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THERAPEUTIC POTENTIAL OF CELL TRANSPLANTATION IN A CASE OF ADULT TRIPLEGIC CEREBRAL PALSY

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ABSTRACT The safety and efficacy of cellular transplantation has been established in pediatric cerebral palsy (CP). However, not many studies have been performed to determine its efficacy in adult CP. Adults with CP are at a higher risk of secondary complications. They have an overall lower fitness level, reduced muscle mass, neuromuscular inefficiency, and limited physical activities. Effects of long-term rehabilitation also eventually plateau in these individuals. To study the effect of cellular transplantation in adult CP, we administered a 27-year-old female, diagnosed as spastic triplegic cerebral palsy, with autologous bone marrow mononuclear cells intrathecally followed by neurorehabilitation. The patient was followed up after 4 and 6 months of transplantation. She was then administered a second transplantation and later followed up after 5 months. Improvements were noted in voluntary control, spasticity, balance and gait. PET CT scan brain was performed before and 6 months after intervention to monitor the outcome of intervention at a cellular level. On the comparative PET CT scan, metabolic improvements were recorded in bilateral paracentral lobule, thalamus, medial temporal and cerebellum regions. No major adverse effects were noted after both the interventions. The results suggest that BMMNC transplantation is potentially safe and effective therapy for adults with CP. Hence, cellular transplantation in adult CP may help address the complications and improve their quality of life.

KEYWORDS : Cerebral Palsy, Cellular transplantation, Autologous, Bone marrow mononuclear cells, Positron emission tomography computed tomography (PET CT), stem cells

INTRODUCTION

Cerebral Palsy (CP) is a non-progressive permanent disorder of movement and posture, resulting from damage to developing fetal or infant brain causing limitations in performing activities of daily living (ADLs) (1). The motor disorders of CP are often accompanied by other secondary problems such as disturbed sensation, perception, cognition, communication, and behavior (2). Population-based studies from around the world estimates the prevalence of CP ranging from 1.5 to more than 4 per 1,000 live births. (3) CP is frequently thought of as a condition that affects only children. Due to the advances in the medical field, there are rarely any deaths in children with CP and almost all children survive till the adulthood. (4) Hence, adults with CP represent a growing population whose health status and healthcare needs are poorly understood, imparting a heavy burden on their families and society. Although the brain pathology initially responsible to cause CP, does not worsen with time but, the neural deficits persist throughout the lifespan leading to physical impairments thereby causing hindrance in their adult life. At present, there is no cure for CP, however many supportive treatments such as rehabilitation, medications and surgery may help alleviate symptoms. These conventional treatments do not address the core brain pathology of CP. The adult cases of CP usually undergo long-term rehabilitation but, their progress eventually plateaus. The treatment strategies should mainly focus on improving the health and mobility in adults with CP, making them functionally independent, which will help them in their employment and family life thus improving their quality of life.

Recently, cellular transplantation is being explored extensively as a potential treatment strategy for various neurological disorders including CP. (5-12) It involves administration of stem cells which carry out the repair process either by directly replacing the damaged or lost neuronal cells or indirectly via paracrine mechanisms. (13) Its benefits have been extensively studied in pediatric cases of CP but, there are not many studies which show its efficacy in adult patients. To study the benefits of cellular transplantation in adult CP, we administered autologous bone marrow mononuclear cells (BMMNCs), intrathecally in a 27-year-old female with CP. These cells are safe, easily obtainable, and do not involve risk of immune rejection and have no ethical issues. Intrathecal route was chosen as it is relatively a less invasive procedure and a direct mode to administer cells to the brain without damaging any neural tissue. (14) The objective of the study was to evaluate the clinical and functional outcome of cellular transplantation, reduce severity of symptoms and to maximize functional independence of in an adult patient of CP. 18 FDG PET-CT scan brain was used to monitor the changes at a cellular level. It helps record changes in the brain metabolism occurring after cellular transplantation.

CASE PRESENTATION

We present a case of 27-year-old female with triplegic cerebral palsy. Patient was born of nonconsanguineous marriage, preterm at 7 months by vaginal delivery. There was no history of seizure or any other neonatal complications. She fell from the bed at the age of 8 months but no major injury was reported. Parents noticed delayed milestones such as head control, rolling, crawling, sitting with or without support, standing, walking and climbing.On clinical evaluation by a pediatrician, she was diagnosed with CP. She

underwent rehabilitation but, her improvements eventually stalled. Before cellular transplantation, a complete neurological evaluation was carried out by experts. She was hypertonic and hyperreflexic, however, the muscle tone in left upper extremity was normal. The voluntary control in the bilateral lower extremities and right upper extremity was poor to fair but good in left upper extremity. Voluntary control of upper and lower abdominals was poor and fair respectively. Sitting balance was good whereas standing and walking balance was fair. She walked with diplegic gait pattern with help of a walker and right upper limb in flexion attitude. There was tightness in bilateral hamstring (-10 degree to extension) and dorsiflexor with swan neck deformity and right-hand fingers stretched upto neutral. Her cognition and memory was normal.

She scored 40/56 on Berg Balance Scale (BBS) and 109 on the Functional Independence Measure(FIM). Magnetic resonance imaging (MRI) brain with diffuse tensor imaging (DTI) revealed gliotic changes in bilateral frontoparietal periventricular white matter resulted from the sequalae of perinatal hypoxic-ischemic insult. Electroencephalography (EEG) record showed generalized (left>right) epileptiform activity. Positron Emission Tomography Computed Tomography (PET CT) of brain demonstrated mild hypometabolism in bilatral cerebellum.

MATERIAL AND METHODOLOGY

The patient selection was based on World Medical Associations Helsinki declaration criteria. (15) The protocol of intrathecal administration of autologous BMMNC was reviewed and approved by the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). Prior to the therapy, the procedure was explained to the patient and her family and a duly filled informed consent was obtained. Pre-intervention routine blood tests, urinalysis and chest x-ray was performed to rule out any active infection and to assess the fitness for anesthesia. Granulocyte colony-stimulating factor (G-CSF) injections were administrated 72 hours and 24 hours prior to BMMNC transplantation, to stimulate CD34+ cells. Since, the EEG was abnormal, she was put on a prophylactic regime consisting of Levetiracetam to avoid any occurrence of seizure episodes.

100ml of Bone marrow was aspirated from the anterior superior iliac spine under local anesthesia. The BMMNCs were then separated using density gradient centrifugation method. These cells were checked for viability and CD34+ markers. The viability of the cells was found to be 96%. Approximately $1.00x10^8$cells were administered immediately post separation, in L4-L5 using a lumbar puncture needle. Fluorescence Activated Cell sorting (FACS) was used to analyze the CD34+ positive cells in BMMNCs and were found to be 2.62%. 1 gm methyl prednisolone in 500 ml Ringer's Lactate was simultaneously injected intravenously to reduce inflammation. Patient underwent extensive neurorehabilitation which included physiotherapy, occupational therapy and psychological counseling and was advised to continue supervised therapy after discharge.

She was followed up regularly at 4 and 6 months after intervention and was reevaluated on BBS and FIM. A repeat PET CT scan was performed after 6 months. She was administered another dose of autologous BMMNCs 6 months after the first one. The protocol for which remained same. For the second procedure the viability of cells was 96%. Approximately $1.36x10^8$cells were administered intrathecally. They consisted of 2.28 % CD34+ positive cells. She was then followed up 5 months after the second dose.

RESULTS

Patient was followed up at regular intervals. 4 months after intervention, improvement was noted in posture, spasticity, voluntary control in right elbow joint, fingers and gait pattern. She could maintain quadruped position for 10-15 minutes which was not possible earlier. Her walking using push knee splint, high boots and walker was increased to 1 hour. No major adverse event was noted.

On follow up after 6 months, her sitting posture improved further along with reduced lumbar lordosis. A significant reduction in spasticity of muscles was recorded. On modified Ashworth scale (MAS), the muscle tone of right glutei muscle reduced from grade 2 to 1 and that of left hip adductor reduced from grade 3 to 2. Her voluntary control improved and synergistic pattern of right upper limb reduced which resulted in better hand opening. Scapulothoracic and trunk pelvic dissociation reactions also improved. Reduced knee flexion tightness and scissoring while walking was observed. On objective scales, BBS score increased from 40 to 41. FIM score remained the same but qualitative changes were noted.

On performing a comparative PET CT, improved metabolism was observed bilateral Paracentral lobule, Thalamus and cerebellum regions. (Figure 1)

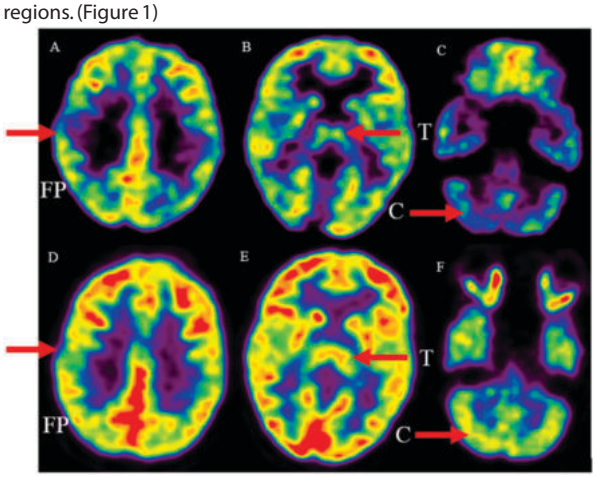

Figure1: (A-C) Pre cell transplantation PET CT scan images showing hypometabolism in bilateral Bilateral fronto-parietal cortex (FP), Thalamus (T), and cerebellum (C) regions respectively. (D-F) Post cell transplantation PET CT scan images demonstrating improved metabolism in these areas

5 months after the second dose of transplantation (11 months after the first), the patient maintained all the improvements. She could now sustain the quadruped positioning for 30 min. Further improvement was recorded in her gait, standing balance, voluntary controls and leg movements. She could perform complete overhead activities. Her grip was also better than before. Knee flexion reduced further and her ground clearance was also better thus improving her walking. BBS and FIM scores remained the same but qualitative changes were recorded.

DISCUSSION

Periventricular leukomalacia (PVL) is one of the major neuropathology of cerebral palsy. It involves diffuse injury of cerebral white matter, with or without focal necrosis. It is characterized by loss of oligodendrocyte precursor cells (OPC) leading to disrupted myelin formation. (16) Neuronal loss, disrupted axonal guidance and synaptogenesis and microglial activation are also often associated with PVL. (17) Cell damage and decreased blood supply to the brain are two of the primary causes of PVL. None of the treatments available for CP addresses this pathology of CP. New treatments for CP should aim at cellular regeneration, replacement, survival and functional reorganization. Injury to the central nervous system stimulates the proliferation of endogenous neural stem cells which further replaces the dead or damaged cells. However, leukomalacia limits this mechanism of self-repair in these individuals due to unavailability of endogenous stem cells. (18)

Cellular transplantation has recently gained attention as a potential therapeutic strategy for CP. In children, the neuroplasticity of the brain is greatest making the recovery of functions of the damaged brain easier. However, the same is not true in adulthood as the neuroplasticity reduces with age. The prolonged damage to the neurons makes functional recovery difficult in adults. Hence,

administration of stem cells may help in stimulating the repair process. It helps in reversal of disease pathology by performing neurogenesis, replacement of lost/damaged neurons, remyelination, immunomodulation, axon sprouting, neural circuit reconstruction, angiogenesis and synaptogenesis. In this study, autologous BMMNCs were used due to their established safety and efficacy. BMMNCs is a mixture of various hematopoietic and non-hematopoietic cells. Studies have shown that using a mixture of cells is more beneficial than using sub-fractions as they impart a cumulative effect (19). BMMNCs, on administration migrate to the damaged areas of the brain and initiate the repair process. (20) They have the ability to differentiate into host cells and replace the lost or damaged cells. In case of CP, they regenerate and replace the lost OPCs and result in remyelination. They release growth factors and neurotrophic factors such as vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) which promotes recovery of lost functions of the brain. These neurotrophic factors are also responsible for neuroprotection. They halt the damage of cells and stimulate endogenous repair. (21) They also induce angiogenesis thus improving blood and oxygen supply to the damaged regions of the brain leading to reversal of hypoxia in CP. (22,23) BMMNCs play an important role in immunomodulation. They produce anti-inflammatory molecules and reduce inflammation along with supressing the microglial activation and subsequently exerting neuroprotective effects in the brain. (24) In this study, we combined cellular transplantation with neurorehabilitation as physical exercise helps in mobilization of the cells to the affected areas of brain. (25) Significant functional improvements were recorded in the patient after intervention. These improvements correlated to the improved metabolism in the areas of brain responsible for those functions as recorded in the PET CT scan. Even though, there were significant functional and objective improvements observed, these improvements did not reflect substantially on the outcome measures. Scores on FIM scale remained same and on BBS improved from 40 to 41. This could be attributed to the fact that the baseline scores before the intervention were already high and the improvements observed were qualitative. These scales are less sensitive to measure small changes occurring in an already high functioning individual. (26)

The patient had been on rehabilitation since the time of her diagnosis but its effect had been plateaued. On supplementing the standard rehabilitation with cellular transplantation, the repair process may have been reactivated leading to improvement in the under-functioning areas.

CONCLUSION

Until now, extensive studies have been conducted demonstrating the efficacy of cellular transplantation in children with CP. However, not many studies have shown its efficacy in adult patients, chronic gliosis being one of the major reasons. This case has demonstrated that autologous bone marrow mononuclear cell transplantation can be safe, feasible and efficacious treatment for adult patients with triplegic CP. Repeated cell transplantation in this case has shown to enhance the clinical outcome. PET CT scan be used as a monitoring tool as it maps the changes at cellular level. In this case, the metabolic improvements on PET CT scan also correlated with the clinical improvements. To obtain optimal outcome of cellular therapy in adult CP, various factors such as ideal cell type, route of administration, volume of cells and number of doses still need to be explored. Nevertheless, intrathecal transplantation of autologous BMMNCs in combination with standard neurorehabilitation can be used as a therapeutic strategy for adult triplegic CP to repair the damaged brain areas and retrieve lost functions.

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