	Good
27	26	M	509880	Tibia	C	Open	Comminuted	Supracutaneous LCP	4.5	60	4	3	1	3	7	6	Nil	Nil	Good
28	30	M	262398	Tibia	C	Open	Comminuted	Supracutaneous LCP	4.5	60	4	3	1	3	7	6	Nil	Nil	Good
29	23	M	481881	Femur	C	Closed	Comminuted	IMIL	4	40	2	3	1	3	7	6	Nil	Nil	Good
30	23	M	481881	Tibia	C	Open	Comminuted	Illizarov	4	60	4	3	1	3	7	6	Nil	Nil	Good