Allogenic Mesenchymal Stromal Cell Therapy for Type III Spinal Muscular Atrophy: Case Report

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Abstract: Rationale: Spinal Muscular Atrophy (SMA) is the most common genetic disorder and presents the most common cause of infant mortality. To date, patient management is symptomatic and focuses on improvement of independence and treatment of complications. Stem cell therapy represents a novel therapeutic option for many neurological diseases. Presenting concerns of the patient: This patient with type III SMA presented with generalized hypotonia and muscle weakness with inability to raise hands and legs, support back or neck, in addition to respiratory distress. Diagnosis: Clinical examination showed hypotonia and loss of reflexes. Creatine kinase levelxxx, electrophysiologyxxx. Interventions: Allogenic mesenchymal stem cells (MSCs) were injected in a dose of xxx intrathecally and a dose of xxx injected systemically. Outcomes: The patient showed improvement of GFM score and upgrading of the GFMC grade from Grade V to Grade III in 3 months. Improved quality of life was reflected in improvement of the PEDI scores. Improvement was noticed in respiration. No complications were encountered. Improvement was maintained until date. Conclusions: Allogenic MSC therapy may present a new therapeutic strategy for SMA patients. Controlled clinical trials are recommended to document the safety and efficacy of the procedure.

Keywords: Neuroregeneration, Spinal Muscle Atrophy, Mesenchymal Stem Cells

1. Introduction

Spinal muscular atrophy (SMA), first described by Werdnig in 1891, encompasses a group of disorders with degeneration of motor neurons in the anterior horn of the spinal cord *(1)*. SMA occurs in an incidence of 1:11,000 live births with a carrier frequency of 1:54 *(2)*. It is the most common genetic cause of infant death. SMA shows a wide spectrum of expression and classified into types I, II, and III based on the age of presentation and motor function *(3)*. Pathogenesis of SMA has been attributed to homozygous loss of survival motor neurone 1 (SMAI) gene *(4)*.

Diagnosis of SMA is usually by clinical presentation. The patient present with hypotonia with absent reflexes *(5)*. Unlike myopathies, serum creatine kinase may be normal or increased *(6)*. Electromyographic examination shows fibrillar pattern and muscle denervation *(7)*. Genetic testing shows homozygous deletion of exon of the SMN1 gene.

2. Case Report

In this report, we describe a female patient, age 23 years. Presentation started at 6 years of age with gradual muscle weakness. The patient was

2.1. Clinical Examination

Marked weakness of all voluntary muscles of the upper and lower limbs, back muscles causing scoliosis and inability to sit without support. Weakness of the neck muscles cause failure of head support. Marked weakness of both proximal and distal limb muscles with inability to raise hands, arms or legs.

Deep tendon reflexes were absent in upper and lower limbs.

2.2. Laboratory Investigations

Creatine kinase: 250 ug/l
ESR: First hour 45mm

2.3. Electrophysiology

Normal sensory nerve conduction
Diminshed nerve signals

2.4. Muscle Biopsy

Marked muscle fiber atrophy

2.5. Therapeutic Intervention

2.5.1. Donor Selection

As the disease is recessively inherited, allogenic mesenchymal stem cells were used. Routine screening for the donor and HLA matching was done. As pure mesenchymal stem cells will be used 4 out of 6 loci matching was accepted.

2.5.2. Sampling

The donor was prepared by 5-day G-CSF mobilization regimen (8). Leukapharesis was done using COBE SPECTRA leukapharesis machine.

2.5.3. Cell Preparation

Mononuclear cells harvested from the leukapharesis machine were counted and MSC isolation was done as follows:

MNCs were plated in a density of 5×10^5 cells in T25 flasks in complete DMEM low glucose medium containing 10% patient's serum, 1% penicillin-streptomycin and 10% L-glutamine. Flasks were incubated at 37ºC in 5% CO2 humidified incubator. After 24 hours, non-adherent cells were removed and medium was replenished. Medium was changed every 3 days and flasks examined under inverted microscope until cells 85% confluence.

Harvest of MSCs was done using 0.05% trypsin-EDTA, harvested cells were counted, and tested for viablity using trypan blue exclusion test. Immunophenotyping was done using MSC identification kit and functional identification kit (R&D). MSC lineage was verified according to the minimal criteria proposed by the International Society for Cellular Therapy (ISCT)(9).

2.5.4. Stem Cell Injection

Figure (1). Diagram Showing Steps of therapy.

Under complete aseptic conditions, isolated MSCs were injected intrathecally in a dose of 100 x10^6 suspended in 5ml sterile saline for injection.

This procedure was repeated after 3 and 6 months (Figure1)

2.5.5. Follow-up

The patient was followed up at 1, 3 and 6 months using:
1. Clinical Examination:
2. Gross Motor Function Scale (10)
3. PEDI quality of life score (11)
4. Quality of life score using the 100 point scale (12)

3. Results

3.1. MSCs Identification

Leukapharesis yield was 230ml with total count of 64 x10^3/cmm. Immunophenotyping of the isolated MSCs revealed 92% positivity for CD90, 90% for CD105, 88% for CD73, 2.3% for CD34 and 0.9% for CD45. Viability of the isolated MSCs was 94%.

3.2. History and Clinical Examination

The patient presented with scoliosis, generalized hypotonia with absent reflexes, inability to support the head, inability to walk, sit unassisted, respiratory distress.

Arm functions were minimal, with inability to hold objects, or raise arm.

No bulbar symptoms were reported.

Creatine kinase level was 250ug/l

Electrophysiologic examination showed pattern of lower motor neurone lesion.

Follow-up 1

After one month of the first stem cell dose, the patient came for re-assessment. Improved head support was noticed, in addition to improved tone of the back muscle with resultant improvement in sitting unassisted.

Improved ability to use the hand and hold objects, but with no ability to raise the arms. Improved respiratory functions were noticed.

Follow-up 2

After 3 months, the patient showed ability to raise the arm, move the legs (at the knee joint), return of reflexes. The patient can sit unassisted, move and turn in bed and pick up objects from the floor.

Follow-up 3

After 6 months of the first dose, and months of the second dose, the patient showed ability to use arm and hand muscles against resistance, improvement in overall independence was clear.

3.3. Gross Motor Function Scale

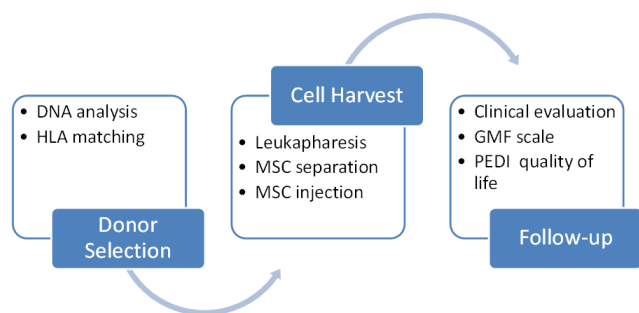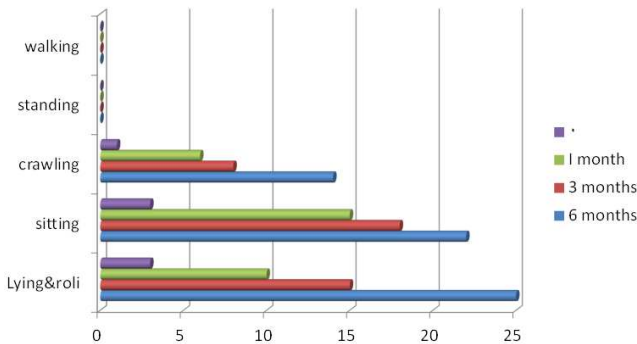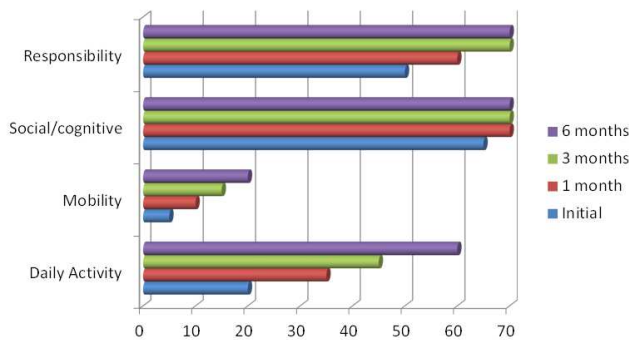Follow-up figures for the GMFS is shown in Table (1) and Figure (2)

Table (1). Gross Motor Function Scale over the follow-up period.

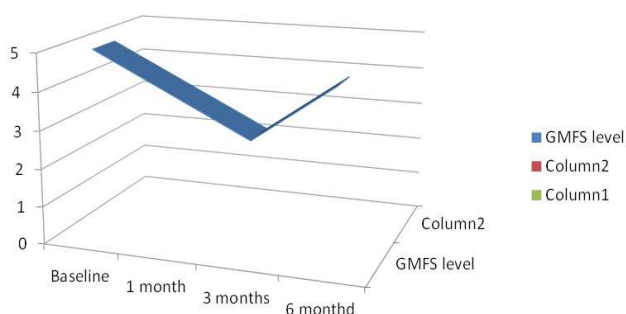| Item	Initial Score	1 month	3 months	6 months
A. Lying and rolling	3 5.88%	10 29.61%	15 29.41%	25 49.02%
B. Sitting	3 5.00%	15 25%	18 30%	22 36.67%
C. Crawling and kneeling	1 2.38%	6 12.29%	8 19.05%	14 32.56%
D. Standing	0 0%	0 0%	0 0%	0 0%
E. Walking, running, jumping	0 0%	0 0%	0 0%	0 0%
Total		11.38%	15.69%	23.67%

Figure (2). Change in GMFS over the follow-up period.

3.4. PEDI Score

Figure (3). PEDI score evaluation over the follow-up period.

3.5. GMFC Grading System

Initial evaluation of the patient revealed Level V GMFC, it changed to level IV at 1 month and to Level III at 3 months, and remained at Level III at 6 months.

Figure (4). GMF scale over the follow-up period.

3.6. Quality of Life

The patient's quality of life showed great improvement. She started to eat independently, read, draw. Objective quality of life assessment was not done using PEDI score .

4. Discussion

SMA is the most common genetic cause of infant death, with a carrier rate of 1:54. With the goal of maximizing independence and improving quality of life, therapeutic options are physiotherapy, splints and health education. Regenerative medicine represents a novel therapeutic approach for neurodegenerative disorders. Several reports document the therapeutic efficacy of stem cell therapy in spinal cord injuries, cerebral palsy, Parkinson's disease, and other neurodegenerative disorders (12,13,14).

This study used allogenic MSCs injected intrathecally in an attempt to induced regeneration in damaged motor neurones. Due to its genetic nature, allogenic stem cells were used in order to introduce normal SMN1gene (15).

Mesenchymal stem cells were obtained from leukapharesis in the first session; while in the following two sessions, bone marrow aspiration was done. Mesenchymal stem cells isolated from mobilized peripheral blood share the neuroregenerative characteristics with bone marrow derived MSCs (16, 17).

One of the great challenges in cellular therapy is the development of objective follow-up tools to evaluate the efficacy of the procedure. This study adopted the recommendations of the NIH So-SMART workshop, using the Gross Motor Function Score and Gross Motor Function Grading System to evaluate motor function. Evaluation of the effect of cellular therapy on the quality of life of the patient was done using PEDI score and personal interview with the patient.

The patient showed improvement of muscle tone and respiration starting from the first month after the first stem cell dose, with gradual improvement until the third month. This improvement was reflected in the GMF scale, PEDI scores and quality of life.

The improvement shown in this case with the great change in the quality of life of the patient prompts the initiation of large scale clinical trials using cellular therapy for SMA. Clinical trial setting should include respiratory function tests as an evaluation measure.

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