Autologous Bone Marrow-derived Mononuclear Cell Transplantation in Duchenne Muscular Dystrophy

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ABSTRACT

Duchenne muscular dystrophy (DMD) is the most common childhood muscular dystrophy. Presently, there is no known cure for the disorder. We report an 18-year-old boy with DMD who underwent autologous bone marrow-derived mononuclear cell transplantation intrathecally as well as intramuscularly in specific muscles. The parameters used to assess the patient pre- and postoperatively were creatine phosphokinase levels, electromyography, magnetic resonance imaging (MRI) musculoskeletal system upper and lower limbs and manual muscle testing. On follow-up at six months, he showed significant functional improvements along with improvements in his muscle strength. Clinically, his MRI also showed muscle fiber regeneration with decrease in fatty infiltration.

Keywords: Duchenne muscular dystrophy, autologous, mononuclear cells

Duchenne muscular dystrophy (DMD) is an X-linked, neuromuscular, recessive disorder, which is caused due to mutation in the dystrophin gene located on the X-chromosome at the location Xp21.2. Hence, it occurs in male children.[1,2] Defect in the dystrophin gene leads to progressive muscle degeneration and eventually loss of ambulation and death. DMD has an early onset with symptoms like difficulty in walking, frequent falls, difficulty in motor skills, muscle wasting, contractures, pseudohypertrophy, respiratory disorders and skeletal deformities in some cases.[3]

Diagnosis of DMD is based on a combination of clinical findings like family history and serum creatine kinase concentration. The muscle biopsy, genetic testing, electromyogram (EMG), nerve conduction velocity (NCV) confirm the diagnosis of DMD.

We hereby present a case of an 18-year-old boy diagnosed with DMD, who underwent autologous bone marrow-derived mononuclear cell (MNC) transplantation. He showed significant improvements functionally as well as clinically.

CASE REPORT

An 18-year-old boy with a family history of DMD was diagnosed at the age of one year with increased creatine phosphokinase (CPK) levels. He showed delayed milestones. He developed bilateral lower limb weakness with difficulty in walking and could walk on toes. He had a history of frequent falls with difficulty in climbing stairs and also in getting up from the sitting position. He stopped walking at the age of 15 years. Gradually, upper extremity weakness also developed with difficulty in overhead activities. On examination, right-sided scoliosis of spine was observed. Functionally, he was dependent on his caretaker for all the activities of daily living. On Functional Independence Measure (FIM), he scored 63, while on Brooke and Vignos scale, he scored 6 and 10, respectively.

Neurologically, he was hypotonic and hyporeflexic. All his sensations were intact. According to manual muscle testing, strength in muscles and force of contraction in them was assessed extensively. He had Grade 1 strength in bilateral upper limb and lower limb proximally and Grade 3 distally in all four limbs. On examination, he was cachexic with poor chest expansion and history of repeated lower respiratory tract infection. His CPK levels were 995 IU/l. Magnetic

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resonance imaging (MRI) of musculoskeletal system showed diffuse fatty infiltration in the muscles of upper arm, fore arm, pelvic girdle, thigh and leg. EMG showed primary muscle disease wherein proximal muscles were more affected than the distal muscles and 2D-Echo was normal with left ventricular ejection fraction (LVEF) - 60%. He was on continuous physiotherapy since he was diagnosed.

He underwent autologous bone marrow-derived MNC transplantation at our center.

MATERIAL AND METHODS

Patient selection and protocol design has been based on the inclusion criterion as per the World Medical Associations, Helsinki declaration. The protocol had been reviewed and approved by the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). The patient was informed about the procedure and a duly filled informed consent was obtained from him and his family. Granulocyte colony-stimulating factor (G-CSF) (300 μg) injections were administered subcutaneously, 48 hours and 24 hours before the therapy. Before the therapy, he underwent extensive evaluation by a team of medical and rehabilitation experts. Detailed evaluation of muscle strength and functional ability was done. Weak group of muscles were selected and then their motor points were plotted with the help of an electrodiagnostic stimulator.

Bone marrow (100 ml) was aspirated from the iliac bone under local anesthesia. The MNCs were separated and approximately 33 x 10^6 MNCs were immediately injected intrathecally in L_4-L_5 space. MNCs (diluted in cerebrospinal fluid) were also injected intramuscularly bilaterally in the glutei, quadriceps, deltoid and abdominals, which were decided prior to the therapy. Solumedrol 750 mg was given intravenously at the time of transplant. Following the transplantation, he underwent intensive neurorehabilitation, which included physiotherapy, occupational therapy as a part of the treatment program. He was put on exercise program emphasizing on strengthening individual muscles that were injected with stem cells so as to facilitate mobility and multiplication of the injected stem cells thereby giving enhanced results.

RESULTS

Our case presented typical symptoms of DMD. The CPK level, EMG and MRI musculoskeletal system of upper and lower limb confirmed the diagnosis. Post-transplantation, he did not report any side effects. Immediately after the therapy, few improvements were observed. His sitting posture was better than before. His CPK levels reduced to 460 IU/l from 955 IU/l.

After four months, he was reassessed. Manual muscle charting (MMT) was carried out and as it is a standard component of the neuromusculoskeletal physical examination, intratestor error was kept at minimum. His hand grip strength had improved significantly. His neck control and movements were better than before. His upper extremity muscles were stronger than before. He could stand on balance board with push knee splints for 15-20 minutes. His trunk balance had also improved.

On comparing the MRI before and after the therapy, improvements were observed in the degree of fatty infiltration with minimal possible muscle regeneration in the vastus medialis, vastus lateralis and semi-tendinosus muscle in the thigh. Similar improvement was also noted in the tibialis anterior, medial and lateral head of gastrocnemius muscle in the leg (Figs. 1-4). In the arm, improvements were noted in the long and lateral head of triceps muscle and biceps brachii muscle.

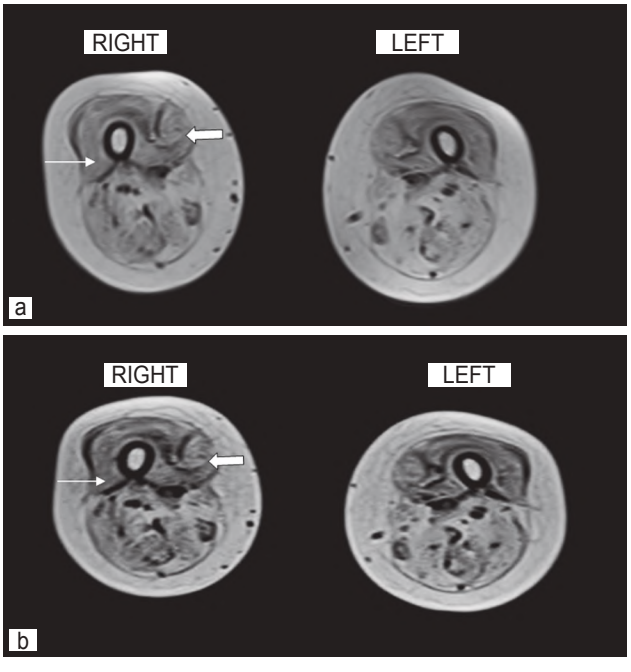

Figure 1. Axial T1W images at the level of upper thigh. **(a)** Pre-stem cell therapy show marked fatty infiltration of the right vastus medials *(thick arrow)* and lateralis muscle (thin arrow), seen as high signal intensity. **(b)** Post-stem cell therapy shows reduced high signal in both the vastus medialis *(thick arrow)* and lateralis *(thin arrow)* suggestive of less fatty infiltration and regeneration of muscle fibers.

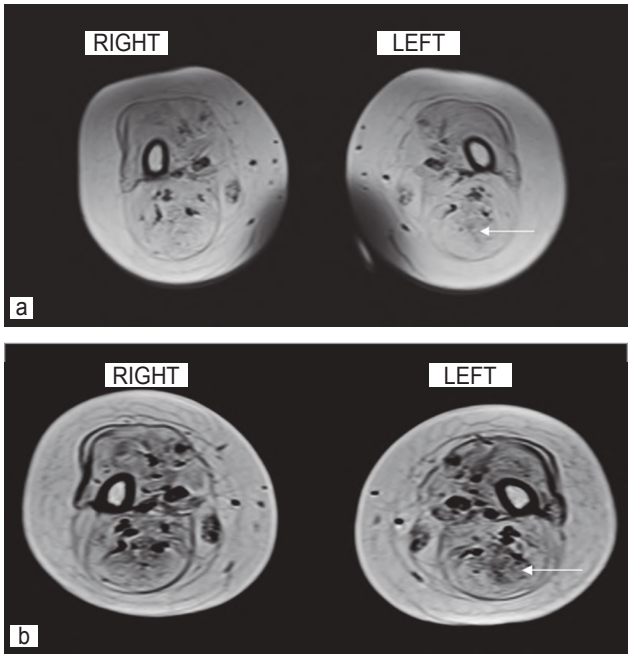

Figure 2. Axial T1W images at the level of upper thigh. **(a)** Pre-stem cell therapy show marked fatty infiltration of the left semitendinosus *(thin arrow)* seen as high signal intensity. **(b)** Post-stem cell therapy shows reduced high signal in the left semitendinosus *(thin arrow)* suggestive of less fatty infiltration and regeneration of muscle fibers.

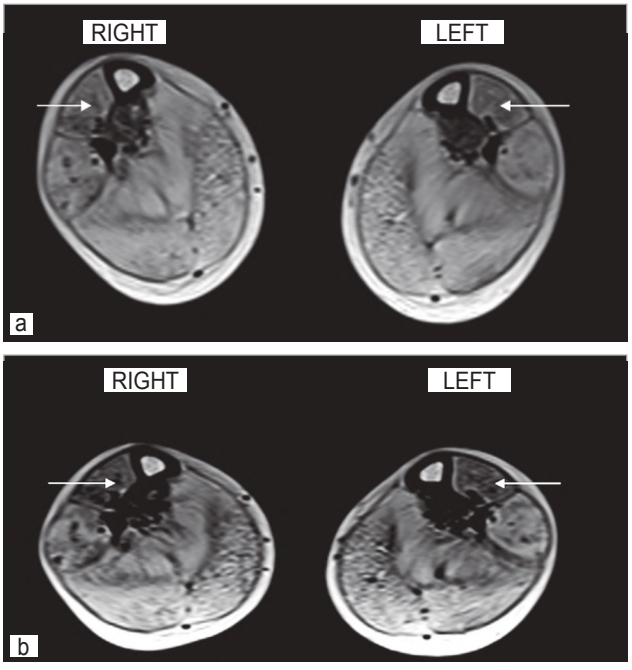

Figure 3. Axial T1W images at the level of the calf. **(a)** Pre-stem cell therapy show marked fatty infiltration of the bilateral tibialis anterior muscle (*thin arrows*) seen as high signal intensity. **(b)** Post-stem cell therapy shows reduced high signal in bilateral tibialis anterior muscle (*thin arrows*) suggestive of less fatty infiltration and regeneration of muscle fibers.

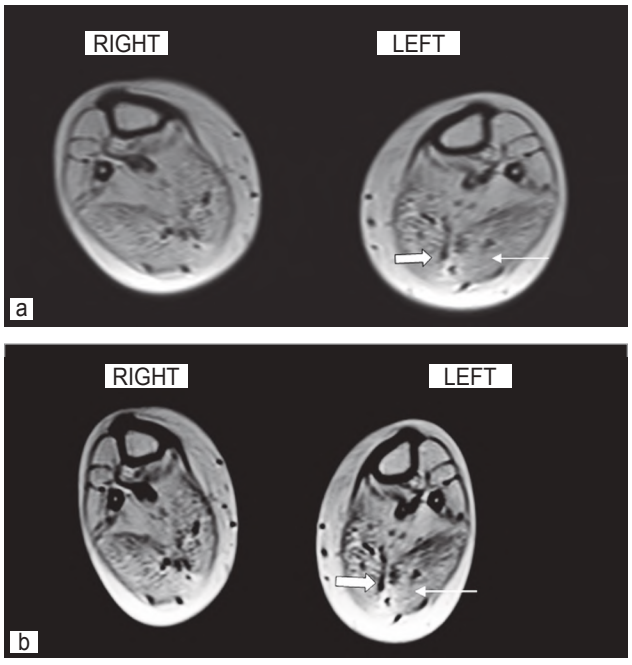

Figure 4. Axial T1W images at the level of the calf. **(a)** Pre-stem cell therapy show marked fatty infiltration of the left medial *(thick arrow)* and lateral gastrocnemius muscles *(thin arrow)*) seen as high signal intensity. **(b)** Post-stem cell therapy shows reduced high signal in left medial *(thick arrow)* and lateral gastrocnemius muscles *(thin arrow)* suggestive of less fatty infiltration and regeneration of muscle fibers.

Histological evaluation was not done since our Ethical Committee "Institutional Committee for Stem Cell Research and Therapy" does not permit invasive tests as biopsy in patients whose muscles are already weakened. Hence, the improvements were enumerated on the basis of MRI, CPK and clinical monitoring.

DISCUSSION

Presently, there is no cure for the disorder. Treatments available currently are palliative. Suitable management provided to the DMD patient can prolong survival and improve the quality-of-life significantly. Use of corticosteroids, physical therapy, surgical interventions, and respiratory management are few of the symptomatic treatments practiced for DMD.[4]

Encouraging experiments have been carried out wherein a number of adult-derived stem cells have been isolated, characterized and transplanted in mouse models for DMD. In humans, Gussoni et al have presented the clinical case of a young DMD patient showing deletion of the exon 45 in the dystrophin gene, wherein 12 years after bone marrow transplantation, showed donor nuclei fused to 0.5% of dystrophic myofibers.[5] We carried out autologous bone marrow-

derived MNC transplantation for the patient since they have no immunologic reaction, no ethical issues and no uncontrollable proliferation as in the case of embryonic stem cells. These MNCs comprised of a variety of cells like hematopoietic stem cells, tissue specific progenitor cells, stromal cells and specialized blood cells in different stages of development. MNCs diluted in CSF were also injected intramuscularly at specific motor points. CSF is known to harbor growth factors which helps the growth of the cortical epithelium and promotes vascularization in the nervous system. In addition, the G-CSF and methylprednisolone administered before and during the transplantation, respectively helped in stimulation of CD34+ cells and also in survival and multiplication of the stem cells.[6]

The extensive neurorehabilitation provided along with MNC transplantation has shown to promote recovery and independence through neurofacilitation. Apart from their individual impact, study shows that exercise enhances the effect of stem cells by helping the mobilization of local stem cells, encouraging angiogenesis and release of cytokines and nerve growth factors.[7]

The present data provides clinical and radiological evidence that autologous bone marrow-derived MNC transplantation along with neurorehabilitation can result in improvements in the case of DMD with no side effects. However, more clinical studies will be needed to prove this definition.

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