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CASE REPORT

EFFECT OF CELLULAR THERAPY IN A CASE OF LIMB GIRDLE MUSCULAR DYSTROPHY

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ABSTRACT

Limb Girdle Muscular Dystrophy (LGMD) is a group of heterogeneous autosomal hereditary neuromuscular disorders resulting in progressive muscular weakness, predominantly in the proximal girdle muscles. Currently the management of LGMD is palliative and does not cure or alter the disease pathology. Hence there is a need of an intervention that may be able to alter the disease pathology. We present a case of a 41-year-old female of LGMD (Delta sarcoglycanopathy) who underwent two intrathecal and intramuscular transplantations of autologous bone marrow mononuclear cells (BMMNCs) along with standard rehabilitation at the interval of 7months. Musculoskeletal Magnetic resonance imaging (MRI-MSK) was conducted before both the transplantations. Over 13 months she showed improvements in functional outcome measures such as Bergs balance score, north star ambulatory assessment and six-minute walk test. Her functional independence measure score was unchanged for the 13 months since intervention. Per the natural course of the disease there is progressive muscle weakness. Maintenance of functional independence, improved muscle strength as well as six-minute walk distance and maintained MRI-MSK parameters over 13 months in this patient suggest that, cellular therapy along with neurorehabilitation may alter or halt the progression of the disease in LGMD.

INTRODUCTION

Limb Girdle Muscular Dystrophy (LGMD) is a group of heterogeneous autosomal hereditary neuromuscular disorders (1). It results in progressive muscular dystrophy (2). This group is associated with mutations in the genes which are responsible for muscle structure and function (1). Patients with LGMD are characterized by muscle weakness and wasting in the proximal muscle groups predominantly. It usually occurs in the hip and shoulder girdle muscles with muscle biopsy exhibiting degeneration. They relatively spare the bulbar musculature; however, exceptions do exist depending on the genetic subtype (3). The prevalence rate for all LGMD subtypes is variable (4). The pathophysiology corresponding to LGMD is different for each form (5).

Due to the variability in the genetic subtypes of LGMD, the management differs for each subtype. However, there is a lack of definite treatment modality for LGMD. A multidisciplinary approach including medical management and rehabilitative therapies available at the moment only improve quality of life but do not alter the disease progression or reverse the disease symptoms (3).

Cellular therapy has emerged as a novel treatment option (6) as stem cells can regenerate and multiply into multipotent cells (7). Autologous bone marrow mononuclear cells (BMMNCs) are easily isolated and available in abundance. BMMNCs have the potential to replace the damaged cells thus aiding in tissue recovery. Animal studies and case reports have highlighted the potential of stem cells in management of LGMD (8). Autologous BMMNCs have been used in other forms of muscular dystrophy like Duchenne muscular dystrophy as well as Beckers muscular dystrophy as a treatment modality; thus, suggesting the safety and efficacy of these cells (9-12). Mesangioblast, iPSCs and other stem cells have been successfully transplanted in mouse model of LGMD showing muscular regeneration (13). Intrathecal and intramuscular transplantation of autologous BMMNCs may be a potential treatment approach in LGMD.

We present a case of a 41 year old female of LGMD (Delta sarcoglycanpathy) who underwent intrathecal and intramuscular transplantation of autologous BMMNCs.

Case Report

A 41 year old female noticed buckling while walking and difficulty in descending the staircase at 14 years of age.

Gradually the weakness progressed to the upper and lower extremity. She found it extremely difficult to get up from the floor. She had a positive family history with her mother being diagnosed with myopathy as well. The patient was diagnosed as LGMDdelta sarcoglynapathy at the age of 35 years on the basis of muscle biopsy, histopathological and immunohistochemical analysisand creatinine phosphokinase serum enzyme levels. The histopathological findings from the muscle biopsy showed variation in fiber size and shape with rounded atrophic and hypertrophic fibers. It showed inflammation with active degeneration, increased adipose tissue and mild increase in collagen. These features were consistent with the findings of muscular dystrophy. Further immune histochemical analysis showed absence of delta sarcoglycans along sarcolemma and reduced expression of alpha and beta sarcoglycans. These features confirmed the diagnosis as delta sarcanoglycopathy.

She presented with complaints of difficulty in walking on uneven surfaces without supports, difficulty in staircase climbing, bilateral lower extremity weakness and buckling of the knees since 14 years of age. She also reported that her frequency of falls was 1-2 times/ 2-3 months. On assessment, we found that she was independent in her bed mobility and activities of daily living (ADL's). Postural examination showed a hyper lordotic lumbar spine with a waddling gait pattern. Bilateral hypertrophy of calf muscles was noted. Gower's sign was present and the time taken was 12 seconds. Neurologically, she was normotonic with diminished ankle reflex bilaterally. The other deep tendon reflexes were normal. Static and dynamic sitting balance was good, whereas her dynamic standing and walking balance was fair. Muscle strength was above the functional level (above Grade 3) in the hip flexors, abductors, internal and external rotators, knee flexors and extensors, ankle and foot, lower abdominals, shoulder girdle, elbow and forearm, wrist and hand muscles. The muscle strength in upper abdominals and back extensors was below the functional level (below Grade 3); whereas in hip extensors and adductors it was at the functional level (Grade 3). The muscle strength was measured with a revised version of modified medical research council grading for manual muscle testing. This revision was done to measure the small changes observed in the muscle strength post transplantation (Appendix I). She maintained a full range of motion in all the joints. Contracture and deformity was absent. Bergs Balance score was 49/56 and North Star Ambulatory Assessment (NSAA) was 20/34. She covered a distance of 231 meters in the six minute walk test. During the 6 minute walk test, her blood pressure was maintained at 110/80 mmHg and the rate of perceived exertion (RPE)was 8. FIM score was 111.

Laboratory investigation revealed that the CPK level were 318 U/L. 2D Echo Cardiography was normal. Musculoskeletal magnetic resonance imaging (MRI-MSK) was suggestive of severe muscular atrophy and fatty replacement in both extremities, consistent with advanced muscular dystrophy. Nerve conduction velocity (NCV) testing suggested that the NCV was normal and Electromyography examination showed early recruitment and myopathic potentials with predominant involvement of proximal muscles suggestive of muscular dystrophy.

Intervention

The ethical principle behind patient selection to provide this intervention was based on the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects (14) The ethical approval for the intervention was obtained from Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). The patient was explained about the procedure and a written informed consent was obtained. She was examined by a team of expert doctorsand therapists prior to the intervention. Pre-surgery routine blood tests, urine analysis, electrocardiogram, 2D-echocardiograph and chest x-ray were carried out for anesthetic and surgical fitness. Musculoskeletal – Magnetic Resonance Imaging (MRI-MSK) was performed which showed severe muscular atrophy with fatty infiltration consistent with the diagnosis of advanced muscular dystrophy. 300 mcg of Granulocyte colony-stimulating factor (G-CSF) injections were administrated subcutaneously 72 hours and 24 hours prior to BMMNC transplantation. This enhances the mobility of BMMNCs, stimulates CD34+ cells and increases their survival and multiplication rate(15).Motor points, the point where the innervating nerve enters the muscle belly, were identified for the muscles that were weakest and most important for functional independence by an experienced physiotherapist; a day prior to transplantation.

On the day of intervention, the patient was positioned in supine, local anesthesia was given in the region of anterior superior iliac spine. Bone marrow of amount 100 ml was aspirated from the iliac bone with the help of a bone marrow aspiration needle. It was then collected in heparinized tubes. Separation of mononuclear cells (MNCs) was carried out by the density gradient method under aseptic condition in a stem cell laboratory. The MNCs were then examined for CD34+ cell marker by FACS analysis and the viability of the cells was calculated. Cell viability was 98% and 1.34×10^8 MNCs were administered intrathecally at the level of L4-L5 and intramuscularly at different motor points in Glutei and Abdominals, using a25 G spinal needle. 1 gm of methyl prednisolone in 500 ml Isolyte P was simultaneously injected intravenously to reduce local inflammatory response and increase the stem cell survival. She was closely monitored during her stay at the hospital for any immediate adverse events. Post transplantation, the patient underwent standard neurorehabilitation including physical therapy, occupational therapy, psychosocial counseling and dietary counseling. Home exercise program was prescribed.

At 7 months after the first procedure she underwent a repeat transplantation in view of the positive outcomes. MRI-MSK was repeated to assess the outcome of transplantation. Cell viability was 99% and 2.38×10^8 MNCs were administered intrathecally and intramuscularly in the motor points of Glutei and Abdominals.

RESULTS

4 months follow up

No adverse events were noted during the follow up period. She showed stability in overhead, gross and fine motor activities, static and dynamic sitting and standing balance, bed mobility and ambulation. Her frequency of falls reduced from 2 episodes/2-3 months to 1 episode in 3 months. She also showed improvements in pseudohypertrophy of the calf muscles. Her maximum inspiratory volume increased from 250

ml to 1300 ml; whereas peak expiratory flow rate increased to 310 L/min. Her muscle strength was maintained as before. The FIM score remained unchanged at 111.

7 months follow up after first transplantation

Her ambulation and walking balance improved as the lurching and waddling reduced. The speed of walking also improved. Maximum inspiratory volume increased to 1500 ml; whereas the PEFR was maintained. Her stamina increased and the frequency of falls was the same as that of before transplantation. Muscle strength increased in hip extensors, abductors, adductors, internal and external rotators, knee flexors and extensors, plantar flexors and extensor hallucis long us bilaterally and abdominals (upper and lower). Her strength was maintained in the back extensors, ankle and foot muscles, shoulder girdle, elbow and forearm, wrist and hand muscles bilaterally (Table 1). The change in muscle strength has been given in Table 1. The distance covered in 6 minute walk test increased from 231 meters to 237.6 meters. FIM score and North Star Ambulatory Assessment (NSAA) remained unchanged at 111 and 20 respectively. Bergs Balance Score improved from 49/56 to 53/56. Gower's sign reduced from 12 seconds to 4.5 seconds.

MRI-MSK showed that muscle structure was maintained, there was no additional fatty infiltration and there was no deterioration as compared to before transplantation.

13 months follow up after first transplantation and 6 months after second transplantation

A follow up of the patient 13 months after 1st and 6 months after 2nd transplantation showed several improvements in function, muscle strength and endurance. Ambulation and walking balance and speed improved. She was more confident of walking outdoors. The balance had improved significantly and it also resulted in increased self-confidence. She could not perform the reach test during earlier evaluations due to fear of fall. In the follow up at 13 months she could reach forward to 26 cms, backward to 13 cms and sideways to 20 cms. Maximum inspiratory volume was maintained at 1500 ml; whereas the PEFR improved to 350 L/min. Her stamina increased and the frequency of falls reduced from 2 to 3 every 3 months to 2 to 3 in 6 months. Muscle strength increased in hip flexors, internal and external rotators. The improved strength at 7 months after stem cells was still maintained. The change in muscle strength has been given in Table 1. The distance covered in 6-minute walk test further increased from 237.6 meters to290 mts.

Table 1 Improvements in muscle strength graded according to manual muscle testing.

Muscle	Pre-Stem cell transplantation	7 months Post Stem cell transplantation	13 months Post Stem cell transplantation
Hip flexors	3++	3++	4
Hip extensors	3	3++	3++
Hip abductors	3+	3++	3++
Hip adductors	3	3+	3+
Hip internal rotators	3+	3++	4
Hip external rotators	3+	3++	4
Knee flexors	3+	3+	3+
Knee extensors	3+	4	4
Plantar flexors	3++	4	4
Extensor hallucis longus	3++	4	4
Upper abdominals	2	5	5
Lower abdominals	3++	5	5

This was a clinically significant increase. North Star Ambulatory Assessment (NSAA) remained unchanged 20. Bergs Balance Score improved from 53/56 to 54/56. FIM score improved from111 to 113. Gower's sign reduced from 4.5 seconds to 3.7 seconds.

Table 2 Outlines the improvements in the outcome measures at 7 months follow up

Outcome measures	Pre-Stem Cell Transplantation	7 months after Stem Cell Transplantation	13 months after Stem Cell Transplantation
6 minute walk distance	231 meters	237.6 meters	290meters
Bergs Balance Score	49	53	54
FIM	111	111	113
North Star Ambulatory Assessment	20	20	20
Gower's sign	12 seconds	4.5 seconds	3.7 seconds
Falls frequency	2 to 3 times / 3 months	2 to 3 times / 3 months	2 to 3 times / 6 months
Inspiratory volume	<250	1500	1500
Peak expiratory flow rate	130	310	350
Reach test	Could not be performed due to the fear of falls	Could not be performed due to the fear of falls	Forward – 26 cms Backward – 13 cms Left and Right – 20 cms

DISCUSSION

The LGMD consists of a range of hereditary neuromuscular disorders which is progressive in nature (2) resulting in muscular dystrophy. "Dystrophic pattern" is the common trait observed in the muscle biopsies of these patients. They are characterized by structural and pronounced variation in the muscle fiberwidth, phases of atrophy, hypertrophy, necrosis, phagocytosis, regeneration, and in the later stages the muscle fiber is replaced by fat and fibrous tissue (1).

LGMD are predominantly classified as LGMD1, autosomal dominant and LGMD2, autosomal recessive disorders (1). Dystrophies that are caused due to sarcoglycan protein deficiency or absence are termed as Sarcoglycanopathies and are of 4 types alpha, beta, gamma and delta. These proteins belong to dystrophin associated glycoprotein complex that act as muscle membrane stabilizers during muscle contraction and regulate muscle development, contractility and vascularization (3,5). A definite diagnosis of the subtype of LGMD can be made only with molecular testing in absence of molecular testing, histopathological and immunohistochemical analysis gives a phenotypic diagnosis (1). The immunohistochemical findings suggested that the patient had delta sarcoglycanopathy, these have autosomal dominant inheritance pattern and therefore genetic counseling is essential.

LGMD is a progressive disorder. No definite treatment is available. Cell transplantation has a unique potential to alter the course of the disease, as stem cells have the ability to differentiate and self-renew into multipotent cells (7). It has been widely used in the management of various incurable neurological disorders (9-12). In this study, we administered autologous BMMNCs intrathecally and intramuscularly to the patient. To alleviate the consequences of cell rejection, graft vs. host disease, an autologous approach was preferred. The targeted route of transplantation of stem cells is of utmost importance. Higher concentration of stem cell in the target area is desirable for maximizing the benefits of cellular transplantation. Hence, regional routes of transplantation of cells should be chosen (16). Systemic routes of transplantation

may trap the delivered cells in the lungs and the total number of cells reaching the target area may not be adequate to produce desirable results (17). Motor points are the points where the innervating nerve enters the muscle, this point has the highest concentration of myoneural synapses. Intramuscular injection at motor points of weak muscles was also administered to the patient to stimulate local satellite cell pool and to strengthen the neuromuscular junction (18). In our patient the muscles that received intramuscular injections showed improvement on manual muscle testing that also translated in function.

The mechanism of action of BMMNCs is twofold; to protect the existing tissue and to replace the damaged tissue. BMMNCs were used as they can be easily isolated and do not have any ethical issues. They promote muscle regeneration, recolonization of muscle fibers by satellite cells, and enhance muscle function, thus resulting into improved functional recovery (19). The genetic mutations in LGMD is irreparable; however, the resultant muscle damage which is progressive in nature can be altered or arrested with stem cell transplantation. The transplanted cells can replenish the satellite cell pool and therefore prevent from exhaustion of stem cells.

Stem cells migrate and home onto the injury site, thereby reducing the inflammation by mediating inflammatory markers (20). Neuroprotection and neuroangiogenesis is brought about by the paracrine effects of BMMNCs which secrete various factors such as a brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and hepatocyte growth factor (HGF) by these stem cells (21-23). This improves the microcirculation and enhances tissue function. MNCs are combination of multiple cell fractions; one of these cells is Mesenchymal cells (MSCs). MSCs secret exosomes that influence angiogenesis, neurogenes is and reduce inflammation (24). MSCs derived exosome secretion is stimulated by inflammation. In a chronic inflammatory condition like that of LGMD, MSCs exert an exosome mediated immune modulation and inhibit fibrotic injury(25). Exosomes also promote formation of new muscle fibers by enhancing myogenesis and angiogenesis in the skeletal muscles (26).

Various studies have also demonstrated beneficial effects of BMMNCs in improving the quality of life of patients with muscular dystrophy (11, 12). Our previous study evaluated the effects of intrathecal and intramuscular transplantation of autologous BMMNCs on the natural course of 65 patients with LGMD. The results showed improvements in the FIM and muscle strength, thereby indicating a plateau phase in the disease progression of these patients (27).

Cellular therapy aims at regeneration of damaged muscle fibers through a targeted delivery of cells (16). In progressive disorders, such as LGMD 2 F, clinical stability in functional activities is also considered as an improvement. Patient in this case report reported progressive functional decline and muscle weakness however post transplantation the functional deterioration halted and independence improved over the 13 months. NSAA score was maintained and FIM score improved from 111 to 113. Furhter improvements in the bergs balance scale, 6-minute walk test, gait pattern and respiratory capacity may therefore be attributed to the beneficial effects of cellular transplantation in combination with exercise. Increased muscle strength in the lower extremities and abdominal muscles has probably led to the improvements in gait pattern and respiratory capacity. LGMD is a progressive condition with increasing weakness of the muscles, increasing functional deterioration and progressive dystrophic changes in MRI-MSK. The improved muscle strength after13 months of transplantation, significantly increased 6-minute walk distance and maintained MRI-MSK is suggestive of altered disease progression.

Studies suggest that exercises aid in the mobility of the local stem cells resulting in angiogenesis (10). It is postulated that a combination of aerobic and strength training improves the walking capacity and postural balance in patients of muscular dystrophy (25). Neurorehabilitation has an anti-inflammatory and angiogenetic effect on various systems of the body, thus complementing the paracrine effects of stem cells (27,28). Exercise also stimulates satellite cells and therefore resonates with paracrine effects of BMMNCs which enhances the environment for better cell survival (29).

Limitation of this study was that the effect of cellular therapy could not be solely assessed based on its effect in an individual alone.

CONCLUSION

This case report demonstrated that in a case of LGMD (Delta sarcoglycanopathy), with progressive muscle loss and functional dependence; autologous BMMNC transplantation has halted the progression as evidenced by the MRI-MSK. It is a novel therapeutic modality and may alter the disease process. Cellular therapy along with neurorehabilitation may have beneficial effects on the progression of the disease in LGMD. However, the evidence for cellular therapy is primitive. Hence, controlled studies or rigorous methodology should be conducted for definitive findings. MRI-MSK could be used as one of the outcomes to measure disease progression.

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