Effect of autologous bone marrow mononuclear cell transplantation coupled with rehabilitation in limb girdle muscular dystrophy – A case report

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ABSTRACT

Cell transplantation is emerging as a potential therapeutic option for LGMD due to its ability to aid in repair and regeneration of dystrophic muscles. Our aim was to study the benefit of autologous bone marrow mononuclear cells (BMMNCs) in LGMD. We administered a 39 year old LGMD male with autologous BMMNCs intramuscularly and intrathecally twice followed by rehabilitation. His muscle power in all limbs was below functional level with proximal weakness more than distal. Functional Independence Measure (FIM) score was 114. Over a period of 18 months, muscle power increased gradually with improvements in functional activities. FIM score was maintained indicating a halt in the progression of disease. 36 months after second dose of transplantation, patient's condition was maintained with no deterioration in quality of life. This report provides early evidence of beneficial effects of autologous BMMNCs coupled with rehabilitation in halting the disease progression and functional improvement in LGMD.

Keywords: autologous, bone marrow mononuclear cells, LGMD, FIM, rehabilitation, quality of life.

INTRODUCTION

Limb girdle muscular dystrophy (LGMD), a form of muscular dystrophy is a heterogeneous group of genetic disorder, which could be either autosomal dominant or recessive [1, 2]. Currently, more than 25 types of LGMDs have been identified according to the affected gene. Its clinical presentation is characterized by progressive weakness and atrophy of proximal limb muscles, and in later stages weakness of distal limb muscles and cardiomyopathy.

There are no established treatments available for LGMD. Different therapeutic approaches are being investigated with their main objective being prolonged survival and improvement in quality of life of the patient. Recently, cell therapy is being studied extensively for various incurable neurological disorders including muscular dystrophies [3, 4]. Many experimental studies have demonstrated halt in the disease progression along with muscle fiber regeneration in animal models [5, 6, 7, 8]. To study the effect of cell therapy, we administered a diagnosed case of LGMD with autologous bone marrow mononuclear cells (BMMNCs). These cells are easily obtainable; they do not involve any ethical or moral controversies. Being autologous, they are safe owing to the absence of immune reaction and rejection in the recipient [9].
These cells once injected, migrate to the areas of muscle damage, survive and differentiate into muscle cells resulting in repair of damaged muscle fibers [6, 8]. Studies have also demonstrated expression of dystrophin and other myoregulatory proteins in the regenerated muscle fibers [7].

This case report presents the positive outcome of intramuscular and intrathecal transplantation of autologous BMMNCs in a 39-year-old male with limb girdle muscular dystrophy.

**MATERIALS AND METHODS**

**Case Presentation**

A 39-year-old male was diagnosed with limb girdle muscular dystrophy at the age of 25 years. He had a strong family history with 4 out of 7 siblings affected with LGMD. His symptoms began at the age of 25 years, with lower extremity weakness, imbalance and frequent falls while walking. However, his upper extremities were spared. Gradually, weakness progressed due to which getting up from floor; squatting and climbing stairs became difficult. He also got fatigued on walking short distances. There were no deformities; and he walked with a foot drop gait. He experienced frequent falls (4 times in 6 months) while walking due to buckling of knees. He walked indoors with support of the wall and required assistance to get up from a chair. Muscular strength was measured by Manual Muscle Testing (MMT), using a scale devised by experienced physiotherapists based on the modified Medical Research Council’s MMT scale (mMRC MMT). As mMRC MMT does not sub-classify grades 1 and 2 according to partial Range of Motion (ROM), in this scale (mMRC MMT-I) grades 1 and 2 were subdivided [Table 1]. This scale could measure slightest of changes in muscle strength in muscular dystrophy patients over time. On examination, he had grade 2+ muscle power in both lower extremities proximally and grade 3+++ in both upper extremities proximally along with pseudohypertrophy of bilateral calves. He was independent in most of activities of daily living (ADLs) and scored 114 on Functional Independence Measure (FIM). Electromyography (EMG) suggested generalized primary muscle disease and Magnetic Resonance Imaging - Musculoskeletal (MRI-MSK) showed extensive diffuse bilaterally symmetric changes of atrophy and fatty infiltration in muscles of upper and lower extremities.

**Intervention:**

The rationale of this intervention is based on World Medical Association’s declaration of Helsinki and thus patient selection was done based on the paragraph 37 of the amended Helsinki declaration stating about use of unproven interventions [10]. The protocol has been reviewed and approved by the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). The patient and his relatives were informed about the procedure and a duly filled informed consent form was obtained from them.

Blood Tests, EMG and MRI-MSK were performed one week before the transplantation. Motor points of the muscles which were below functional level were identified by electrical muscle stimulation and marked on the skin for the purpose of intramuscular injections of stem cells. Granulocyte colony stimulating factor (G-CSF) injections were administrated 72 hours and 24 hours before BMMNCs transplantation [11]. On the day of transplantation, 100ml of bone marrow was aspirated from the iliac bone. Mononuclear cells (MNCs) were obtained after density gradient separation. Fluorescence activated cell sorting (FACS) analysis showed 91% viability of the cells. Half of the cells were injected intrathecally in L4-L5 space using a lumbar puncture needle, and the remaining cells were then diluted in cerebrospinal fluid (CSF) and injected intramuscularly in the motor points of bilateral deltoit, glutei, quadriceps, hamstrings, peronei, tibialis anterior, back extensors and abdominal muscles bilaterally. Approximately 1.48x10⁸ MNCs were injected by both the routes. Intravenous administration of Methylprednisolone 1 gm in 500 ml Ringer lactate was carried out simultaneously to improve stem cell multiplication and survival.

After the transplantation, the patient was put on a personalized physical rehabilitation program which included physiotherapy to strengthen the weak muscles and improve endurance, occupational therapy to improve function and independence, and psychological counseling for motivation. After one week, on discharge the patient was given a home program to continue rehabilitation at home. Follow up assessments were done periodically, at six and eighteen months. After eighteen months, he underwent a second transplantation of autologous BMMNCs.

**RESULTS**

No adverse events were reported after cell transplantation. On follow up after six months, his standing posture had improved due to decreased hyperlordosis of lumbar spine owing to improved trunk stability. He required less base of
support while walking. Knees became more stable, and there was no buckling of knees anymore. This improved his standing and walking balance. He could lift his leg about 4-5 inches up and could stand on one leg for 30 seconds, owing to improvement in standing balance. His ankle movements had improved as there was increase in muscle power of ankle muscles. He could independently get up from a low chair for which he required assistance earlier. Climbing down stairs was easier and required less support. FIM score was maintained at 114. Muscles of lower limbs showed increased power on MMT [Table 2].

18 months after intervention, frequency of falls had markedly reduced (earlier it occurred 4 times in 6 months, and after intervention it occurred 2 times in 6 months). He could balance himself while walking on uneven surfaces and hyperlordosis of spine while standing and walking had also reduced considerably. His stamina improved furthermore so he could work for longer time. Climbing up stairs was easier and caused less fatigue. Pseudohypertrophy of the calves had reduced and the calves became softer.

FIM score was maintained at 114.

At 3 years follow up post second dose of cell transplantation, all the functional improvements were maintained. The patient could perform his daily activities in a similar manner as on follow up before second procedure of cell transplantation, without any deterioration in quality.

**DISCUSSION**

LGMDs are associated with continuous cycles of muscle fiber necrosis followed by regeneration of fibers, until the exhaustion of muscle regenerative capacity [12]. Studies are thus aiming at facilitating the regeneration potential of muscles. Cellular therapy has effectively demonstrated its myogenic potential in various forms of muscular dystrophies [13 – 17]. Adult bone marrow cells are one of the safest and widely used cells. In our case we administered BMMNCs due to their properties of self renewal, migration and differentiation. Bone marrow cells have the ability to differentiate into muscle cells [18, 19]. Animal studies have demonstrated the biological progression of marrow cells into muscle satellite cells which in turn progress to myoblasts eventually giving rise to mature muscle fibers [20].

The underlying mechanism of action of these cells mainly includes activation of local satellite cells, angiogenesis for repair, and reduction of inflammation and immune response. They also carry out repair and regeneration by producing a variety of cytokines, chemokines, growth factors and extracellular matrix (ECM) molecules, known as paracrine activity. These growth factors are known to promote stem cell proliferation, cytoprotection and migration. These effects control the muscle cells’ apoptotic process and help in regeneration over time [21 – 24].

BMMNCS is a mixture of cells of hematopoietic as well as non hematopoietic lineages, the latter including the side population cells, mesenchymal stromal cells (MSCs), multipotent adult progenitor cells, hemangioblasts, endothelial progenitor cells (EPCs) and tissue-committed stem cells [25]. Collectively these cells are known to exert a greater therapeutic effect rather than individual sub fractions [26].

Studies have provided evidence of migration and homing of MSCs in the injured tissues after transplantation and localization of engrafted MSCs have found to be more in the severely injured tissues [18]. MSCs also exert their immunomodulatory effects by inhibiting T-cell proliferation thereby making a room for initiation of reparative process [19].

Motor point is the point/location of the skin above the muscle at which least intensity of electrical impulse produces a maximal contraction of muscle [27]. Because of the presence of highly efficient nerve-muscle synapses at this point, motor points of weaker group of muscles were chosen for the purpose of intramuscular injections.

The dystrophin–glycoprotein complex (DGC) interacts with neuronal nitric oxide synthase (nNOS) which is involved in synthesis of nitric oxide at the neuromuscular junctions. Nitric oxide is an important signaling molecule involved in regulation of synaptic functions in the central nervous system and release of acetylcholine from nerve terminals at the neuromuscular junction. The DGC is composed of cytoskeletal proteins, the dystroglycan complex and the sarcoglycan (SG) complex. LGMDs result due to genetic mutations causing defects in these cytoskeletal proteins important for synthesis of nitric oxide. Thus, reduction or loss of nNOS and in turn nitric oxide contributes to weakening of neuromuscular transmission and muscle fiber degeneration in LGMDs. Therefore, intrathecal
BMMNCs transplantation was carried out to strengthen the neuromuscular junction and to improve synaptic transmission [28,29].

Sharma et al, in their study using autologous BMMNCs transplantation demonstrated the safety and efficacy of the intervention in modifying the disease process in muscular dystrophies. A total of 150 patients with muscular dystrophy including LGMDs were administered autologous BMMNCs via intramuscular as well as intrathecal routes, and on a mean follow up of 12 ± 1 months, symptomatic and functional improvements were observed in 86.67% of cases, supported by radiological evidence of decrease in fatty infiltration and muscle regeneration [30].

Rehabilitation interventions seek to promote recovery and independence through neurofacilitation. Exercise enhances the effect of stem cells by helping the mobilization of local stem cells, encouraging angiogenesis [31,32]. Therefore, the patient was put on a rehabilitation program to augment the effect of cellular therapy.

Following cellular transplantation, muscle power had improved and thus, the patient was able to carry out daily activities with much ease. The changes seen after cell transplantation were maintained for over a period of 54 months, which suggest a halt in the progression of the disease. Prior to cell transplantation, inspite of regular rehabilitation and standard treatment, patient’s condition was deteriorating. Whereas, after cell transplantation coupled with rehabilitation, functional improvements were observed which were maintained for a long period of time; with FIM score also remaining the same. These findings suggest the cytoprotective and myogenic actions of BMMNCs transplantation.

Several studies suggest benefits of repeated cell transplantation on repopulating the damaged tissues [33,34]. Therefore, to further enhance the improvements and to supplement the dystrophic muscles with additional number of stem cells, cell transplantation was repeated after 18 months. The second dose of cell transplantation helped maintain the condition of patient for more 36 months indicating a halt in the disease process.

This is a single case report without control. But, since the patient’s condition was deteriorating in spite of standard treatment and rehabilitation; functional improvements and halting of disease progression were obtained only after the cell transplantation, we may say that the patient served as a self control in this study. The other limitation of this study is that the results obtained were effects of cell transplantation coupled with rehabilitation, thus, solitary effect of cell transplantation could not be gauged. But, since rehabilitation was going on prior to cell transplantation also and the procedure of cell transplantation helped in halting of disease progression, suggest that cell transplantation played a crucial role.

#### Tables

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

<table>
<thead>
<tr>
<th>mMRC-MMT grade</th>
<th>Description</th>
<th>mMRC-MMT (I) grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Movement</td>
<td>0</td>
<td>No movement</td>
</tr>
<tr>
<td>1</td>
<td>A flicker of movement is seen or felt in the muscle</td>
<td>1</td>
<td>A flicker of movement is seen or felt in the muscle</td>
</tr>
<tr>
<td>2</td>
<td>Muscle moves the joint when gravity is eliminated</td>
<td>1+</td>
<td>Muscle moves the joint through up to 1/3rd of the ROM when gravity is eliminated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1++</td>
<td>Muscle moves the joint more than 1/3rd less than 2/3rdof the ROM when gravity is eliminated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-</td>
<td>Muscle moves the joint more than 2/3rd but less than the full ROM</td>
</tr>
<tr>
<td>3-</td>
<td>Muscle moves the joint against gravity, but not through full mechanical range of motion</td>
<td>2+</td>
<td>Muscle moves the joint up to 1/3rd ROM against gravity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2++</td>
<td>Muscle moves the joint &gt;1/3rd, &lt;2/3rd of ROM against gravity</td>
</tr>
</tbody>
</table>
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

4+ Muscle moves the joint against gravity and moderate to maximal resistance from 1/3rd to 2/3rd of ROM (Barely detectable weakness)

5- Barely detectable weakness

5 Normal strength

5 Muscle moves the joint against gravity and moderate to maximal resistance though complete ROM (Normal Strength)

<table>
<thead>
<tr>
<th>Muscle group tested</th>
<th>mMRC-MMT (I) before first cellular therapy</th>
<th>mMRC-MMT (I) 6 months after first cellular therapy</th>
<th>Muscle group tested</th>
<th>mMRC-MMT (I) before first cellular therapy</th>
<th>mMRC-MMT (I) 6 months after first cellular therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip</td>
<td>2++</td>
<td>3</td>
<td>Extensors</td>
<td>2++</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2++</td>
<td>3+</td>
<td>Abductors</td>
<td>2++</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>2++</td>
<td>3</td>
<td>Adductors</td>
<td>2++</td>
<td>3</td>
</tr>
<tr>
<td>Knee</td>
<td>2+</td>
<td>3+</td>
<td>Flexors</td>
<td>2+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2++</td>
<td>Extensors</td>
<td>2+</td>
<td>2++</td>
</tr>
<tr>
<td>Ankle</td>
<td></td>
<td></td>
<td>Tibialis Anterior</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

CONCLUSION

As the number of cases of LGMD is increasing, it is essential to establish a standard approach. Autologous BMMNCs transplantation has shown great potential as a therapeutic strategy for various muscular dystrophies. This case has effectively demonstrated the positive outcome of autologous BMMNCs serial transplantation along with rehabilitation in LGMD. It helps in halting the progression of the disease and improves the quality of life of the patients. However, more detailed studies involving rigorous methodology are required to establish its potential in LGMD.

REFERENCES