Fibular hemimelia treated by autologous osteoblasts: a case report

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INTRODUCTION

Fibular hemimelia (FH) is the complete or partial absence of the fibula with an incidence of seven to 20 per 1,000,000 live births.[1] Femoral and tibial hypoplasia results in limb-length discrepancy (LLD), which becomes an obvious deformity by adulthood with grossly compromised quality of life.[2] Several classification systems have been developed for FH; the most common is by Achterman and Kalamchi[3] describing the amount of fibular discrepancy in patients. It is now reported that the LLD does not correlate with the amount of fibula deformity.[2] Thus, the more recent Paley[2] classification system gives priority to foot and ankle deformity that is associated with FH. Various studies reported amputation to be a successful method in the form of foot ablation and prosthetic fitting in treating FH. Similarly, different limb reconstruction procedures also showed favorable results in many studies.[1] Both treatments aim to achieve functional correction of the affected limb, with a plantigrade, stable, and flexible foot. Amputation is recommended for more severe FH cases. Limb reconstruction procedures are used in mild to moderate cases,[2] and multiple corrective surgeries need to be planned over several years in childhood. The most common corrective surgery is distraction osteogenesis (DO) with the use of an Ilizarov apparatus as an external fixator[4]; however, recurrence rates are high with this technique.[5] Surgical procedures are limited by long treatment times that are not well tolerated by patients as well as associated bone and soft-tissue complications.[6,7] There is a need for alternative or adjunctive treatment options in this area.

Reports suggest that bone-marrow extract used along with DO for reconstructive bone surgeries may shorten treatment duration and provide better patient satisfaction compared with conventional reconstruction procedures.[8–11] However, the number of osteoprogenitor cells that can differentiate into osteoblasts lowers the bone marrow aspirate, and the cell culture may have potential benefits.[12,13] Based on these assumptions, the authors decided to use autologous osteoblast cell therapy. Autologous cultured live osteoblasts were obtained by differentiation and expansion by using a small aliquot of bone marrow along with current standard care in a patient with congenital FH. Required ethical approval and permission from the Kirkee Military Hospital Ethical Committee and consent from the patient were obtained.

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CASE REPORT

A 17-year-old patient presented to our hospital with a LLD of 15 cm on the left side that resulted in an obvious limp when walking. Type 2 fibular deficiency per the Achterman and Kalamchi[3] classification was reported, and the clinical examina-

FIGURE 1. Preoperative radiograph of right leg (A) and left leg (B) (→: knee deformity; Δ; uniformly dense short tibia with absent fibula).

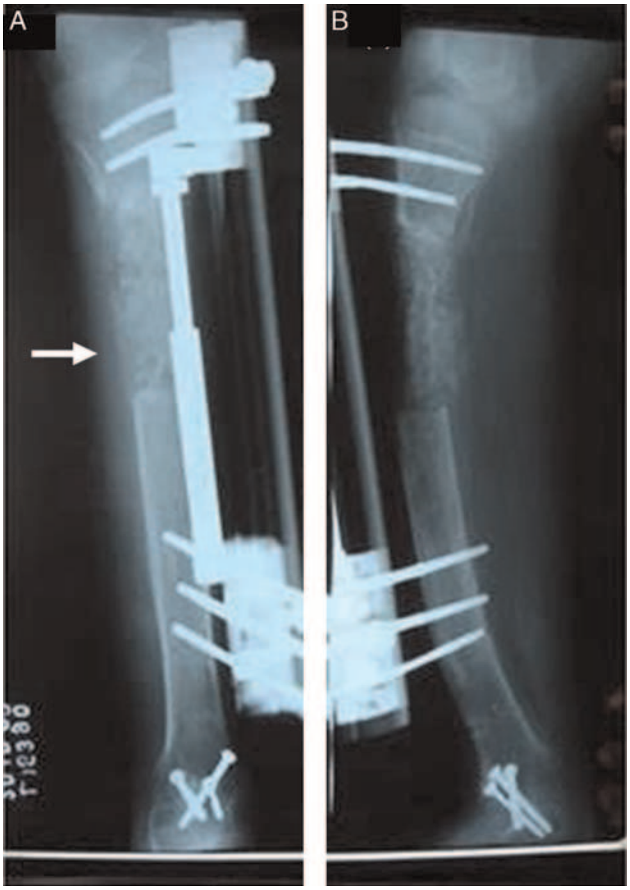

FIGURE 2. Arrow showing the new bone formed 6 wk after distraction and insertion of stem cells with allograft. Anteroposterior (A) and lateral (B) views.

tion revealed complete absence of the fibula and the fourth and fifth rays. The patient provided history of multiple corrective surgeries that were performed since the age of 5 yr that included an attempt towards limb lengthening using bifocal Ilizarov fixator followed by intraarticular arthrodesis of the ankle. At presentation, the patient had a 20-degree flexion deformity in the knee with complete flexion up to 135 degrees associated with LLD. No neurovascular deficit was seen (Figure 1A). Basic radiology confirmed the patient's history of absence of the fibula as well as the arthrodesed ankle with two screws (Figure 1B). There was no asymmetry in the tibia, and it was uniformly dense with fused epiphysis. The patient and his family were counseled for the entire course of treatment, which included cell therapy.

Osteoblast cell therapy is a two-step process. The first step involves harvesting bone marrow by biopsy, and the second includes implantation of autologous osteoblast cells that were cultured using the bone marrow. A percutaneous bone marrow aspiration from the iliac crest was done and was collected in a transport media containing anticoagulant that was transported under temperature-monitored conditions and processed at a Good Manufacturing Practice (GMP)-certified cell processing facility (Regrow Biosciences Pvt. Ltd., Mumbai, India) to obtain a predefined osteoblast culture. These osteoblasts were cultured for approximately 4 wk under stringent laboratory conditions and multiplied to up to 48 million osteoblasts. The *ex-vivo* culture of osteoblasts using bone-marrow from the patient involves

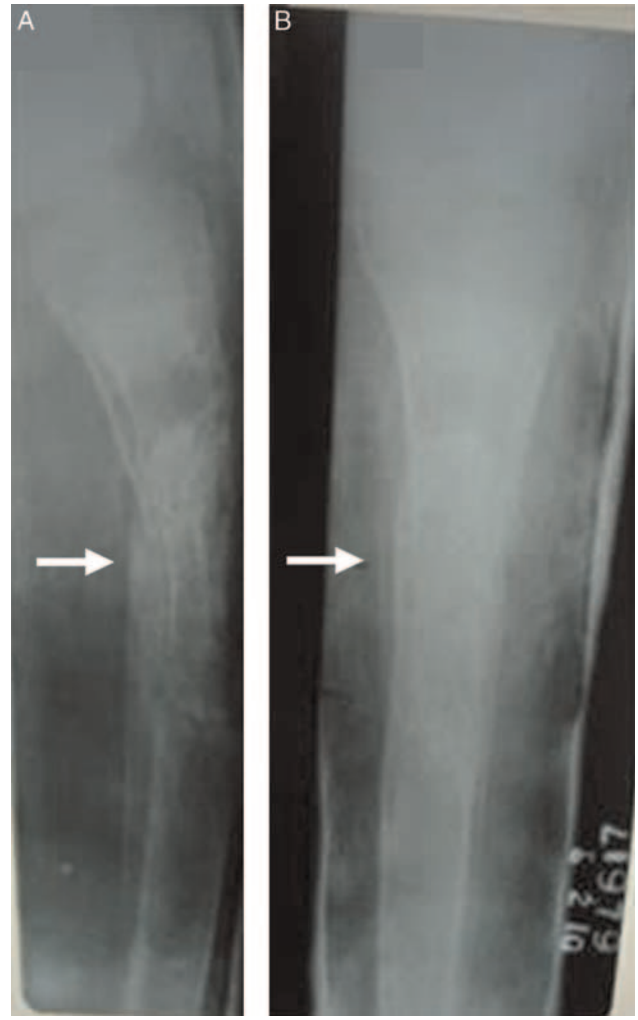

FIGURE 3. Radiograph at 10 wk after starting of distraction while limb is in cast showing new bone (see white arrow) at the distraction site. Anteroposterior (A) and lateral (B) views.

isolation of osteoprogenitor cells and osteogenic differentiation, which is followed by expansion. Immunophenotypic characterization was done to ensure that the cultured cells test positive for osteoblast biomarkers. After 5 wk, not less than 48 million viable osteoblasts were received for implantation.

Surgical intervention was in the form of tibial corticotomy in the proximal one-third region using Ortho-Fix (Ortho-Fix- Pitkar, Limb Reconstruction System (LRS), Orthofix, Lewisville, Texas). It was the first intervention as an attempt at limb lengthening. Two weeks after corticotomy a lengthening of 10 cm was achieved. The patient underwent another surgery in which cultured cells were implanted using a gel (Tisseel kit from Baxter, Deerfield, Illinois) along with an allograft in the gap that was created by the distraction. The patient was kept nonweight-bearing on the left lower limb. Four weeks after the second surgery, new bone had developed that had consolidated well (Figure 2A and B). The Ortho-Fix was removed and was replaced by an above-knee cast for the next 4 wk (Figure 3). At the end of 10 wk, the patient had a LLD of 5 cm with residual flexion deformity at the knee of 5 degrees (Figure 3). He was given a patellar tendon weight-bearing caliper with a shoe raise of 5 cm.

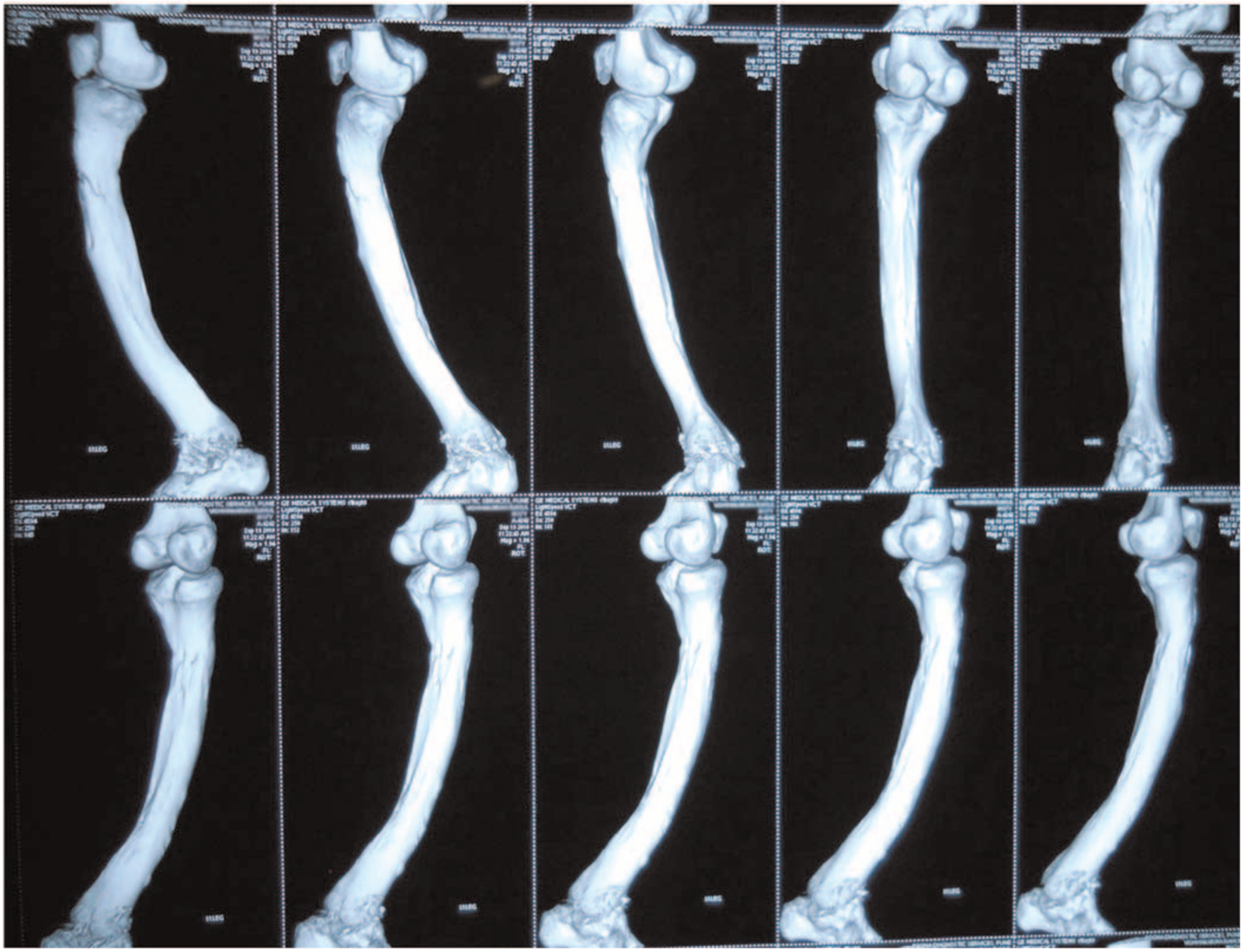

FIGURE 4. CT scan at 1-year after surgery (three-dimensional reconstructions).

No procedural complications were observed in the patient. One year after surgery, his CT scan evaluation showed well consolidated bone at the site of distraction (Figure 4). At the 10-year follow-up after the osteoblast implantation, the patient was found to be doing very well. The radiograph evaluation showed well incorporated bone at the site (Figure 5A and B). Additionally, the patient sustained a fracture in the distal third of tibia during the 10-year follow-up period, which suggested that the proximal end in which the bone distraction was done was strong enough to take that impact. The patient did not require any surgery during this period and could perform all routine activities without any pain as well as performing stationary cycling and other passive low-demand sports. However, running and jogging were the inherent limitations.

DISCUSSION

In this case report, a 17-year-old patient with FH was treated successfully by osteoblast cell therapy. Ten years after surgery, the patient could perform daily activities without pain. No complications that were associated with the surgical procedure were seen in the patient.

Recent advancement in biomechanics of fibular hemimelia has led to more acceptance of the reconstruction procedures over amputations.[2,14] DO is currently a standard acceptable method for bone lengthening but has some limitations that affect its use. Expensive devices, high rates of complications, slower recruitment of mesenchymal stromal cells (MSCs), and derailed osteogenic differentiation limit new bone formation after DO. To overcome these limitations and increase patient satisfaction, the authors used autologous osteoblasts for bone regeneration in this patient. Gessmann, *et al*.[15] reported the use of bone marrow derived MSCs for limb lengthening in six patients with an average lengthening of 82.4 mm achieved. They also found that transplantation of autologous bone marrow mesenchymal stem cells (BM-MSCs) positively affected early osseous consolidation in DO.[16] Unfortunately, only one clinical trial appears to be registered with Clinical Trials Registry of the National Institutes of Health in the United States.[17]

Bone regeneration using implantation of autologous osteoblasts rather than MSCs would be preferable.[18,19] For reconstructive regeneration of additional length of healthy bone, supplemental orthobiologics, especially *ex-vivo* cultured and

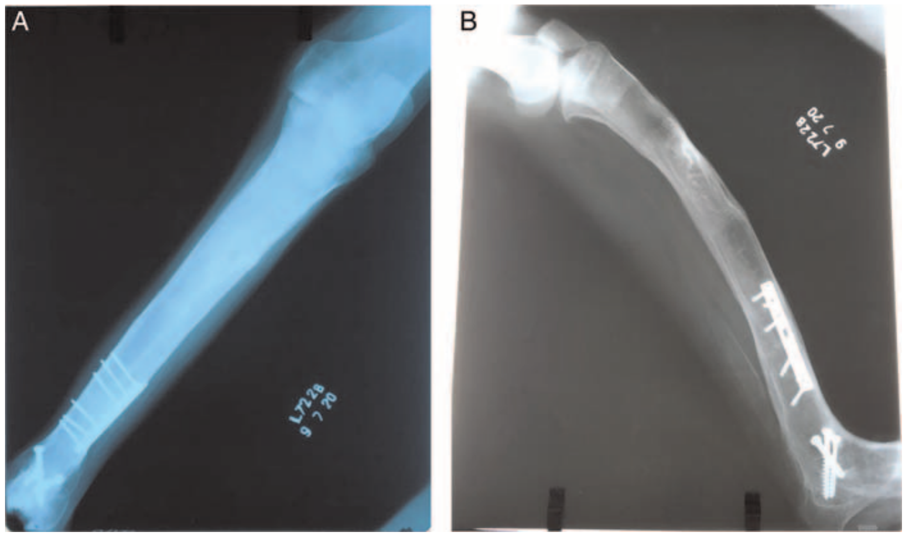

FIGURE 5. Radiograph 10 yr after surgery showing well-incorporated bone. Anteroposterior (A) and lateral (B) views.

expanded osteoblast cells, were found to be successful. Osteoblasts are an integral part of the bone remodeling process, and their recruitment would counter osteoclastic activity. This would help regain balance required for bone remodeling, healthy bone regeneration, and effective limb lengthening. With this hypothesis, the collective experience and thought process of the authors led to the use of autologous *ex-vivo* cultured osteoblast, distraction, and crushed allograft in this patient with congenital FH. In this case report, no complication was observed in the patient, which supported low complication rates with osteoblast implantation compared with other treatments.[18] As this is a single case study, the results should be interpreted with some degree of caution. Future clinical trials with adequate sample sizes would help evaluate its efficacy.

CONCLUSION

Clinical outcome in this patient was satisfactory; there were no complications during the procedure, less time was required for fixator use, and the patient could perform routine activities without any difficulty. The total length of correction achieved in terms of increased leg length was 10 cm. The quality of regenerated bone was as good as normal bone. Nine and a half years of follow-up showed no evidence of any related event that required medical attention. Clinical outcomes of this case report suggest that autologous osteoblast cell treatment could be an effective adjunct to DO for limb lengthening.

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