Autologous Bone Marrow Mononuclear Cell Transplantation Improves Function in a Case of Becker's Muscular Dystrophy

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Abstract

Becker's muscular dystrophy (BMD) is a genetic disorder characterized by progressive muscle degeneration and weakness with no definite cure. Cell therapy is emerging as a promising treatment option for BMD. Autologous bone marrow derived mononuclear cells (BMMNCs) transplantation has been shown to be safe and effective in previous pre-clinical studies. We treated a 21 year old boy suffering from BMD with autologous bone marrow mononuclear cells intrathecal as well as intramuscular transplantation. Pre-therapy clinical assessment showed reduced muscle power in all four limbs and trunk. He was modified independent for all the activities of daily living (ADLs) and scored 102 on Functional Independence Measure (FIM). Musculoskeletal Magnetic Resonance Imaging (MRI-MSK) revealed severe muscular atrophy and fatty replacement in the muscles. Repeat clinical assessment at follow up revealed maintained muscle power along with improvement in some muscles. North Star Ambulatory Assessment score and FIM score was maintained for over nineteen months. Repeat MRI-MSK revealed no increase in fatty infiltration in the muscles indicating halting of the progression of the disease. Thus, this report suggests the role of BMMNCs transplantation in stabilizing the condition of BMD patients. Larger clinical trials with scrupulous methodology may be suggested.

Keywords: *Becker's muscular dystrophy, cell therapy, Musculoskeletal Magnetic Resonance Imaging, Functional Independence Measure, autologous, bone marrow, mononuclear cells*

Introduction

Becker's muscular dystrophy (BMD), an X-linked recessive form of muscular dystrophy, caused by a mutation in the dystrophin gene, leads to absence of or defect in the dystrophin protein which causes muscle cell membrane instability in turn leading to muscle degeneration and necrosis, which is eventually replaced by adipose and connective tissue [1, 2]. Individuals with BMD experience difficulty in running, hopping, jumping, gradually progressing to difficulty in staircase climbing and walking with frequent falls [3]. Upper extremity and trunk muscle weakness also sets in. There is typical toe-walking and use of Gower's maneuver for getting up from the floor. Abnormal bone development occurs, leading to skeletal deformities of the chest and back like scoliosis and kyphosis. Loss of muscle mass, pseudohypertrophy, muscle cramps and contractures at various joints is seen [3]. BMD is also associated with cardiomyopathy and respiratory problems which includes signs and symptoms like arrhythmia, dyspnoea, fatigue, low exercise endurance, low lung volumes and capacities, and swelling of the legs and feet [4]. Dystrophin deficiency in the brain may cause cognitive and behavioral deficits like difficulty in attention focusing, verbal learning, memory, and emotional interaction [5].

The diagnosis of BMD is established upon the clinical findings, increased creatine phosphokinase (CPK) levels, electromyography and nerve conduction velocity (EMG & NCV) studies, Magnetic Resonance Imaging-Musculoskeletal (MRI-MSK); while genetic analysis and muscle biopsy confirm the diagnosis [6-8].

There is no identified cure for BMD. The standard medical, surgical, and rehabilitative interventions just manage the symptoms, preserve residual function and independence of the patient, but, do not modify the course of the disease [9]. Research is ongoing in the field of cellular therapy for muscular dystrophies.

Preclinical cellular therapy studies show evidence of muscle regeneration and improved function [10-12]. Autologous bone marrow mononuclear cells (BMMNCs) transplantation is one of the most advancing and interesting treatment approaches in cell therapy. This therapy has been known to activate the satellite cells and other factors responsible for muscle repair and regeneration process [13].

Sharma et al in their study using autologous BMMNCs transplantation in 150 patients with muscular dystrophy, showed symptomatic and functional improvements along with objective improvement on EMG and MRI-MSK [14]. Yang et al in a similar study used double transplantations of autologous bone marrow mesenchymal stem cells (BMSC) and umbilical cord mesenchymal stem cells (UMSC) in 82 cases of muscular dystrophy and showed a positive outcome with improvement in ADLs [15]. Sharma et al published reports of successful treatment of patients suffering from BMD with autologous BMMNCs transplantation and showed muscle regeneration on MRI-MSK with functional improvements [16, 17].

We present a case of a 21 year old boy suffering from BMD treated with autologous BMMNCs transplantation.

Case Report

We present a case of a 21-year-old male treated with cellular therapy. At the age of 4 years he started having difficulty climbing staircase. Gradually he started having difficulty in running and jumping with frequent falls while walking and running. At the age of nine years he started toe walking, experiencing fatigue and losing balance while walking after which he sought medical advice and was diagnosed as a case of Becker muscular dystrophy based on the clinical presentation, serum CPK levels and genetic testing supported by a strong family history of muscular dystrophy. Despite standard rehabilitation, the condition of the patient was deteriorating.

A detailed neuromuscular examination was done by a team of medical & rehabilitation experts. He had bilateral pectoral wasting, scapular winging, and pseudohypertrophy of calves and tongue. He had waddling type of gait, walking on toes on right side with left foot inversion and also hyperlordotic spine, with fear of fall and falls once in a week. Gower's sign was present. He had decreased tone in lower extremities. Muscular strength was measured by manual muscle testing, using a scale devised by our experienced physiotherapists based on the modified Medical Research Council's manual muscle testing scale (mMRC MMT). Since mMRC-MMT does not sub-classify grades 1 and 2 according to partial Range of Motion (ROM), in our scale (mMRC MMT – I) grades 1 and 2 were subdivided. This helped us to measure the subtle changes in the strength as observed in patients with BMD (Table 1). His upper extremity strength was above functional level (Grade 3), while lower extremity and trunk were below functional level. His right pinch and grip strength was 9 lbs and 30 lbs, and left 10 lbs and 35 lbs on the pinchometer and hand dynamometer respectively. Right tendoachiles contracture with a lag in range of 25 degrees, and left tendoachiles tightness which was stretchable up to neutral was present. Functionally the patient had fair sitting and standing balance. The patient had difficulty in rolling. Also he experienced early fatigability after walking for 15-20 steps. Inspiratory capacity was 2000 ml. The patient had a score of 102 on Functional Independence Measure (FIM) scale, 13 on North Star Ambulatory Assessment (NSAA) scale, 4 on Brooke and Vignos scale (Brooke - Grade 1, Vignos – Grade 3) and 25 on Berg Balance scale. Assessment by speech therapist revealed mild tongue hypertrophy with lisping; rate of speech being slow, with mild effort in speech after speaking for 20 minutes. Maximum Phonation Duration (MPD) was 15 sec.

Various investigations were done. Serum CK-MB (72 U/L), CPK (2770 U/L) were elevated. EMG revealed small amplitude, short duration motor unit potentials, increased interference pattern and early recruitment in bilateral tibialis anterior, gastrocnemius, vastus medialis, extensor digitorum communis, right biceps, triceps and deltoid muscles indicating a primary muscle disease. Motor NCV revealed low amplitude motor and F waves of bilateral common peroneal, axillary and musculocutaneous nerves. The genetic testing was done which showed dystrophin gene deletion in exons 45 and 60. MSK spectroscopy revealed pronounced peaks of extramyocellular and intramyocellular lipids with negligible other muscle metabolites. MSK DTI revealed markedly reduced muscle bulk. The FA values in the medial compartment thigh muscles were 0.389 on the

right side and 0.385 on the left side. MRI-MSK thus revealed severe muscular atrophy and fatty replacement in muscles of lower extremities, while moderate in muscles of upper extremities. 2D Echocardiography Colour Doppler revealed mild to moderately dilated LV, left ventricular ejection fraction (LVEF) of 36%, trivial mitral regurgitation, and pulmonary hypertension [Pulmonary artery pressure (PAP) = 35 mmHg].

Materials and Methods

Patient selection for the treatment was done based upon World Medical Association's Revised Declaration of Helsinki [18]. The ethical approval was obtained from the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). A duly filled informed consent was obtained from the patient and parents.

A week prior to the autologous BMMNCs transplantation, patient's serological, biochemical & blood tests, and chest X-ray were done to ensure pre-operative fitness. Motor point, the point where the innervating motor nerve enters the muscle, was identified by electrical muscle stimulation and marked on the skin. Motor points were marked for the muscles which were below functional level as well as antigravity muscles by an experienced physiotherapist for the purpose of intramuscular injections of stem cells. Granulocyte colony stimulating factor (GCSF) was administered subcutaneously 48 hours and 24 hours prior to the MNCs transplantation to stimulate CD34+ cells and enhance their survival and multiplication [19].

On the day of transplantation, 110 ml bone marrow was aspirated from iliac bone under local anaesthesia, using bone marrow aspiration needle and was collected in heparinized tubes. Density gradient method was used to separate the MNCs from the aspirate. Fluorescence activated cell sorting (FACS) analysis showed 98 % viability of the cells and CD34+ count to be at $4.32x10^8$. The separated cells were diluted in cerebrospinal fluid (CSF) and injected intrathecally in L4-L5 space and also intramuscularly in the motor points of the deltoid, biceps, rhomboids, glutei, quadriceps, tibialis anterior, peronei, back extensors and abdominal muscles bilaterally. Intravenous administration of Methyl prednisolone 1 gm in 500 ml Ringer lactate was done simultaneously to reduce immediate inflammation.

Following the transplantation, the patient underwent extensive physical rehabilitation which included strengthening exercises, suspension exercises with thera-bands and weight cuffs for muscles of limbs and trunk, balance training, functional reeducation in terms of transfers, and breathing exercises with incentive spirometry. Speech therapy included oral motor exercises to improve tongue flexibility, lips puckering and pursing; deep breathing exercises followed by slow rate of speech to improve clarity. The patient was discharged at one week and was advised to continue the rehabilitation at home. Follow up assessments were done at three, six, nine and thirteen months. MRI-MSK and 2D Echo tests were repeated at thirteen months. In view of improvements seen after stem cell therapy (as discussed in results), cellular transplantation was repeated thirteen months after the first transplantation. The clinical reason behind repeating the transplantation is discussed in discussion part. The procedure of transplantation was the same as of first transplantation.

Results

At one week after the first procedure of cell therapy, rolling, sit to stand and vice versa had slightly improved. Walking balance and stamina had improved. Tongue flexibility had improved with reduction in efforts while speaking. MPD had increased to 20 sec.

At three months after the first procedure, fear of falls had reduced with improvement in walking balance and speed. Number of falls had reduced to once in a month. Improvement in bilateral shoulder muscles, upper abdominal and back extensor muscles' strength was noticed (mentioned in table).

At six months after the first procedure, improvement was seen in dynamic walking balance. He could now climb up an elevation with one person assistance, which was not possible earlier. Power in bilateral peronei,

forearm supinators and pronators, palmar and dorsal interossei, and lower abdominals had improved (mentioned in table). Supine to sit and getting up from floor and chair required less effort. Waddling while walking had reduced. Fatigue levels had reduced. Inspiratory capacity improved to 2500 ml (previously 2000 ml). Right pinch strength improved from 9 lbs to 11 lbs.

At nine months after the first procedure, writing speed had increased. Getting up from floor was easier and required less effort and support.

At thirteen months after the first procedure, sitting and standing static as well as dynamic balance had improved. Stamina while performing exercises had improved. Score on Berg Balance Scale had increased from 25 to 27, on FIM from 102 to 103, while North Star Ambulatory Assessment score, and Brooke and Vignos scales score were maintained. Follow up MRI-MSK revealed no increase in fatty infiltration in the muscles of upper and lower extremities. 2D Echocardiography showed improvement in LVEF from 36% to 45% and normalization of pulmonary artery pressure.

At three months after the second procedure (sixteen months after the first procedure), pseudohypertrophy of the calves was reduced. Patient had no falls thereafter. There was further improvement in upper limb overhead activities, sitting, standing and walking balance. Stamina had also improved.

At six months after the second procedure (nineteen months after the first procedure), standing dynamic balance had improved further, and the Berg Balance Scale score also improved from 27 to 28. The rest of the aspects of function were well maintained.

Cell therapy produced no adverse effects.

Discussion

BMD is caused by a mutation of the dystrophin gene which codes for protein dystrophin. Dystrophin protein forms a structural and connective framework of the muscle tissues. Absence of or mutation of dystrophin leads to instability of muscle cell membranes and excess calcium influx causing water to enter into the mitochondria leading to its breakdown [20]. Mitochondrial damage triggers a complex process of numerous cellular events leading to cell membrane damage and eventually muscle cell death, followed by muscle fiber necrosis and replacement by adipose and connective tissue [21].

Skeletal muscles exhibit a tremendous potential of self-repair in response to damage which is dependent on the tissue specific stem cells called satellite cells. These cells differentiate into myocytes or self-renew to maintain their pool of satellite cells [22, 23]. However, continuous cycles of damage and repair in muscular dystrophy exhaust this pool of resident stem cells and render the muscle unable to repair itself anymore, which is eventually replaced by adipose and connective tissue [24]. Recent studies and research are, thus, aiming at aiding and improving the regeneration potential of muscles through newer techniques like stem cell transplantation. Various forms of stem cells possessing myogenic and neurogenic potential have been identified [25]. Autologous BMMNCs transplantation is safe and modifies the disease process in various neurological disorders [26]. These cells on interacting with the muscle environment, have been shown to contribute in muscle regeneration by exhibiting the paracrine effects - stimulating various growth factors, activating the satellite cells and angiogenesis, reducing inflammation and immune response, and controlling the apoptotic process, in various animal models [27-30].

Sharma et al carried out autologous BMMNCs transplantation in 150 patients diagnosed with muscular dystrophy. On follow up 86.67% cases showed symptomatic and functional improvements, six patients showed decrease in fatty infiltration and muscle regeneration on MRI-MSK, and nine showed improved muscle electrical activity on EMG [14]. Yang et al carried out a study to find out safety and efficacy of double

transplantations of autologous bone marrow mesenchymal stem cells (BMSC) and umbilical cord mesenchymal stem cells (UMSC) in 82 cases of muscular dystrophy. Both the types of cells were injected intramuscularly as well as intravenously. At follow up of 3 to 12 months, 82.9% cases showed a positive outcome with improvement in ADLs in 87.8% of the cases [15]. Sharma et al treated a 39 year old male with BMD with three autologous BMMNCs transplantations. At follow up, a gradual increase in the muscle strength was noted and the patient who was wheelchair bound started ambulating with push-knee splints independently. His FIM score increased from 93 to 105 and there was no increase in the fatty infiltration as seen on the MRI-MSK for over 2 years [16]. Sharma et al treated a 28 year old male suffering from BMD with autologous BMMNCs transplantation. At six months follow up repeat MRI-MSK showed regeneration of muscle fibers. This case report is one of the initial evidences of muscle regeneration following cellular therapy in case of BMD [17].

BMD is also associated with intellectual and cognitive impairment with patients having difficulty in learning, low IQ, behavioral and emotional issues [31, 32]. Several studies have shown presence of dystrophin in the synapses and myelin forming Schwann cells, absence or abnormality of which can lead to demyelination and degeneration in the nerves [33, 34]. Thus, we targeted the repair of both muscles as well as the nerves via intramuscular and intrathecal route of autologous BMMNCs transplantation respectively [35]. The intrathecal injection of BMMNCs also helps in axonal growth and sprouting, forming synaptic connections thereby strengthening the neuromuscular junction [36]. This improves the signal transmission in the damaged muscle. CSF is known to support viability and proliferation of cortical cells, and was, thus, used as a diluting medium [37, 38]. Physical activity and endurance training promote increased oxygen consumption, improve exercise performance and daily function, and thus, promote fitness and facilitate the efficacy of stem cell transplantation [39, 40]. Therefore we combined cellular therapy with intensive physical rehabilitation.

Resistance training increases muscular strength and endurance in patients with BMD [41]. Muscle power in all the muscles did not deteriorate over sixteen months, but was maintained. Slight improvement in muscle power of bilateral proximal upper extremity and peronei muscles was seen. Interestingly these were the muscles which were given intramuscular injection of BMMNCs.

Inspiratory resistive training enhances respiratory muscle endurance in muscular dystrophy patients [42, 43]. Incentive spirometry and breathing exercises coupled with endurance training improved his inspiratory capacity and stamina, and brought down his fatigue levels. A study by Leslie et al showed an average rate of decline in ejection fraction of 1.56% in childhood onset DMD and BMD [44]. A significant improvement in LVEF and pulmonary artery pressure was also seen. This may be attributed to improved endurance due to rehabilitation.

The Brooke and Vignos scales and the FIM are easy to assess functional rating scales in progressive muscle diseases, the scores of which did not deteriorate till nineteen months post therapy; which indicates that the disease pathology did not progress but was halted [45]. The NSAA, a validated functional rating scale used as a secondary outcome measure for muscular dystrophy also showed maintained scores nineteen months post therapy demonstrating the reparative effect of stem cell therapy [46].

MRI-MSK serves as a valuable outcome tool for measuring therapeutic effects at follow up [47]. BMD is associated with progressive muscle degeneration, atrophy and fatty infiltration. Post cellular therapy MRI-MSK showed no increase in muscle atrophy or fatty infiltration as compared to pre-therapy MRI, suggesting reduction and/or control over muscular apoptotic process following cellular therapy. Cellular therapy has shown reduction in serum creatine kinase levels in various studies [17, 29]. Repeating the procedure of cell therapy ameliorates the condition, helps maintain the improvements achieved and solidifies the improved state of the disease. Cellular transplantation may fill the gap between muscle degeneration and regeneration, thereby altering the disease progression in muscular dystrophy.

This is a single case report without control. But, this patient's condition was deteriorating despite the standard treatment, and the progression of the disease halted only after the cell transplantation. Therefore, patient served

as a self-control in this study. The other limitation is that the effect is of combination of cell therapy with rehabilitation. So, solitary effect of cell therapy could not be measured. But, since prior to cellular therapy, rehabilitation was ongoing and addition of cell therapy brought about the stabilization of the condition, suggest that cell therapy played a vital role.

Conclusion

This case report suggests that cellular therapy combined with rehabilitation may have the potential of repairing and regenerating muscle fibers in BMD. Since this is an observation in a single patient, it may recommend further research and large clinical trials for substantiating the effects of cellular therapy in regenerating muscle fibers and altering the disease progression in case of BMD.

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Table 1: Comparison of the grades of the scales mMRC-MMT and mMRC-MMT (I)

mMRC-MMT grade	Description	mMRC-MMT (I) grade	Description
0	No Movement	0	No movement
1	A flicker of movement is seen or felt in the muscle	1	A flicker of movement is seen or felt in the muscle
2	Muscle moves the joint when gravity is eliminated	1+	Muscle moves the joint through up to 1/3rd of the ROM when gravity is eliminated
		1++	Muscle moves the joint more than 1/3rd less than 2/3rdof the ROM when gravity is eliminated
		2-	Muscle moves the joint more than 2/3rd but less than the full ROM
		2	Muscle moves the joint through complete ROM when gravity is eliminated
3-	Muscle moves the joint against gravity, but not through full mechanical range of motion	2+	Muscle moves the joint up to 1/3rd ROM against gravity
		2++	Muscle moves the joint >1/3rd, <2/3rd of ROM against gravity
		3-	Muscle moves the joint more than 2/3rd but less than complete ROM
3	Muscle cannot hold the joint against resistance but moved the joint fully against gravity	3	Muscle moves the joint through complete ROM against gravity
3+	Muscle moves the joint fully against gravity and is capable of transient resistance, but collapses abruptly	3+	Muscle moves the joint against gravity and moderate resistance up to 1/3rd of ROM
		3++	Muscle moves the joint against gravity and moderate resistance from 1/3rd to 2/3rd of ROM
4-	Same as grade 4, but muscle holds the joint only against minimal resistance	4-	Muscle moves the joint more than 2/3rd but less than complete ROM against gravity and moderate resistance
4	Muscle holds the joint against a combination of gravity and moderate resistance	4	Muscle moves the joint against gravity and moderate resistance though complete ROM
4+	Same as grade 4 but muscle holds the joints against moderate to maximal resistance	4+	Muscle moves the joint against gravity and moderate to maximal resistance up to 1/3rd of ROM
5-	Barely detectable weakness	4++	Muscle moves the joint against gravity and moderate to maximal resistance from 1/3rd to 2/3rd of ROM (Barely detectable weakness)
5	Normal strength	5	Muscle moves the joint against gravity and moderate to maximal resistance though complete ROM (Normal Strength)

Table 2: mMMRC-MMT (I) scale grading for all the muscles as examined before stem cell transplantation

Muscle group tested	mMRC-MMT (I) score on the right side	mMRC-MMT (I) score on the left side
Hip		
Flexors	2+	2
Extensors	2-	1+
Abductors	2+	2+
Adductors	2-	2
Internal rotators	1+	1
External rotators	1	1
Knee		
Flexors	3+	3
Extensors	2++	2
Ankle		
Dorsiflexors	3+	3+
Plantar flexors	4	4
Invertors	3++	3++
Evertors	2+	2+
Shoulder		
Flexors	3+	3+
Extensors	3+	3+
Abductors	3+	3+
Adductors	3+	3+
Internal rotators	3+	3+
External rotators	3+	3+
Elbow		
Flexors	3+	3+
Extensors	3++	3++
Forearm		
Supinators	3++	3++
Pronators	3++	3++
Wrist		
Flexors	4	4
Extensors	4	4
Hand		
Lumbricals	4	4
Palmar interossei	3++	3++
Dorsal interossei	3++	3++
Trunk		
Upper abdominals	3+	
Lower abdominals	2+	
Back extensors	1+	

Table 3: Changes in the muscle strength after the first and second cellular transplantation as measured by mMRC-MMT (I)

Muscle group tested	mMRC-MMT (I) bilaterally before first cellular therapy	mMRC-MMT (I) bilaterally 6 months after first cellular therapy	mMRC-MMT (I) bilaterally 13 months after first cellular therapy	mMRC-MMT (I) bilaterally 6 months after the second cellular therapy
Hip				
Flexors	2	2+	2++	2++
Extensors	1+	1++	1++	1++
Abductors	2++	2+	2+	2++
Adductors	2	2	2	2
Internal rotators	1	1+	1++	2
External rotators	1	1	1	1+
Knee				
Flexors	3	2++	2++	2+
Extensors	2+	2++	2++	2++
Ankle				
Dorsiflexors	3+	3++	3++	3++
Plantar flexors	4	4	4	3++
Peronei	2+	3+	3+	3+
Shoulder				
Flexors	3+	3++	3++	3++
Extensors	3+	3++	3++	3++
Abductors	3+	3++	3++	3++
Adductors	3+	3++	3++	3++
Internal rotators	3+	3++	3++	3++
External rotators	3+	3++	3++	3++
Forearm				
Supinators	3++	3++	4	4
Pronators	3++	3++	4	4
Wrist				
Flexors	4	4	4	4
Extensors	4	4	4	4
Palmar interossei	3++	3++	4	4
Dorsal interossei	3++	3++	4	4
Trunk				
Upper abdominals	3+	4	4	3++
Lower abdominals	2+	2+	2++	2+
Back extensors	1+	2+	2+	2++

Table 4: Changes in the scores of standard scales after the first and second cellular transplantation

Scale used	Score before first cellular therapy	Score 6 months after first cellular therapy	Score 13 months after first cellular therapy	Score 6 months after the second cellular therapy
Brooke scale	1	1	1	1
Vignos scale	3	3	3	3
North Star Ambulatory Assessment	13	13	13	13
Berg Balance scale	25	25	27	28
Functional Independence Measure	102	103	103	103