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ORIGINAL ARTICLE

# Ilizarov method in combination with autologous mesenchymal stem cells from iliac crest shows improved outcome in tibial non-union

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**KEYWORDS**

Ilizarov procedure;
Mesenchymal stem cells;
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**Abstract** Autologous bone grafting and ilizarov technique are the preferred mode of treatment for bone nonunion, studies suggest that bone marrow derived mesenchymal stem cells can be effective in treatment of tibial non-union where there is length of bone defect. The current study investigates the beneficial clinical outcome of combining the ilizarov procedure with intraosseous injection of autologous mesenchymal stem cells. The open-label study enrolled 25 patients with infected tibial non-union at the Shanghai Fengxian District Central Hospital, Shanghai, China between April 2010 and July 2014. Patients were randomised to undergo the ilizarov procedure with ($n$ = 11) or without ($n$ = 13) intraosseous injection of bone marrow derived mesenchymal stem cells. All participants were followed prospectively until union was achieved (primary end point). The mean length of the bone defect in the Ilizarov group and Ilizarov group plus MSC group was 6.09 and 5.84 cm respectively. The mean time from the original injury to the time of the treatment for tibial non-union was 5–22 months (mean 13.5 months) for the Ilizarov group and 6–21 months (mean 13.5 months) for Ilizarov plus MSc group. All 24 patients were followed up for 12–34 months (mean 16 months). Both groups achieved the primary endpoint of stable union of the tibial fracture. No adverse events were observed in any of the group. Our study demonstrates that using autologous bone marrow derived mesenchymal stem cell as an add-on therapy to the ilizarov procedure shows significant clinical benefit in fixation of tibial non-union.


## 1. Introduction

Nonunion of the tibia is frequently observed in the bone speciality setting and often leads to bone and soft tissue defect with impaired quality of life in patients. Failure to correct this condition may lead to chronic systemic infection or in prolonged instances may even lead to amputation of the limb. In general, any fracture that does not show union after 5–6 months is

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regarded as a nonunion and requires surgical intervention (Rodriguez-Merchan and Forriol, 2004). There are high chances of infection in an open fracture, and may lead to severe complications such as osteoporosis, systemic infection, and permanent restriction of joint movement (Patzakis and Zalavras, 2005). Estimate suggests that around 10% of the patients with compound fracture of long bone develop nonunion or delayed union which results in severe impairment and multiple instances of surgeries. The treatment of bone nonunion poses a treatment challenge and requires a certain level of expertise for successful treatment (Han et al., 2015; Kiratipaiboon et al., 2015).

Conventional treatments, include autologous bone grafting or artificial bone and metal grafting; however these procedures are associated with operative wounds, bone defects, incorrect union or nonunion and effective for bone defects less than 4 cms (Motsitsi, 2008; Farmanullah et al., 2007). Ilizarov external fixation technique, devised by Gavriil Abramovich Ilizarov in 1951, is a popular procedure for the treatment of bone nonunion, bone lengthening, and bone deformity correction. This procedure has gained increasing popularity in recent years and is extensively used by clinicians worldwide for the treatment of compound fracture nonunion. The procedures use metallic wires that are inserted percutaneously and attached to metallic ring to provide tension and support to the bone (Ilizarov, 1989; Abdel-Aal, 2006; Saridis et al., 2006). Illizarov procedure is also effective for the treatment of long bone nonunion and improves early weight bearing along with increasing the stability of the bone union. However, the procedure requires a specialist to carry out the procedure and also carries the risk that the bone can be re-fractured if it takes a long time to heal and carries the risk of infection at the site of wire insertion.

Other alternative procedures include use of bone marrow derived mesenchymal stem cell injection to facilitate fast healing of the bone (Connolly et al., 1991). The osteogenic property of the bone marrow was first shown by Giujon et al. in a rabbit model (Hernigou et al., 2005a). Thereafter a number of studies have confirmed the osteogenic potential of the bone marrow derived stem cells and bone marrow as a whole. Studies have highlighted the use of mixing autologous with allogenic bone marrow can also be useful in treatment of bone nonunion (Burwell, 1985; Lindholm and Urist, 1980; Salama and Weissman, 1978; Desai et al., 2015). Distal portion of long bone is usually rich in haematopoietic bone marrow; however, it has been shown that the red bone marrow is responsible for the physiologic activity of the bone union. Various studies have confirmed the role of bone marrow derived osteogenic precursor cells in promoting healing of the bone. Parenteral administrations of the bone marrow derived stem cells have been successfully shown to promote healing of bone nonunion with minimum systemic complications (Hernigou, 1998).

There is currently a lack of scientific evidence to demonstrate beneficial clinical outcome of combining the ilizarov procedure with intraosseous injection of autologous mesenchymal stem cells. Therefore the current study has been undertaken to evaluate the beneficial effect of the two procedures. Our study tests the hypothesis that the combination of the two procedures may have beneficial outcome on the quality of union and reduce the time taken to achieve bone union.

## 2. Materials and methods

### 2.1. Patient enrolment

A total of 25 patients with infected tibial non-union at the Shanghai Fengxian District Central Hospital, Shanghai, China hospital between April 2010 and July 2014 were enrolled in the study. The study protocol was approved by the institutional ethics committee of the Shanghai Fengxian District Central Hospital, China and performed in accordance with the declaration of Helsinki and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (E6) guidelines. A written informed consent for participation in the study was obtained from all participating patients. A total of 31 patients were initially screened for the study; however, only 25 qualified for the study based on the inclusion criteria. Four patients could not be included in the study due to concomitant systemic diseases or age more than 50 years; two patients refused to participate in the study and opted for the Ilizarov procedure outside the study. Patients were enrolled based on the following inclusion criteria:

- Radiographically confirmed compound fracture of tibia with diagnosis of tibia non-union.
- Male or female participants between ages of 18 and 50 years.
- The non-union gap should be between 3 and 12 cm.
- Subject or legal guardian is willing and able to understand, sign and date the study specific Patient Informed Consent, which has been approved by the Institutional Review Board.
- Agrees to participate in post-operative evaluations and required rehabilitation regimen.

Patients with following criteria were excluded from the study:

- Age of the participant is more than 50 years.
- If female, participant is pregnant, plans on becoming pregnant during the duration of this clinical outcomes collection study or lactating.
- Participant has an implanted unipolar pacemaker.
- Participant has active cancer.
- Participant has severe peripheral vascular disease (ABI $< 0.4$)
- Participant is an established case of chronic diabetes.

A total of 25 patients with tibial non-union were randomised to undergo the Ilizarov procedure in combination with intraosseous injection of mesenchymal stem cells ($n = 13$) or the Ilizarov procedure alone ($n = 12$). All participants were followed prospectively until union was achieved (primary end point). In all patients, calcium and vitamin D levels were checked after the initial procedure and treatment was started if found to be low. All patients had vitamin D levels of 30 μg/dL or more prior and during follow-up. At the time of enrolment both anteroposterior (AP) and lateral radiographs were taken. The radiographs were reviewed by three independent orthopaedicians trained in orthopaedic trauma surgery. Only patients whose radiograph was approved

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by the consensus of three experts were included in the study. The length of bone defect (in cm) was measured as the largest span between the two cortical fragments at the site of the fracture.

### 2.2. Extraction and harvesting of mesenchymal stem cells (MSCs)

All patients allocated to the Ilizarov procedure plus intraosseous injection of MSC group were subjected to extraction procedure of obtaining bone marrow concentrate from the iliac crest. The patient under lumbar epidural anaesthesia was placed in a supine position on the operating table. The affected long bone and the iliac crest of the same side were preferred for bone marrow extraction. A small horizontal incision (0.5 cm) was made at the anterior iliac crest to prevent any iatrogenic injury to the iliac bone during insertion or withdrawal of the trocar needle. Using a 16 gauge trocar needle an incision was made into the cancellous bone between the inner and outer tables of the iliac bone. Using a 50 ml syringe bone marrow aspirate was withdrawn. The trocar needle was gently tilted to 45° after every 10 ml of bone marrow extracted. Care was taken to keep a distance of 2 cm apart during multiple insertions and only 30 ml of bone marrow was withdrawn at a time to prevent dilution with peripheral blood. The extracted bone marrow was transferred through commercially available mesenchymal stem cell extractor system, BMAC system (Bone Marrow Procedure Pack; Harvest Technologies, Plymouth, MA) from the aspiration syringe. The MSC harvest was $72 \times 10^6$ cells per ml (total Nucleated Cells/mL).

### 2.3. Ilizarov surgical procedure

Ilizarov procedure was performed under epidural anaesthesia and preoperative parenteral cephalosporin was administered to each patient. The standard ilizarov procedure was followed. Ilizarov frames of stainless steel material (Suzhou Sunan Zimmered Medical Instrument Co., Jiangsu, China), consisting of three rings only for all patients, with 1.8-mm k-wire and 3.5-mm Schanz were used. In majority of the patients 4–6 extension of 1 mm/day were performed. The ilizarov fixation was removed only after callus mineralisation. Two to three weeks of weight bearing was prescribed before the external fixation was removed.

### 2.4. Intraosseous injection of autologous MSCs

Under biplane fluoroscopy the trocar needle was placed in the non-union gap (Fig. 1). About 20 ml of MSC rich marrow was injected at a rate of 20 ml/min. Care was taken to minimise the leakage of the marrow and the position of the trocar needle was changed if required. The perforation was closed using surgical suture.

### 2.5. Statistical analysis

The primary end point of this study was time for union of the tibial bone. Statistical significance was accepted for *p*-values of $< 0.05$. All analyses were performed using SigmaStat ver. 4.0 (Systat Software Inc., San Jose, CA, USA). To compare the intergroup variation of the data a unidirectional analysis of variance was performed. LSD-t test was performed for multiple comparison.

## 3. Results

### 3.1. Patient characteristics

A total of 24 patients (17 male, 7 female; mean age 33.04 years) with tibia non-union were enrolled in the study; 12 patients were allocated to the Ilizarov procedure for union of tibial fracture and 13 patients were allocated to Ilizarov procedure in combination with intraosseous injection of autologous mesenchymal stem cells (Fig. 2). The mean length of the bone defect in the Ilizarov group and Ilizarov group plus MSC group was 6.09 and 5.84 cm respectively. Patient demographics and clinical profile are represented in Table 1. The mean time from the original injury to the time of the treatment for tibial non-union was 5–22 months (mean 13.5 months) for the Ilizarov group and 6–21 months (mean 13.5 months) for Ilizarov plus MSc group. All 24 patients were followed up for 12–34 months (mean 16 months). Both groups achieved the primary endpoint of stable union of the tibial fracture. No adverse events were observed in any of the groups; however 2 patients in the Ilizarov group and 3 patients in the Ilizarov plus MSC group reported minor infection at the pin site, which was controlled by systemic and topical antibiotics. One patient in the Ilizarov group reported skin allergy which was controlled with oral antihistamine.

There was a significant decrease in the time taken for union of the tibia fracture in MSC plus the Ilizarov group as compared to the Ilizarov group alone ($P = 0.006$). The mean duration for bone union was 6.45 months in patients undergoing Ilizarov procedure alone and 3.92 months in patients undergoing a combination of the Ilizarov procedure and intraosseous injection of autologous MSC. The external fixation index (days/cm) in the Ilizarov plus MSC group was 22.83 compared with 34.13 in the Ilizarov only procedure group ($P = 0.016$) (Fig. 3). There was also a significant decrease in the duration of hospitalisation in patients who underwent the Ilizarov procedure in combination with intraosseous injection of autologous MSC ($P = 0.025$). Paley score was excellent in majority of the patients in both groups. Mean values of clinical and demographic parameters are represented in Table 2. The count of MSC in extracted bone marrow was 45 x $10^6$ cells whereas the CFU count per millilitre in aspirate was $210 \pm 126$.

## 4. Discussion

The current study gathers evidence from a randomised clinical study on the beneficial effect of adding autologous mesenchymal stem cell procedure to the established Ilizarov procedure. In recent years the Illizarov procedure has become treatment of choice for infected bone nonunion; however it requires the patient to have restricted movement due to the bulky metallic frame and inserted wires. Adding a complimentary therapy/procedure to reduce the duration of Ilizarov would be much welcomed by patients. There may be several causes of bone union, such as high impact injury, damage to blood supply, infection of the open wound and low levels of calcium. Infection is a major risk associated with the ilizarov procedure and may cause osteoporosis, restriction of joint movement and

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**Study Schema: CONSORT depicting the flow of participant in each stage of the trial**

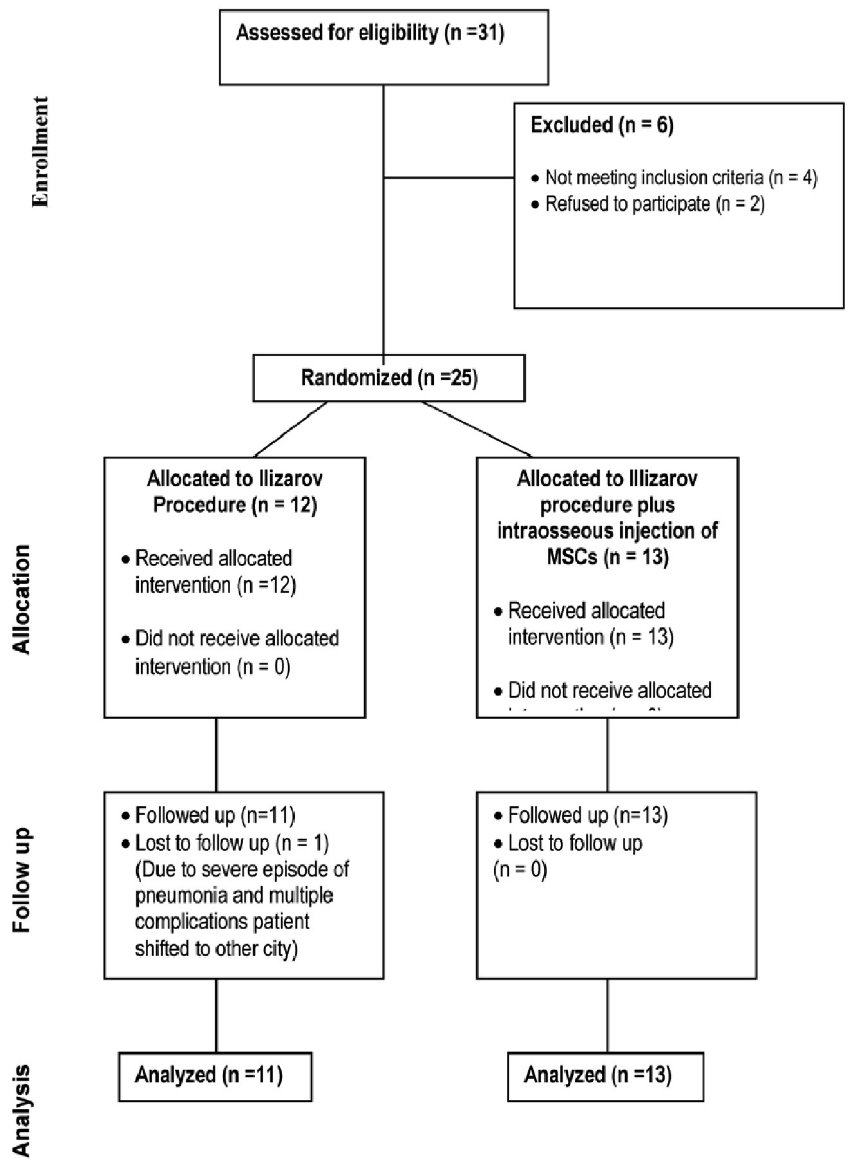


**Figure 1** (A) Bone marrow aspirate was obtained from posterior iliac crest using a trocar needle and then passed through mesenchymal stem cell extractor system (B) Intraosseous injection of MSC rich bone marrow under biplane fluoroscope (C) Illizarov external fixator surrounding the tibial bone (D) X-ray of tibia after the Ilizarov fixator was placed.

cutaneous deficiency (Green et al., 1992). As per clinical Classification of post-traumatic tibial osteomyelitis by May, bone graft is preferred for bone defects <6 cm, for defects >6 cm bone grafting possesses several challenges (May et al., 1989).

The conventional osteodistraction procedure was first developed in 1950. The procedure requires generation of muscle, aponeurosis, nervous tissue and integumentary tissue, due to the distraction of the bone. In due course of time the physical stress causes remodelling and bone union. Ilizarov technique is an expansion of this procedure and addresses much of the complications and drastically improves the quality of the union (Ilizarov, 1989). Ilizarov procedure also enables free access to the site of injury to monitor rate of healing and infection. Some of the significant highlights of this procedure are: The tension of the wire needs to be proportional to the diameter of the wire, that keeps the bone in place; in a week's time extension of the bone should be visible; period X-ray examination should be performed to monitor the bone union and fixation; partial weight bearing activity may be resumed during the extension phase. The recommended tension and wire diameter for the ilizarov fixator is $1–2 \times 10^3$ N and 2 mm respectively (Mullins et al., 2003). During the long period of extension phase the steel wires may become weak and break, therefore the time required for keeping the external fixator on a patient's bone becomes critical.

Our study addresses this concern as we used autologous MSC to combine with the ilizarov procedure to reduce the time taken to achieve bone union. We included all patients with the

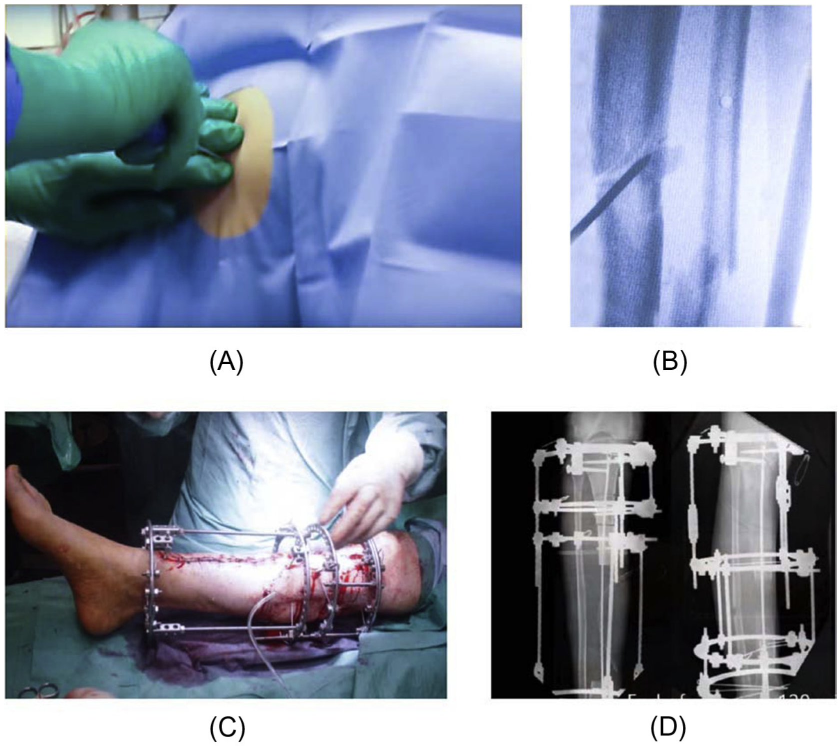


**Figure 2** Study schema showing flow of patients in each stage of the trial.

**Table 1** Demographics and clinical profile of each patient from both groups.

| S. No. | Group | Patient ID | Age (Years) | Sex | Length of bone defect (in cm) | Duration of follow-up (months) | Duration of Hospitalisation (months) | Time taken for union of fracture (months) | Duration of external fixation (months) | External fixation index (days/cm) | Paley score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ilizarov Group | 1XA | 34 | M | 3 | 12 | 2 | 3 | 4 | 30 | Excellent |
| 2 | | 1 KB | 42 | M | 9 | 18 | 4 | 6 | 8 | 20 | Excellent |
| 3 | | 1NC | 19 | F | 7 | 20 | 5 | 6 | 7 | 25.7143 | Good |
| 4 | | 1TD | 37 | M | 4 | 15 | 2.5 | 4 | 5 | 30 | Excellent |
| 5 | | 1XE | 23 | M | 5 | 12 | 6 | 8 | 7 | 48 | Good |
| 6 | | 1RF | 40 | M | 3 | 12 | 3 | 5 | 5.5 | 50 | Excellent |
| 7 | | 1XG | 49 | F | 7 | 32 | 6 | 9 | 11 | 38.5714 | Excellent |
| 8 | | 1JH | 22 | F | 9 | 12 | 4 | 11 | 12 | 36.6667 | Excellent |
| 9 | | 1XI | 28 | M | 10 | 28 | 6 | 8 | 9 | 24 | Good |
| 10 | | 1SJ | 43 | F | 4 | 14 | 5 | 7 | 7.5 | 52.5 | Excellent |
| 11 | | 1FK | 31 | M | 6 | 14 | 2.5 | 4 | 6 | 20 | Excellent |
| 1 | Ilizarov + MSC | 1LA | 26 | M | 4 | 12 | 2 | 3 | 3.5 | 22.5 | Excellent |
| 2 | | 1QB | 35 | F | 9 | 12 | 3 | 4 | 4.5 | 13.3333 | Excellent |
| 3 | | 1YC | 48 | M | 3 | 12 | 1.5 | 4 | 5 | 40 | Excellent |
| 4 | | 1MD | 18 | M | 3 | 12 | 2 | 3 | 3.5 | 30 | Excellent |
| 5 | | 1YE | 27 | M | 6 | 14 | 3 | 3 | 4 | 15 | Excellent |
| 6 | | 1GF | 31 | M | 8 | 12 | 5 | 6 | 7 | 22.5 | Excellent |
| 7 | | 1YG | 36 | F | 4 | 12 | 3 | 5 | 8 | 37.5 | Excellent |
| 8 | | 1PH | 28 | M | 6 | 12 | 2 | 4 | 5 | 20 | Excellent |
| 9 | | 1CI | 41 | M | 7 | 20 | 2 | 3 | 4 | 12.8571 | Excellent |
| 10 | | 1YJ | 39 | M | 11 | 34 | 6 | 6 | 6.5 | 16.3636 | Good |
| 11 | | 1BK | 29 | M | 7 | 18 | 1.5 | 3 | 4 | 12.8571 | Excellent |
| 12 | | 1UL | 30 | M | 3 | 12 | 2 | 3 | 4 | 30 | Excellent |
| 13 | | 1HM | 37 | F | 5 | 13 | 3 | 4 | 5 | 24 | Excellent |

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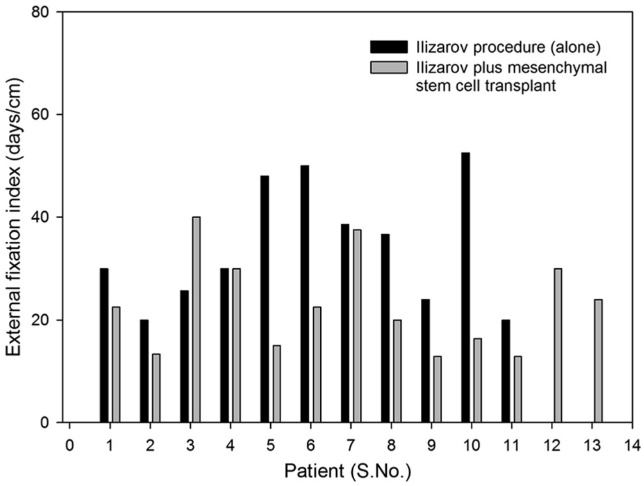


**Figure 3** External fixation index (days/cm) of each patient from both groups.

**Table 2** Comparative clinical and demographic parameters between the two groups.

| | Ilizarov procedure Group | Ilizarov + MSC | *P* value |
|---|---|---|---|
| Mean age | 33.45 | 32.69 | |
| Mean bone defect (in cm) | 6.09 | 5.84 | |
| Male | 7 | 10 | |
| Female | 4 | 3 | |
| The time of follow-up (months) | 17.82 | 15 | $P = 0.325$ |
| The time in hospital (months) | 4.18 | 2.7 | $P = 0.025$ |
| Time taken for union of fracture (months) | 6.45 | 3.92 | $P = 0.006$ |
| Duration of external fixation (months) | 7.45 | 4.92 | $P = 0.011$ |
| External fixation index (days/cm) | 34.132 | 22.83 | $P = 0.016$ |

size of the bone defect within 3–12 cm, which gave us the scope to test our hypothesis in varied types of non-unions. Our study also demonstrates a reduction in the duration of achieving bone union by 2.5 months which is statistically significant. There is no such study available that utilises MSCs for the bone defect range that we used in our study. In another study by Desai et al. the time for bone union was found to be 4.7 months; however this study used MSCs as a standalone procedure (Desai et al., 2015). Other parameters which showed statistically significant benefit were duration of hospitalisation (months) and external fixation index (days/cm). The combination of commercially available demineralised bone matrix with mesenchymal stem cells have shown comparable results in bone union in both preclinical and clinical studies but it does not show a significant reduction in the time taken for healing (Hernigou et al., 2005b; Sen and Miclau, 2007). Studies also suggest that combination of allogeneic graft of rhBMP-2 with autologous bone marrow shows a rate of around 90%, however our study achieved 100% results with significant reduction in the number of parameters.

## 5. Conclusion

Our study has successfully demonstrated that using autologous bone marrow derived mesenchymal stem cells as an add-on therapy to the ilisarov procedure shows significant clinical benefit in fixation of tibial nonunion. The study utilised a commercially available device for isolation of mesenchymal stem cells; further characterisation of the stem cell population needs to be performed. The study also warrants a larger clinical trial in different types of bone unions to highlight the beneficial outcome of combination approach.

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