PROSPECTIVE ANALYSIS OF OUTCOME IN THE MANAGEMENT OF POST-TRAUMATIC BONE DEFECTS IN TIBIA BY MASQUELET TECHNIQUE

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MADRAS MEDICAL COLLEGE INSTITUTE OF ORTHOPAEDICS AND TRAUMATOLOGY RAJIV GANDHI GOVERNMENT GENERAL HOSPITAL, CHENNAI-3.

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CERTIFICATE

This is to certify that this dissertation titled "*PROSPECTIVE ANALYSIS OF OUTCOME IN THE MANAGEMENT OF POST-TRAUMATIC BONE DEFECTS IN TIBIA BY MASQUELET TECHNIQUE* " is a bonafide record of work done by **DR.P.GOWTHAM RAM**, during the period of his Post graduate study from Aug 2016 to September 2018 under guidance and supervision in the Institute of Orthopaedics and Traumatology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfilment of the requirement for **M.S.ORTHOPAEDIC SURGERY** degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2019.

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DECLARATION

I declare that the dissertation entitled "*Prospective analysis of outcome in the management of post-traumatic bone defects in tibia by Masquelet technique* " submitted by me for the degree of M.S.Orthopaedics is the record work carried out by me during the period of **August 2016 to September 2018** under the guidance of **PROF.N.DEEN MUHAMMAD ISMAIL., M.S.Ortho., D.Ortho.,** Director and Professor of Orthopaedics, Institute of Orthopaedics and Traumatology, Madras Medical College, Chennai.

This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.S.ORTHOPAEDICS (BRANCH-II) examination to be held in April 2019.

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INTRODUCTION

Bone defects result from compound fractures of long bones mostly due to high velocity road traffic accidents. Incidence of open fractures is about 4.5million per year in India¹.

Early 20th century when there was extensive soft tissue damage, bone loss and contamination at the fracture site, the patients had to undergo an amputation as a life saving measure². Bone loss may occur from fragment extrusion at the time of injury or during debridement of an open fracture when devitalised bone segments are removed, thereby creating a bone defect. The tibia is the most common site of bone loss because it is subcutaneous and prone to open fracture and fragment extrusion³. Bone defects more than 2 cm are unlikely to unite spontaneously following skeletal stabilisation alone³.

Management of bone defects is very challenging. Small size defects of less than 2cm can be easily managed by non-vascularised cancellous bone grafting. Autograft is considered to be the standard bone defect filler for treating bone loss of less than 2cm as it has osteoconductive, osteoinductive and osteogenic properties at the site of injury.

Before the advent of bone transport procedures for large bone defects, shortening of the limb or a segment of fibula was used to manage

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the defect in tibia⁴. But since fibula shares only 15% of the weight- bearing load, this technique did not give good functional results⁵.

Bone transport is a widely accepted technique for the management of very large bone defects .The Ilizarov technique is the one commonly used to manage intermediate and large bone defects. The distraction osteogenesis technique using Ilizarov frame requires specialized training, steep learning curve and is usually done in tertiary centres where other specialities such as vascular, plastic surgery departments are available⁶.

The other options available for large bone defects are vascularised bone grafting from rib, fibula or iliac crest which is a highly demanding technique, where there is a need for microsurgical anastomoses procedures⁷. The major drawback of this procedure is graft site morbidity and pedicle length limitations⁸. These treatment procedures are time consuming, leads to disuse muscle wasting of involved limb, patient undergoing mental stress and economic loss⁹.

The *Masquelet* technique assures a simple, alternative and a viable management for large bone defects up to 25cm as it can be done in a health centre without much facilities and expertise¹⁰. This technique was invented accidentally as they were not sure about the membrane formation around the spacer at the defect site. But the newly formed membrane was not removed, to limit blood loss and surgical devitalisation. As the membrane

was retained it benefited in increased bone healing through neovascularisation and graft resorption prevention^{2,11}. The induced membrane technique¹¹ was developed in 1986 to reconstruct large defects with autografts. In this technique a cement spacer is placed in a posttraumatic bone defect.

Its presence helps in preventing fibrous ingrowth into the bone defect, and induces the formation of biomembrane around it. Bone graft placed within this chamber of induced membrane incorporates into functioning bone. The membrane prevents cancellous bone resorption and promotes cancellous bone corticalization. The ideal period to perform bone grafting is after 1 month of cement spacer application. Even after the failure with this procedure bone transport techniques are possible as mentioned before.

Our study was intended to review the clinical, radiological and functional outcome of bone defects in tibia managed by Masquelet technique.

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AIMS AND OBJECTIVES

Management of bone defects in tibia by Masquelet technique in cases admitted in Institute of Orthopaedics and Traumatology, Rajiv Gandhi Government General Hospital over a period of 24 months from August 2016 to August 2018 after obtaining institute ethical committee approval.

This is a prospective study of efficacy of Masquelet technique in the management of compound fractures of tibia associated with bone defects upto 5 cm with or without infection and with or without soft tissue loss.

REVIEW OF LITERATURE

Management of the bone defects in long bone compound fractures is a challenging problem.

Court-Brown and colleagues found in their study of 33 cases, that more than 2 cm bone loss and 50% of tibial circumference had a 100% incidence of nonunion and frequent need for bone grafting after debridement of devitalised bone. The limitations of bone grafting alone techniques are graft resorption even if the defect site is vascularised.

Before the advent of bone transport procedures, shortening of the limb or a segment of fibula was used to manage defects in tibia⁵.

In the 1950s, *Ilizarov*⁶ developed a method of salvaging limb after severe bone loss by bone transport and distraction osteogenesis technique. But bone transport is a lengthy procedure and challenging experience for the patient. Problems associated with this method are non union at the docking site, pin site infection, high rate of revision surgeries (23.8%)

In 1978, *Ian taylor*¹² described about the use of vascularised fibula graft for reconstructing long bone defects. The procedure requires a lot of pre planning such as recipient vessels coordination, pedicle length, and method of fixation. The ideal candidates are patients for whom

conventional bone grafting technique has failed, or those who have bone defects >6 cm. But only experienced surgeons do it in tertiary centres.

In 1986, *A.C Masquelet¹¹* developed a novel technique for large bone defects based on formation of biomembrane. This technique allows reconstruction of wide defects even if the defect site has been infected or irradiated. Using this simple technique the French surgeon has managed bone defects up to 25 cm in length.

Masquelet and Begue¹ initially retained the membrane at the time of second procedure only to prevent blood loss since the membrane was hypervascular.

Pelissier et al¹³ from their study reported that the biomembrane contain growth factors, osteoinductive factors and BMP-2, therby helping in bone regeneration.

Gerber et al^{14} in their study on sheep model concluded that polylactide membrane protects the cancellous graft against resorption and permits rapid and stable regeneration of bone defect.

*Viateau and his colleagues*¹⁵ tried this technique in sheep model and observed that induced membrane alone cannot heal the defects and bone grafts have to be placed inside the membrane to obtain bony union.

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*Karger et al*¹⁶ reported their experience with Masquelet technique for 84 posttraumatic bone defect cases with a 90% union rate. For the stage II procedure, they placed grafts between the tibial defects such that it pressed upon the fibula to reduce the union time.

T.*M.* Wong and his colleague¹⁷ treated 9 patients with this technique and achieved 100% success rate. They used antibiotic coated cement spacer either 2gm vancomycin or gentamycin per 40 gm cement powder.

*Biau et al*¹⁸ managed successfully a 16 cm defect in femur following tumor resection by this technique stabilized with an interlocking nail in a 12 year old child.

*McCall et al*¹⁹ performed study on 21 patients with large segmental bone defects and used Reamer –Irrigator – Aspirator(RIA) system for harvesting bone graft. They concluded that the RIA technique helped in obtaining large quantity of bone grafts and donor site morbidity being minimal.

Huffman et al^{20} showed that this technique can be successfully used to manage post traumatic midfoot bone defect in a patient who sustained ballistic injuries. They used RIA system to harvest bone graft. *El-Alfy and Ali*²¹ in their study on segmental skeletal defects of 17 patients found that the time taken for graft maturation and consolidation is relatively long.

*Gupta et al*²² In their study on 9 patients with gap non-union of the tibia, observed that this technique improved the bone union and thus it is useful and simplified method for developing countries.

Mario Ronga et al^{23} concluded in their case report that Masquelet technique is the effective way of treating severe acute tibial bone loss.

*Apard et al*²⁴in their study of 12 patients with post traumatic tibial bone loss, using intramedullary nail as stabilisation device arrived at a conclusion that early weight bearing was possible , when to compared to the usage of external fixator. The intramedullary nail also helped in proper bone alignment.

Olesan et a^{25} in their study on eight patients with posttraumatic bone defects managed by Masquelet technique found out that using nail for skeletal stabilization allows for bone graft stimulation axially and secondary stress fractures prevention.

 $Pouton^{26}$ in his study used fasciocutaneous flap as one stage procedure to cover lower leg soft tissue defects.

*Franken et al*²⁷ managed 52 patients with post-traumatic soft tissue defect of lower leg by local , musculocutaneous flap in early stage of trauma and came up with good outcome.

*Bhattacharya et al*²⁸ managed post traumatic soft tissue leg defect in 69 patients with transposition flap either on proximally or distally based perforators and showed good outcome with flap loss only in 4 patients.

SURGICAL ANATOMY OF LEG

OSTEOLOGY

There are two bones in the bony framework of leg. The main weight bearing bone is tibia which carries 85% of load. The cortical thickness of tibial diaphysis is more than metphysis of the proximal and distal tibia. It is the second largest bone next to femur. The fibula is located lateral and posterior to the tibia and they are connected to each other by interosseous membrane

Tibia has three surfaces which are medial, lateral and posterior surfaces. The medial surface being subcutaneous gets more commonly exposed during injury while the lateral and posterior surfaces are well covered by muscles.



TIBIA AND FIBULA ANATOMY²⁹

PROXIMAL TIBIA

The proximal tibia expands through the transverse plane, with the medial and lateral condyle which are flattened at the horizontal plane. The medial condyle is larger than the lateral condyle and is better supported over the shaft. The knee joint is formed by the upper surfaces of the condyles which articulate with the femur. The medial tibio femoral joint forms the major weight bearing part of the knee joint. The intercondylar area separates the medial condyle from the lateral condyle. There are rough depressions for the attachment of the cruciate ligaments and the menisci at the front and back of the intercondylar eminence. There is a horizontal groove for the semimembranosus muscle attachment in the posterior surface of the medial condyle. Patellar ligament lies attached to the tibial tuberosity, which is a bony prominence in the front of the upper part of tibia, 2.5cm distal to the knee joint line.

At the level of tibial tuberosity lies the head of fibula posterolaterally , which paves way to identify the common fibula nerve (peroneal nerve) , that winds around the neck of fibula.

PROXIMAL TIBIA CROSS-SECTION²⁹



MIDDLE TIBIA

The triangular tibial shaft is bordered by the anterior, medial and lateral which is commonly known as the "*Interosseous border*". The deep facia of the leg gets affixed to the anterior border. The tibial collateral ligament of the knee joint gets affixed to the upper part of the medial border. The soleus and the flexor digitorum longus muscles originate from the middle-third of the medial border. The interosseous membrane gets affixed to the lateral border. The tendons of the Sartorius, gracilis and the semitendinosus covers the upper part of the medial surface, the rest of the medial surface remains subcutaneous. The tibialis anterior muscle originates from the groove in the upper one-third of the lateral surface of tibia. The tendons of the tibialis anterior, extensor hallucis longus and extensor digitorum longus arranged in this order from medial to lateral, which covers the lower one-third of the lateral surface. A part of the soleus, flexor digitorum longus and tibialis posterior muscle originates from the upper part of the posterior surface.

MID TIBIA CROSS-SECTION²⁹



LOWER THIRD OF TIBIA

The ankle joint is formed by the lower one- third of the tibia, fibula and the talus. The lower articular surface is smooth and quadrilateral and articulates with the talus. The medial malleolus which is pyramidal process forms the medial surface. The tendons of the extensor muscles cover the anterior surface.

The inferior interosseous ligament gets affixed to a rough depression on the lateral surface. Paving a passage for the tendon of flexor hallucis longus, the posterior surface is a shallow grove.

LOWER TIBIA CROSS-SECTION²⁹



JOINTS FORMED BY TIBIA

The tibia form a part of four joints in the leg namely knee joint, superior and inferior tibiofibular joint and ankle joint.

BLOOD SUPPLY

The tibia has got medullary and a periosteal blood supply. The posterior tibial artery gives a branch in the proximal part of tibia to form the nutrient artery which supplies a rich network of medullary vessels. The nutrient artery has two ascending branches and one descending branch which divide into many smaller branches to the endosteal surface. When soft tissue damage occurs at the time of injury, the periosteal blood supply from the anterior tibial artery branches gets disrupted.

BLOOD SUPPLY OF TIBIA²⁹



OSSIFICATION CENTERS OF TIBIA

It gets ossified from three centers; two secondary centers for both epiphysis and a primary center for the diaphysis. The upper epiphysis center appears before birth at close to 34 weeks gestation while the lower epiphysis centre appears in the 2^{nd} year of life. The upper epiphysis fusion with tibial shaft occurs at about twenty years while the lower epiphysis fusion occurs at eighteen years.

FIBULA

The Fibula forms only 15% of the weight bearing load. The Fibula articulates with the lower aspect of the lateral condyle of the proximal tibia and in the lower end gets affixed to the tibia by a fibrous joint. Fibula head is quadrangle shaped with an articulating surface for tibia on the medial side. On the lateral side there are two attachments - Biceps femoris tendon and Fibular collateral ligament of the knee joint. There are four borders for Fibula body – the antero-lateral, the antero-medial, the postero-lateral and the postero-medial.

BLOOD SUPPLY TO THE FIBULA

The Fibular artery gives of the main nutrient vessel which supplies middle-third of Fibula shaft. The anterior tibial artery branch supplies the proximal head and epiphysis of the fibula.

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OSSIFICATION CENTRES FOR FIBULA

The Fibula is developed from three centres – two for the epiphysis and one

for the shaft. Ossification starts from second year of life in the lower end and starts from fourth year of life at the upper end. The lower epiphysis gets fused with the body first at the 20^{th} year and followed by the upper epiphysis fusion at the 25^{th} year.

INTEROSSEOUS MEMBRANE

It is a broad and fibrous sheet of connective tissue that separates Tibia and Fibula. The fibres are predominantly oblique and most descend laterally.

Through the opening in the proximal end of the membrane, the anterior tibial artery passes and distally the fibular artery perforates. It becomes continuous with the introsseous ligament of the distal tibiofibular joint. Apart from linking the tibia and fibula, it additionally provides enough space for attachment of muscles.

INTEROSSEOUS MEMBRANE²⁹



COMPARTMENTS

The leg is consists of four compartments which are the anterior compartment, Lateral compartment, Superficial Posterior compartment and Deep posterior compartment.

COMPARTMENTS OF THE LEG²⁹



ANTERIOR COMPARTMENT

Anterior compartment muscles are tibialis anterior, Extensor hallucis longus, Extensor digitorum longus, and peroneus tertius, that are ankle dorsiflexors. It contains anterior tibial artery and nerve.

LATERAL COMPARTMENT

The lateral compartment muscles are peroneus longus and peroneus brevis, which evert the foot. There are no major vessels in the lateral compartment. It contains the common peroneal nerve which winds around the neck of fibula and is at risk for injury during fasciotomy.

POSTERIOR COMPARTMENT

The superficial posterior compartment muscles are gastronemius, soleus and plantaris muscle. It contains short saphenous vein, Peroneal communicating branch of the common peroneal nerve, Medial cutaneous nerve of the calf and sural nerve

The deep posterior compartment muscles are Popliteus, Flexor digitorum longus, Flexor hallucis longus and Tibialis posterior. It contains posterior tibial artery and nerve.

TIBIA MUSCLE ATTACHMENTS²⁹



TYPES OF BONE HEALING

Endochondral ossification

The resident chondrocytes mature and are replaced by osteoblasts as vessels invade the cartilage callus. This is associated with motion at the fracture site as in cast treatment or fractures with incomplete stability.

Direct bone healing (Intramembranous ossification)

Attempt to re-establish an anatomically correct and biomechanically competent lamellar bone structure without a cartilaginous intermediate. There is direct deposition of osteoid by mesenchymal cells. There will not be visible callus formation and fracture unites by passage of osteoclast led cutting cones followed by osteoblasts that restore the cortical bone lamellar architecture.



CUTTING CONES

Healing process dependent on gap size

<0.01mm – contact healing

0.01mm – 1mm – gap healing

Intramembranous ossification starts at outer end and grows inward



Endochondral ossification starts from the inner edges of the fracture site to

its middle



STAGES OF INDIRECT BONE HEALING

Hematoma formation

Hematoma is composed of debris from surrounding marrow, periosteum and muscle, platelets, RBCs, immune cells from torn blood vessels. The fracture hematoma contains IL-6, IL-8 and abundant amount of tumor necrosis factor –alpha(TNF-a) which help in repair process. It becomes template for callus formation.IL-6 stimulates angiogenesis and differentiation of osteoblasts to osteoclasts.

HEMATOMA FORMATION



Soft callus formation

This stage begins by around 3 weeks where cartilage or osteoid becomes the predominant tissue in the callus replacing hematoma and fibrous tissue. The cartilage contains fibrin rich granulation tissue. Type 1 and type II collagen restores mechanical stability to the fracture ends. It allows intermembranous ossification to start subperiosteally at the fracture site.

SOFT CALLUS



Hard callus formation

There is resorption of soft callus which is replaced by hard callus through mineralization. The conversion of cartilage to a calcified matrix occurs in this stage. The dominant cells are osteoblast and osteoclast. The osteoblast deposits woven bone which further strengthens the callus. This stage gives more stability to the fracture site and starts to allow endochondral ossification.

HARD CALLUS



Revascularization stage

This stage is important for promotion of fracture healing. Promoted through 2 pathways

- 1. Angiopoietin pathway vessel in-growth from surrounding vessels
- VEGF pathway main vascularization regulator, forms new vessels that proliferate within the callus (vasculogenesis)

Remodelling stage

This is an ongoing process that may take years to complete. Hard callus provides biomechanical stability but does not return normal bone biomechanic properties. All woven bone becomes lamellar bone at the end. There is a balance between hard callus resorption by osteoclasts and lamellar bone lay down by osteoblast. Remodelling is influenced by Wolff's law which states that strengthening of the internal and cortical architecture of bone occurs in response to the forces or demands placed on it

Bone graft healing- creeping substitution

Creeping substitution, the process of bone remodelling by which slow complete resorption of the graft by osteoclasts occurs with simultaneous osteoblastic new bone formation by creation of new vascular channels resulting in new haversian systems. This is the method by which strong cortical bone is formed from graft. Vascular ingrowth into the graft gradually occurs as a result of end-end anastomoses of host vessels with those of the graft, with resorption and replacement of necrotic bone graft with viable bone. Creeping substitution starts at the graft-host juncture, then moves along the cortical graft axis.

STAGES OF MASQUELET TECHNIQUE

FIRST STAGE

The first stage includes radical debridement of the defect, skeletal stabilization and insertion of an antibiotic coated polymethylmethacrylate (PMMA) cement spacer into the bony defect. This spacer has a main role in preventing the ingrowth of fibrous tissue and a biological role by inducing a foreign body reaction surrounding the spacer which will support the subsequent bone grafting procedure.



Intraoperative photograph showing cement spacer application between the bone defect

BIOMEMBRANE

Experimental study at the AO Development Institute of Davos found out that the membrane prevented the resorption of the contained cancellous bone graft. The induced membrane has a appearance similar to fascia and is highly vascularised and it has got two parts. Masquelet and Begue¹¹ in their study found out that smooth surface produced by PMMA spacer led to smooth lining of the inner part of the membrane which is like synovium and the outer part consist of fibroblast, collagen and myofibroblasts. The fibroblast present in the outer part originates from the mesenchymal tissue and plays a significant role in laying down extracellular matrix and tissue healing. The biomembrane contains abundant number of osteoprogenitor cells and also secretes growth factors (such as BMP-2, VEGF and TGFbeta1). Bone morphogenetic protein-2 reaches its peak value by 4 weeks. Vascular endothelial growth factor (VEGF) present in biomembane plays an important role in remodelling of the bone¹⁷. These factors help in bone formation by stimulating bone marrow cell proliferation and differentiation to osteoblastic lineage, bone remodelling and metabolism. The membrane is 0.5 to 1mm thick. This pseudomembrane is impermeable, acts as a barrier to outward growth factors diffusion and has osteoinductive factors. The membrane being a source of stem cells and vascular cells supports revascularization and bony integration.



Intra-operative photograph showing vascular induced membrane



Peroperative photograph demonstrating the mature biomembrane of 1mm thickness.
SECOND STAGE

The second stage after 4-8 weeks involves removal of the cement spacer while preserving the biofilm. An interval of 4-6 weeks is required for development and maturation of a biologically active membrane. *Aho and his colleagues*³² studied that one month old membrane has got high osteogenic factors as compared to a two month-old membrane. The induced membrane is incised carefully and the cement spacer is taken out with an osteotome taking care not to break the bone ends or cause damage to the biofilm. The intramedullary canal is curetted and debrided and the bone ends should be decorticated. All non-viable tissues are excised.



Per-operative photograph showing cement spacer removal during stage II surgery

The gap is filled with fresh cancellous autograft and the membrane is sutured with absorbable suture so that the grafts are contained inside the defect. If autograft is not adequate enough , demineralised allograft can used in a ratio of 1:3.The biomembrane wrapped around the cancellous bone has a protective effect against bone resorption. The cancellous bone is capable of forming bone even without stress to the bone.



Intra-operative photograph showing stage II surgery, filling the defect with morcellized iliac crest autografts

CLASSIFICATION

GUSTILO ANDERSON'S CLASSIFICATION OF COMPOUND FRACTURES

Туре	Wound	Level of contamination	Soft tissue injury	Bone injury
Ι	<1cm	clean	Minimal	Simple, minimal communition
II	>1cm	Moderate	Some muscle damage	Moderate communition
IIIA	>10cm	High	Severe crush injury	Segmental or severe communition Soft tissue coverage of bone possible
IIIB	>10 cm	High	Very severe soft tissue cover loss	Periosteal stripping and bone exposure Bone cover poor; Requires soft tissue reconstruction
IIIC	Usually>10cm	High	Open fracture with vascular injury requiring repair regardless of the size of the wound	Usually requires soft tissue reconstruction

CLASSIFICATION OF BONE DEFECTS

Significant bone loss occurs only in a small proportion of all fractures. The majority of these fractures are Gustilo grade IIIB injuries. The tibia is the most common location of bone loss after fracture because of its subcutaneous position. The most common site of involvement is the diaphysis region of the bone. The bone defects are classified according to its anatomical location such as diaphyseal , metaphyseal or articular. The defect extent is measured in term of bone length.

Karger's classification of bone defects¹⁶:

Type I (<2cm)

Type II (2-5 cm)

III (5-10 cm) and

IV (>10 cm)

In our study we have taken into account only type II bone defects

Bone defects classification according to $Azi \ et \ al^{30}$:

Complete- if all four cortices are missing or

Partial- if atleast one area of cortical continuity is present between proximal and distal segments.

MANAGEMENT

INITIAL MANAGEMENT

Preoperatively soft tissue and bony defect must be assessed for future reconstructive surgeries.



Clinical photograph showing exposed bone with severe soft tissue contamination

WOUND DEBRIDEMENT

In operating room patient in supine position, prepare the local parts and allow for a wide exposure of the affected extremity.

Start wound lavage with at least 9 litres of saline to clear debris, blood clots and nonviable tissues then do debridement starting at the skin and remove all devitalized and contaminated soft tissues. Excise all devitalized bone fragments without soft tissue attachments, identify the fracture ends and apply an external fixator. Debridement should extend until viable bony ends are seen, which is determined peroperatively using the "paprika sign" (punctate bleeding after drilling with a 2.5 mm drill bit)



Photograph showing wound status after debridment and fit for soft tissue cover



Per-operative photograph demonstrating defect size measurement after excising all infected and necrotic bone.

EXTERNAL FIXATOR APPLICATION

Skeletal stabilization is started after discarding the instruments utilized for wound debridement. Before inserting pins pre-drilling must be done to minimize thermal necrosis. Place pins through the intact soft tissues and perpendicular to long axis of the bone through small longitudinal incisions. Use pins size ranging from 4.5-6.0mm diameter. The pin size diameter should be less than one third of bone diameter to prevent fracture. A minimum of two pins per fragment should be placed. In femur pins are inserted anterolaterally anterior to iliotibial tract whereas in tibia pins are inserted anterior or antermedially along the subcutaneous border of tibia.

Frame rigidity can be increased by connecting the pins to the two AO rods on the top of each other.



Immediate post op x ray after debridement of devitalized bone segments and skeletal stabilization .

Technique

Usually the most proximal and distal pins are inserted first. Reduce the fracture using bone clamps placed through the open wound. The inner pins should be placed at least 1cm away from the fracture site. Then connect the pin clamps to the rod and tighten all connections.



Intraoperative photograph showing bone defect with cement spacer in-situ

Antibiotic cement (PMMA) spacer application

Higher viscosity cement elude antibiotics more effectively than low viscosity cement. The cement spacer provides structural support. The amount of antibiotic to be used is controversial. Our preference is 4g of vancomycin powder per 40 g cement package. A metallic strainer is used to crush the antibiotic powder. Then antibiotic powder is mixed with cement powder. The monomer is added to the powder and then mixed with spoon. When the cement becomes doughy, it is then rolled by hands to create a rectangular or square shape approximately same length as the bone defect.



Intraoperative photograph demonstrating cement spacer preparation

SOFT TISSUE MANAGEMENT

In patients with skin loss over the fracture site exposing bone, flap cover is planned along with stage I Masquelet technique. Wounds with good muscle bed and not exposing the bone are covered by split skin grafting technique. When the subcutaneous part of tibia is exposed it is usually covered by rotational flap. In our cases patient underwent rotational fasciocutaneous flap with either proximal or distal base. For soft tissue defects around the proximal tibia rotational gastrocnemius flap is done as medial gastrocnemius has good blood supply.

Types of fasciocutaneous flap

- 1. Antegrade (superiorly based)
- 2. Retrograde (inferiorly based)
- 3. Islanded perforator based flaps
- 4. Deepithelized turn over flaps
- 5. Fasciocutaneous with adipofascial extension

Preoperatively all flaps are marked after planning in reverse.

After raising adequate length of the flap, it is transposed to the defect site and is sutured in a single layer with 3 '0' Ethilon after placing a suction drain beneath the flap. Plaster of paris slab immobilization is done if external fixator stability is not sufficient.



Photograph showing inferomedially based transposition fasciocutaneous flap done for the posttraumatic soft tissue defect in lower leg.

Proximal 1/3rd leg

Superiorly based fasciocutaneous flap based on the perforators of the anterior tibial or posterior tibial or peroneal artery.

Middle third leg

Superiorly based rotational flaps on the peroneal perforators or a inferiorly based flap on the perforators of the lower posterior tibial artery.



Photograph showing superiorly based transposition flap done for middle third soft tissue defect in leg.

Distal third leg

Distally based or cross leg fasciocutaneous flap or Posterolateral malleolar

flap or Reverse sural artery flap can be used.

STAGE II Bone grafting technique

It is performed after 4-6 weeks only after evaluating for clinical signs of infection by serial monitoring of ESR, CRP and pin tract infection. Patient in supine position, incision is made along the crest starting 2cm from the ASIS extending laterally depending on the amount of graft needed.

We usually harvest tricortical graft stripping muscles from both inner and outer tables of the ilium. The graft is then chopped into pieces.



METHODS AND MATERIALS

Place of study	:	Institute of Orthopaedics and Traumatology(IOT),		
		Rajiv Gandhi Government General Hospital,		
		Madras medical college, Chennai		
Type of study	:	Prospective study		
Sample size	:	20		
Period of study	:	2016 -2018		

The study was started after obtaining approval from IOT ethical committee and Madras medical college ethical committee. All patients signed an informed consent form before participating in the study.

INCLUSION CRITERIA

All patients in the age group of 18-60 years with posttraumatic bone defect (<5cm) of tibia with or without infection and with or without accompanying soft tissue defect

All patients giving consent for the study

EXCLUSION CRITERIA

- 1. Age 18< >60
- 2. Patients with neurovascular injury to the limb.
- 3. Defect size > 5 cm
- 4. Patients with bone defect of etiology other than trauma.
- 5. Persistent infection after stage I and
- 6. Patients not giving consent for the study

PREOPERATIVE EVALUATION

All patients were subjected to the following investigations

- Detailed clinical examination and pre op clinical photos
- X-RAYS of the affected extremity both AP and LAT view
- Complete haemogram
- Renal function tests
- Pus culture and sensitivity
- ESR and CRP

IMPLANTS AND INSTRUMENTS

In most of the cases AO external fixators and limb reconstruction system were used. Bone cement and heat stable antibiotics such as vancomycin were used.

AO EXTERNAL FIXATOR

It includes

- 1. AO Rod
- 2. Universal clamp
- 3. Tube –tube clamp
- 4. 4.5mm schanz pin
- 5. 5.5 mm schanz pin
- 6. T clamp



Photograph showing external fixator instruments.



Photograph showing Polymethylmethracylate (PMMA) cement powder and monomer

LIMB RECONSTRUCTION SYSTEM (LRS)SET



POSTOPERATIVE PROTOCOL

- Drain is removed on the 2ndpost operative day with collection being less than 20 ml for a period of 8 hours. Post operatively intravenous antibiotic for 3weeks and oral antibiotics for another 3 weeks based on culture and sensitivity.
- 2. Suture removal done on 12thpost operative day
- Second stage II procedure is done after control of infection by serial monitoring of ESR and CRP

- 4. Physiotherapy of the adjacent joints is started from the next postoperative day and non weight bearing walking with walker
- Non weight bearing period until radiological union (3-4 months)
 followed by partial weight bearing with external fixator support
- 6. Progressive return to full activity usually after 1 year.

FOLLOW UP

Patients are followed at every 6 weeks after stage II procedure for first 6 months and every 3rd month thereafter. At each visit the patient is subjected to the following,

- 1. Clinical examination of tenderness and abnormal mobility.
- 2. The patient is examined for any pin tract infection (PTI) and any flap necrosis.
- Radiographic assessment AP and LAT view of the affected extremity to look for graft integration and consolidation with the host bone at both the ends.
- 4. Assessment of implants(external fixator) for,
 - a. Loosening
 - b. Infection
 - c. Failure
- 5. Functional assessment- clinical outcome is measured using ASAMI scoring system.

ASAMI SCORING SYSTEM: BONE UNION OUTCOME

	T ·	
Excellent	Union	
	no infection	
	deformity<7 °	
	limb length discrepancy <2.5 cm	
Good	Union + any two of the following:	
	a) Absence of infection	
	b) Deformity <7 °	
	c) Limb length inequality <2.5cm	
Fair	Union + only one of the following:	
	a) Absence of infection	
	b) Deformity <7 °	
	c) Limb length inequality <2.5cm	
Poor	Non-union/refracture/union + infection + deformity	
	$>7^{\circ}$ + limb length inequality >2.5 cm	

ASAMI SCORING SYSTEM : FUNCTIONAL OUTCOME

Excellent	Active
	no limp
	minimum stiffness (loss of 15° knee extension/ 15°
	dorsiflexion of ankle),
	no reflex sympathetic dystrophy (RSD)
	insignificant pain
Good	Active with one or two of the following:
	a) Limp
	b) Stiffness
	c) RSD
	d) Significant pain
Fair	Active with three or all of the following:
	a) Limp
	b) Stiffness
	c) RSD
	d) Significant pain
Poor	Inactive (unemployment or inability to return to
	daily activities because of injury)
Failure	Amputation
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OBSERVATIONS

In our study we analysed the functional results of compound fractures with bone defects managed by Masquelet technique in 20 patients. All patients were male with an average age of 36 years (range 18-55).



Male predominance was noted from the above bar diagram.



The injury has occurred in both left and right leg in the ratio of 3:4.



We have treated patients with an average age of 35.5 years (range 18-55 years). We found that young adult age group, who are the major working population of the society are sustaining road traffic accidents.



We analysed the mode of injury in our cases and found that 16 cases sustained their injury from road traffic accident and 4 cases sustained injury due to accidental fall from height.



All patients sustained open fractures and were classified according to Gustilo and Anderson classification. Out of 20 cases, 8 cases of grade IIIA and 12 cases of grade IIIB compound injuries were found showing a predominance of injuries with extensive soft tissue damage and bone exposure were involved in this study.



55% of cases had defects in Mid $1/3^{rd}$, 25% of cases had defects in Lowe $1/3^{rd}$, 20% had defects in Upper $1/3^{rd}$.

BONE DEFECT SIZE



From the above graph we infer that maximum number of cases were treated with a bone defect size of around 3cm.



In our study, out of 20 cases, 12 cases required flap cover along with stage I surgery and in 8 cases primary skin closure was possible.



From the above chart we observed that the average time for bone union was 10 months with a range from 6 months to 14 months.

RESULTS

All patients were followed up clinically and radiologically every month for first 6 months and then every third month thereafter for 1 year.

The mean duration between trauma and first stage surgery was 30 days (range 10-90). Average follow up period is 12 months (range 7-17 months). In our study size of bone defect varied from 2 cm to 5cm.

All affected legs were skeletally stabilized with AO external fixators/ Limb reconstruction system.

The cement spacer used in all cases was Polymethymethracylate (PMMA). 12 out of 20 cases required soft tissue repair by rotational fasciocutaneous flaps either proximally or distally based pedicles.

The mean interval between the first and second stage was 43.8 days (range 32-56). The culture swabs taken during stage II were negative in 19 out of 20 cases.

We confirmed union clinically by absence of pain and abnormal mobility at the fracture site .In all the patients, successive radiographs showed improved bone graft integration and maturation among themselves and with the host bone. The defect site radio opacity increased with every follow up radiograph.



17 out of 20 patients with bone defect united in the mean time of 10 months (6 to 14 months). The limb length discrepancy was not more than 1cm in the healed cases. The average period of non-weight bearing was 4 months (range 3-5 months) post bone grafting. Full weight –bearing walk was commenced at an average period of 12 months (range 10-14 months).



Out of 20 cases 50% patients had excellent results, 25% had good results, 10% was fair and 15% had poor results.

COMPLICATIONS

The complications we faced in our study was persistent infection in 2 cases after stage II surgery who subsequently underwent bone transport technique with some modifications in external fixator, malalignment in 2 cases but they refused for further surgical correction, non-union of the graft with the host bone for which additional procedure, bone grafting was done in two cases after which bony union was achieved. There was also shortening of the limb observed in 5 cases.



DISCUSSION

Management of long bone defects is a very challenging task.

Masquelet two - staged technique offers a simple alternative method of treatment which can be done easily without any specialized instruments. As in original study we observed induced biomembrane in all cases in the fracture gap and its surrounding areas after cement spacer removal and graft integration after stage II procedure. We did stage II procedure after ruling out infection clinically and radiologically. *Aho and his colleagues*³¹ inferred in their study that biomembrane is well formed and reach its peak activity after 1 month of stage I procedure.

Stevens et al^{32} in his study concluded that the induced membrane is regarded as the "invivo complex bioreactor" providing uninterrupted blood supply for the bone graft thereby resulting in rapid healing of bone defects. *Pelissier et al*¹³ in their study found out that the pseudomembrane is rich of growth factors, highly vascularised and contains abundant amount of osteogenic factors. *Viataeu et al*¹⁵ did immunohistochemical study on biomembrane and found out cells expressing transcription factor CBFA1 and type-1 collagen rich extracellular matrix. The induced membrane known as "in situ growthfactors delivery system" is capable of enhancing the bone graft healing³³. However we were not able to perform any

quantitative analysis of growth factors in the induced membrane due to unavailability of such facilities in our centre. Many authors have combined autograft with allograft or BMP for larger defects. Huffman et al²⁰ used reamer-irrigator-aspirator technique to harvest cancellous graft from ipsilateral femur and it has been found that level of growth factors such as insulin-like growth factor I, platelet derived growth factors are high in femoral cancellous graft as compared to iliac crest and platelet concentrations. However we harvested only cancellous autograft from iliac crest without any sophisticated instruments as it has got osteogeneic, osteoinduction and osteoconduction properties, easily available and from bilateral side for large defects in two cases for structural support. Apard et al^{24} managed posttraumatic tibia bone defects using intramedullary nail as fixation device to start early weight bearing but we used unilateral AO external fixator in 13 cases for skeletal stabilization as described by the original technique of Masquelet since it was easy to proceed with soft tissue cover and stage I procedure at the same sitting as in all cases the wounds were grossly contaminated and limb reconstruction system(LRS) was used in 7 cases, in case of change of plan to bone transport technique due to graft resorption. Giannoudis et al^{34} has reported unvarying results with the use of plates and nails for stabilization. This technique can be successfully used to manage metaphyseal as well as epiphyseal bone defects and not just diaphyseal bone defects³⁵. We observed good results in two cases with bone defects in metaphysis region. This technique being a two- stage procedure is the main disadvantage as the patients have to undergo second anaesthesia and hospitalization. The main complications were infection and graft non-union. Graft consolidation failure was taken into consideration when there were no signs of increased radio opacity on follow up X-rays for three consecutive months as suggested by El-Alfy and Ali³⁶.

CONCLUSION

The Masquelet's technique is a safe alternative in the management of bone defects. This two staged technique is useful in infected gap non union where repeated debridement procedures are necessary to clear out infection. It can be performed without any sophisticated instruments.

In case if this technique fails, there are many other alternative treatment methods such as bone transport, vacularised bone transfer from fibula, vascularised iliac crest grafts to treat the bone defects but these alternate techniques involve complex procedures.

Patients should be counselled regarding the need for further additional procedures because of the complex nature of the injury.

CASE ILLUSTRATION

Case-1

Name: Mr. A

Age/Sex: 19/M

IP No. 90469

Diagnosis: Grade IIIB compound fracture both bones Right Leg.

Bone defect Size: 3cm

Pre-OP



POST-OP

Post-Stage I



Post-Stage II



12 Weeks POST-OP



1 year and 8 months POST-OP



FUNCTIONAL OUTCOME








Case-2

Name: Mr. B

Age/Sex: 22/M

IP No. 94971

Diagnosis: Grade IIIA compound fracture distal both bones Right Leg.

Bone defect Size: 2.5cm

PRE-OP



POST OP X-RAYS

POST STAGE I



POST STAGE II



12 Weeks POST OP



1 year and 6 months follow up



FUNCTIONAL OUTCOME







Case-3

Name: Mr. C

Age/Sex: 42/M

IP No. 11054

Diagnosis: Grade IIIA compound fracture both bones Right Leg.

Bone defect Size: 4cm

PRE-OP



POST OP X-RAYS

POST STAGE I



POST STAGE II



12 WEEKS FOLLOW UP



1 year follow up



FUNCTIONAL OUTCOME





Case-4

Name: Mr. D

Age/Sex: 40/M

IP No. 76791

Diagnosis: Grade IIIB compound fracture distal both bones Left Leg.

Bone defect Size: 3cm

PRE OP



POST OP X-RAYS

POST STAGE I



POST STAGE II



12 WEEKS FOLLOW UP



1 year follow up



FUNCTIONAL OUTCOME







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MASTER CHART

Case no.	Age (years) /sex	Fracture type	side	Mode of injury	Level of bone gap	Bone defect size (cm)	Soft Tissue procedures	Number of surgeries	Delay since injury	Delay between two stages(days)	Complications	Secondary interventions	Union time (months)	Limb length discrepancy
1	20/M	Gustilo IIIB	R	Bike accident	Mid 1/3rd	3cm	FCF	4	3 months	40 days	Nil	Nil	6	0
2	22/M	Gustilo II	R	Bike accident	Distal 1/3rd	2.5cm	PC	3	20 days	32 days	Toe drop	Nil	10	0
3	40/M	Gustilo IIIB	L	Bike accident	Lower 1/3	3cm	FCF	4	15 days	45 days	Varus mal- alignment	Nil	12	0.5
4	42/M	Gustilo IIIA	R	Fall from Height	Mid 1/3	4cm	PC	3	10 days	56 days	Nil	Nil	9	0
5	22/M	Gustilo IIIB	L	Bike accident	Mid 1/3	5cm	FCF	5	1 month	42 days	Nonunion	Bone grafting	14	1
6	54/M	Gustilo IIIB	L	Fall from height	Mid 1/3	5cm	FCF	5	20 days	NP	-		-	NP
7	18/M	Gustilo II	L	Bike accident	Lower 1/3	3cm	PC	3	12 days	35 days	Nil	Nil	8	0
8	22/M	Gustilo IIIB	R	Bike accident	Mid 1/3	4cm	FCF	3	50	40days	Shortening	Nil	11	0.5
9	24/M	Gustilo IIIB	L	Bike accident	Mid 1/3 rd	3cm	FCF	4	2 months	49 days	Nil	Nil	11	0

Case no.	Age (years) /sex	Fracture type	side	Mode of injury	Level of bone gap	Bone defect size (cm)	Soft Tissue procedures	Number of surgeries	Delay since injury	Delay between two stages(days)	Complications	Secondary interventions	Union time (months)	Limb length discrepancy
10	45M	Gustilo IIIA	L	Fall from height	Proximal 1/3rd	2cm	PC	3	15 days	45 days	Valgus malalignment	Nil	12	0
11	55/M	Gustilo II	L	Pedestrian vs 4 wheeler	Prox 1/3	3cm	PC	5	10 days	38 days	Nonunion	Bone grafting	13	0
12	40/M	Gustilo IIIB	R	Bike accident	Proximal 1/3	4cm	FCF	4	2 months	50 days	Shortening	Nil	10	0.5
13	55/M	Gustilo II	L	Pedestrian vs 2wheeler	Mid 1/3	3cm	РС	3	1 month	55 days	Nil	Nil	9	0
14	35/M	Gustilo IIIA	R	Car crash	Lower 1/3	3cm	РС	3	20 days	40 days	Nil	Nil	11	0
15	45/M	Gustilo IIIB	L	Bike vs 4 wheeler	Mid 1/3	4cm	FCF	5	15 days	NP	Infection	Bone transport	-	NI
16	27/M	Gustilo IIIB	R	Bike accident	Mid 1/3	3cm	FCF	4	2 months	42 days	Shortening	Nil	10	1

Case no.	Age (years) /sex	Fracture type	side	Mode of injury	Level of bone gap	Bone defect size (cm)	Soft Tissue procedures	Number of surgeries	Delay since injury	Delay between two stages(days)	Complications	Secondary interventions	Union time (months)	Limb length discrepancy
17	50/M	Gustilo IIIb	R	Car crash	Prox 1/3	4cm	FCF	3	20 days	45 days	Infection	Bone transport	-	NI
18	22/M	Gustilo IIIB	R	Fall from Height	Mid 1/3	3cm	FCF	3	15 days	47 days	Nil	Nil	11	0
19	50/M	Gustilo IIIB	R	Pedestrian Vs 2 wheeler	Lower 1/3	3cm	FCF	3	20 days	42 days	Nil	Nil	10	0
20	44/M	Gustilo IIIA	R	Car crash	Mid 1/3	3cm	PC	3	22 days	40 days	Nil	Nil	10	0

NP- not performed second stage surgery due to persistent infection, NI-Not included because treated with bone transport technique, PC-primary closure, FCF-fasciocutaneous flap

PROFORMA

SI.No:

Patient name:

Age / Sex: IP.No :

Occupation:

Address:

Phone no.:

Date of injury :

Mode of injury : Side :

Right/Left

Fracture classification

AO :

Simple / compound (Grade)

Level of fibula fracture :

Associated injuries :

PRE OP WORK UP

Pus c/s

CBC:

ESR: CRP:

BONE LOSS:

(Initial or late after debridement)

Bone gap length:

Bone cement used: PMMA

Antibiotic used:

Interval between injury& surgery

DO external fixator application :

stage 1 Masquelet technique DOS:

stage 2 (bone grafting)DOS: DO Removal of external fixator: DO weight bearing : Post op Varus/ Valgus angulation Cast support: Time of union: If non union or delayed union any secondary procedure done: Functional outcome

PATIENT CONSENT FORM

Study Detail : "Management of bone defects in tibia by Masquelet technique "

Study Centre : Rajiv Gandhi Government General Hospital, Chennai.

Patient's Name :

Patient's Age :

Identification Number :

Patient may check ($\sqrt{}$) these boxes

a) I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction.

b) I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected.

c) I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.

d) I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms.

e) I hereby consent to participate in this study.

f) I hereby give permission to undergo detailed clinical examination, radiographs clinical photographs and blood investigations as required.

Signature/thumb impression

Patient's Name and Address:

Signature of Investigator

Investigator's Name: (Dr. GOWTHAM RAM)

INSTITUTIONAL ETHICS COMMITTEE MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013 Telephone No.044 25305301 Fax: 011 25363970

CERTIFICATE OF APPROVAL

То

Dr. P. Gowtham Ram, Post Graduate in M.S. Orthopaedics Institute of Orthopaedics and traumatology, Madras Medical College ,Chennai

Dear ,

The Institutional Ethics Committee has considered your request and approved your study titled **"MANAGEMENT OF BONE DEFECTS BY MASQUWELET'S TECHNIQUE "** NO.19092016 .

The following members of Ethics Committee were present in the meeting hold on **06.09.2016** conducted at Madras Medical College, Chennai 3

1. Prof. C. Rajendran, MD.	Chairperson		
2. Prof. Dr. M.K. Muralidharan, M.S, M.Ch., MMC , Ch-3	Deputy Chairperson		
3. Prof. Sudha Seshayyan, MD., Vice Principal, MMC.Ch- 3.	Member Secretary		
4. Prof. B.Vasanthi, MD., Prof of Pharmacology, MMC,	Member		
5. Prof. P.Raghumani.MS., Professor of Surgery, Inst. of surge	ery Member		
6. Prof. R.Padmavathy, MD., Professor, Inst. of Pathology, MMC	C,Ch Member		
7. Tmt.J.Rajalakshmi, Junior Administrative Officer, MMC, Ch	Layperson		
8. Thiru.S.Govindasamy., B.A.B.L., High Court, Chennai-1	Lawyer		
9. Tmt.ArnoldSaulina, MA., MSW.,	Social Scientist		

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary - Ethics' Comm MEMBER SECRETARY NSTITUTIONAL BYHICS COMMITTEE. - Ethics Committee MADRAS MEDICAL COLLEGE CHENNAL-000 003

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9 100% the antero-itateral, the antero-medial, the postero-itat	teral, and the postero-medial;				
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PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled "PROSPECTIVE ANALYSIS OF OUTCOME IN THE MANAGEMENT OF POST-TRAUMATIC BONE DEFECTS IN TIBIA BY MASQUELET TECHNIQUE" of the candidate Dr.P.GOWHTAM RAM with registration Number 221612001 for the award of M.S. ORHTO in the branch of II. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 8 percentage of plagiarism in the dissertation.

Guide & Supervisor sign with Seal

Prof.N.Deen Muhammad Ismail, M.S.Ortho., D.Ortho., Director and Professor, Institute of Orthopaedics and Traumatology, Madras Medical College, Chennai-600003.

ஆய்வு தலைப்பு ராஜீவ் காந்தி அரசு பொது மருத்துவமனைக்கு வரும் கால் எலும்பில் ஏற்பட்ட முறிவினையும் அதன் பாதிப்பையும் பல்வேறு வைத்திய முறைகளின் கீழ் ஒப்பிட்டு அறிதல்.

கால் எலும்பில் ஏற்பட்ட முறிவினையும் அதன் பாதிப்பையும் பல்வேறு வைத்திய முறைகளின் கீழ் ஒப்பிட்டு அறிவதே இந்த ஆராய்ச்சியின் நோக்கமாகும்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில்தான் இருக்கிறது. மேலும் நீங்கள் எந்த நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின் வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த சிறப்புப் பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவில் தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

ஆராய்ச்சியாளா் கையொப்பம்

பங்கேற்பாளா் கையொப்பம்

தேதி: நாள்: