Efficacy of Autologous Bone Marrow Derived Mononuclear Cells In The Treatment Of Neurodeficits In Down’s Syndrome: A Case Report

Alok Sharma1, Nandini Gokulchandran1, Hemangi Sane1, Samson Nivins2, Pooja Kulkarni2, Vrushali Mane2, Maitree Maheshwari3, Prerna Badhe4

1Department of Medical Services and Clinical Research, NeuroGen Brain & Spine Institute, India.
2Department of Research & Development, NeuroGen Brain & Spine Institute, India.
3Department of Psychology, NeuroGen Brain & Spine Institute, India.
4Department of Regenerative laboratory services, NeuroGen Brain & Spine Institute, India.

ARTICLE INFO

ABSTRACT

Down’s syndrome (DS) is the most common genetic neurodevelopmental disorder with a presence of extra copy of chromosome 21. The characteristic features include facial dysmorphology, low muscle tone and intellectual disability. Although, different approaches to ameliorate the neurosymptoms have been attempted, still there is no treatment available to reverse the neurodeficits in DS. Cellular therapy is an emerging therapeutic modality and has to be explored to address the core neurodeficits in DS. In this study, autologous bone marrow derived mononuclear cells were administered intrathecally, followed by intensive neurorehabilitation in a 24-year-old female with DS. Her chief complaints were poor command following, memory and social interaction, age inappropriate behavior, sensory issues, presence of rocking and grimacing, and unclear speech. PET/CT and FIM were used as an objective measure to monitor the changes following cell therapy. No adverse events were recorded throughout the duration of follow-up. At 1-year follow up, after cell therapy, there were significant improvements in clinical and functional symptoms indicated by changes recorded on PET/CT and Functional Independence Measure. PET/CT showed improved brain activity in bilateral anterior cingulate, prefrontal cortex, medial temporal cortex and cerebellum. Clinical improvements were improved cognition, problem solving, command following, sitting tolerance, reduced aggressive behavior and sensory issues. Improvements were also recorded in the functional status of the patient with FIM score improving from 64 to 91. In view of functional and clinical improvements seen, a second dose of stem cell was administered following 1-year. At eight months follow-up after the second dose, there were further improvements in brain activity and clinical symptoms. Her FIM improved from 91 to 94. Cellular therapy has opened a new line of therapeutic management by improving the neurodeficits in DS. It is a safe and feasible treatment alternative and can improve the quality of life of the patient when administered in combination with standard rehabilitation techniques.
INTRODUCTION:
Down’s syndrome (DS) is the most common genetic cause of intellectual disability [1, 2]. According to world health organization, the estimated prevalence of DS is between 1 in 1000 to 1 in 1100 live births worldwide. It is characterized by features such as learning disabilities, craniofacial abnormalities and hypotonia which are common to all affected individuals [3, 4]. Moreover, DS is associated with a number of other anomalies like cardiac defects, Alzheimer’s diseases (AD) and leukemia [5]. It is caused by trisomy of chromosome 21 [3]. The trisomy fetuses are at increased risk of miscarriages and other medical complications [6]. Its incidence is influenced by maternal age and widely differs with population [7-10]. Studies suggest that, life expectancy could be between 10 to 56 years [5, 11, 12]. Due to recent advancement in medical science, the life expectancy in DS has increased dramatically. A downside to this is long term dependency of the patient on the care giver affecting the quality of life of the patient as well as the care giver. Hence, the development of treatment strategies should focus on making the individual functionally independent thus improving the quality of life of the patient and the caregiver.

Previous MRI studies on DS, showed abnormalities in frontal cortex, superior temporal cortex, fusiform gyrus, medial temporal cortex, cingulate gyrus, cerebellum and brainstem [13, 14, 15, 16]. Moreover, functional neuroimaging studies suggest that abnormal connectivity, and numerous distributed brain networks associated with distinct functional domains in DS [17, 18]. These structural abnormalities and abnormal connectivity may constrain hypothesis of neurodeficits in DS.

Currently, there is no core treatment available to reverse the neurodeficits in DS. A variety of therapies including physical, speech-language, occupational, emotional and behavioral intervention have been used for the benefit of the patients.

To explore the effect of cellular therapy in DS, we administered a 24-year-old female patient with autologous BMMNCs, intrathecally and clinically evaluated her at regular intervals. Positron emission tomography computerized tomography (PET/CT) brain was used to monitor the changes occurring at the cellular level.

CLINICAL PRESENTATION
Herein, we present a 24-year-old female, known case of DS. She was a pre-term 32 weeks delivery, born of non-consanguineous marriage by C-section in view of cephalopelvic disproportion. She had delayed cry at birth and weighed 1.5 kgs. Neo-natal complication of jaundice was reported at day 5 of life and required NICU stay for 15 days. In addition, she was also detected with hypothyroidism. She was diagnosed as DS at the time of birth due to the typical facial characteristics. She has a familial history of DS, as her mother’s paternal cousin was also affected. All her motor milestones were delayed by few months. She started walking at the age of 3 years and started speaking monosyllables by the age of 4 years. She started special schooling at the age of 6 years. Currently, she could read English alphabets with difficulty, but could write and read sentences in her mother tongue. She could also perform simple mathematical problems but was poor in recognizing colors, shapes, time and money. She was on medication, since birth for hypothyroidism. There was no history of seizures noted.

On clinical assessment, she was found to have poor command following, poor memory, age inappropriate behavior, lack of awareness and judgment skills, occasional laughing without reason, deficit in social interaction, sensory issues, presence of rocking, grimacing, and unclear speech. She showed lack of interest in learning new tasks and activities, poor in academic and visual perception skills, and poor sitting tolerance. She was partially dependent for the activities of daily living. Moreover, she didn’t participate in doing any household chores. Her Functional Independence Measure (FIM) score during the time of admission was 60.

EEG, Brain MRI and PET/CT brain scan was performed as a part of pre-operative investigation protocol. EEG showed normal pattern during awake state with absence of epileptic activity. MRI revealed gliotic changes in the right posterior temporal cortex with absence of significant abnormality in cerebral hemisphere, brain stem and cerebellum as shown in Fig 1. No abnormalities were localized in the ventricular system, cortical sulci, basal cisterns. No mid-line shift and cerebellar fissures or focal lesion were noticed. Brain PET/CT showed severe hypometabolism in bilateral cerebellum, and medial temporal cortex with minimal reduction in orbitofrontal cortex and anterior cingulate cortex.
MATERIALS AND METHODS

Patient selection was based on World Medical Associations Helsinki declaration [21]. The complete protocol has been reviewed and approved by the Institutional committee for Stem-cell Research and Therapy (IC-SCRT). The parents were informed about the procedure and a duly filled informed consent form was obtained from them. Patient underwent autologous BMMNC transplantation after pre-investigation protocol. 300 mcg of G-CSF injections were administrated 72 hours and 24 hours before stem-cell transplantation, to stimulate CD34+ cells and increase their survival and multiplication [22]. Bone marrow was aspirated from the iliac crest in the operation theatre with aseptic precautions. Mononuclear cells (MNCs) were separated using density gradient separation method. The viable count was performed using trypan blue dye and was found to be 96%. The MNCs were checked for CD34+ by FACS analysis and the count was found to be 6.30%. Approximately 1.45 x 10^8 MNCs were immediately injected intrathecally at L4-L5 level using a lumbar puncture needle.

The patient was given a personalized rehabilitation therapy as a part of the protocol which included behavioral therapy, occupational therapy, speech therapy, psychological intervention and special education. She was followed up at 4 months, 7 months and 1 year. A comparative PET/CT Brain was performed 1 year after cellular therapy.

The second dose of cellular therapy was administered after one year. The purified mononuclear cells were found to have a viability of 97 %. During the procedure a total of 1.46 x 10^8 MNCs were injected with CD34+ cell count of 3.44%. Eight months after the second dose, a comparative PET/CT Brain was performed again.

RESULTS

No adverse events were recorded throughout the duration of follow-up. At follow-up of 4 months, she showed improvement in following three step commands, awareness, judgment, play behavior, identification of the colors and clarity of speech. She had started to do all the household chores, when instructed and could perform her toilet activities independently. Moreover, she showed interest in learning new activities and tasks.

During 7-month follow-up, she showed further improvement in cognition, problem solving and command following. She showed improvement in sitting tolerance, identifying and recognizing shape and color, right and left side discrimination, figure ground perception and fine motor skills. Besides, she showed improvement in academic learning and visual perceptual skills. Aggressive behavior and sensory issues reduced significantly. FIM score improved from 60 to 64.

At follow-up after one year, all the improvements sustained. She showed improvement in attention, command following, identification of colors, objects and shapes and attentive to the surrounding. She could do all household chores including serving food, chopping vegetables, etc. She could perform mathematical calculation and started showing interest in singing and dancing. Her FIM score further improved from 60 to 91. She was independent for the activities of daily living.

Comparative PET/CT scan was performed after 1 year to assess the outcome of intervention on the metabolic activity of the brain. As compared to previous PET/CT scan before cellular therapy, post PET/CT scan revealed improved brain activity in bilateral anterior cingulate, prefrontal cortex, medial temporal cortex and cerebellum. (Fig. 2).

In view of functional and clinical improvements seen, a second dose of stem cell was administered after 1-year. At eight months follow-up after the second dose, she showed further improvements in awareness, social judgment, speech and attention. She could now remember songs which she learnt at her music class and also initiate social interactions. She was receptive to primary emotions. Her FIM score further improved from 91 to 94.

A Comparative PET/CT scan was performed again after eight months of the second transplantation. As compared to previous PET/CT scans, it showed further improvement in brain activity of bilateral anterior cingulate, prefrontal cortex, medial temporal cortex and cerebellum.
Fig. 1. Representative Transaxial T2-Flair and T2- weighted sequences of MR brain sections of DS patient. Gliotic changes are seen in the right Posterior temporal cortex (PT).

Fig. 2. Representation of Axial, coronal and sagittal slice of 18F-FDG-PET image of the patient with DS. (Top row) PET image before stem cell therapy, (Middle row) PET image after one year following cell therapy and (Bottom row) PET image after twenty months of stem cell therapy, showing improved brain activity in bilateral Anterior cingulate cortex (ACC), Medial temporal cortex (MT) and Cerebellum (C) as compared with the previous PET image. The arrow head indicates the regions of interest.

DISCUSSION
Down’s syndrome is the trisomy of chromosome 21 and most commonly identified genetic form of Intellectual disability. The additional copy of chromosome 21 elevates the expression of many genes encoded on this chromosome [23, 24]. This elevated expression of genes causes imbalance between chromosome 21 and other genes have been proposed to cause the phenotypic alterations in DS [23, 24].

Neuropathological studies showed delayed and disorganized cortical lamination; fewer synapses; smaller hippocampal dentate gyrus and lower number of granule, Purkinje cells and cerebellar granule cell density. Moreover, animal models on DS had documented the excessive inhibition and alteration of synaptic transmission; microglial activation; increased levels of proinflammatory cytokines and impairment of synaptic plasticity in cholinergic interneurons [25-36].

In addition, neuromorphological studies on DS show increased parahippocampal volume and decreased frontal, amygdala, prefrontal, posterior cingulate, hippocampal and cerebellar volume as compared to age matched controls [27, 37, 38]. It had been hypothesized that, extra copy of chromosome 21 in DS delays the mitotic cell cycle of neuronal precursors, there by affecting neurogenesis in DG and lateral ventricle [39, 40]. Alterations in several neurotransmitters and receptors such as GABA, Excitatory transmitters, Neurotrophins, Dopamine, histamine have been reported in animal models of DS [41-45]. These impairments are responsible for the defects in behavior, cognition and memory in DS.
The discovery of the therapeutic potential of stem cells provide a new outlook for the treatment of incurable neurological disorders [46-49]. Stem cells can differentiate into variety of highly specialized cells and maintain homeostasis in physiological and pathological conditions. They are a mixture of cells which includes hematopoietic stem cells, MSCs, tissue specific progenitor cells and stromal cell. These cells have a tendency to migrate and provide trophic support to stimulate self-repair systems such as endogenous neurogenesis, angiogenesis and synaptogenesis [50-52]. Beside trophic factors, these cells also release exosomes to deliver functional proteins and microRNAs to neuronal cells [53]. Additionally, these cells also reduce inflammation and enhance recovery process [54]. These cells rescue purkinje cells and inhibit microglia proliferation in central nervous system [55, 56]. These repaired neurons may enhance processing of neuronal information and contribute to the functional improvement of the patient. These distinct characteristics of stem cells may induce functional recovery in DS. This case of DS showed significant improvement clinically and functionally, following two doses of autologous BMMNC transplantation. The functional changes in the brain were monitored using PET/CT scan where 2-deoxy-2-[fluorine-18] fluoro-D-glucose ($^{18}$F-FDG) was used as a tracer for measuring the synaptic activity. PET/CT performed after one year and twenty months showed significant improvement in brain activity as compared to PET/CT performed before transplantation. The regional correlation between associated brain regions and clinical improvements are shown in Table 1. All the regions have led to the functional independence of the patient. Improvement seen in functional independence of the patient was also indicated by improved FIM score after first (60 to 91) and second transplantation (91-94).

<table>
<thead>
<tr>
<th>Brain regions showing improved activity on PET/CT</th>
<th>Clinical improvements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cingulate cortex and cerebellum</td>
<td>Cognitive behavior, problem solving, decision making and moderating social behavior</td>
</tr>
<tr>
<td>Medial temporal cortex and thalamus</td>
<td>Ability to identify colors, objects and shapes</td>
</tr>
<tr>
<td></td>
<td>Alertness</td>
</tr>
</tbody>
</table>

Autologous BMMNC transplantation does not repair the genetic defect or alter the genetic make-up in DS, but it indeed repairs the neurological deficits caused by the genetic impairment. It works synergistically with other standard rehabilitation therapies to restore the lost functions. Therefore, it helps to make the individuals with DS functionally independent and allow their amalgamation into the outside world. This also reduces the burden on the caregivers.

Cell therapy has opened new avenues for therapeutic applications in DS. To our knowledge, this is one of the initial studies demonstrating the efficacy of cell therapy in DS. Though, this is a single case study it directs towards the potential of resolving neurodeficits in DS. It is a safe, feasible and a promising approach to improve the quality of life. However, extensive studies are warranted to elucidate the mechanisms in future to establish its therapeutic potency in DS.

**REFERENCES**

31. Ishihara, K., et al., Enlarged brain ventricles and impaired neurogenesis in the Ts1Cje and


54. Kim, Y.J., et al., Neuroprotective effects of human mesenchymal stem cells on
