18 FDG PET CT scan maps the effect of intrathecal transplantation of autologous bone marrow mononuclear cells (BMMNCs) in cerebral palsy.

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Abstract:

PET CT scan is one of the most sensitive neuroimaging techniques used to study brain metabolism. We used this technique to map changes occurring in the brain at a cellular level after intrathecal transplantation of autologous bone marrow mononuclear cells (BMMNCs) combined with neurorehabilitation in Cerebral Palsy (CP). CP is a group of non-progressive heterogeneous neurological disorders resulting in damage to the brain at or around birth. This damage leads to neurological deficits. The pathophysiology of CP is multifactorial. Hence, the management of CP should be multidisciplinary. Cellular transplantation has gained popularity in the treatment of CP. We administered a one and half year old female of dystonic CP with autologous bone marrow mononuclear cells (BMMNCs) twice in the period of 1 year and followed her up for 3 years. A comparative PET CT scan was performed after 1 year of intervention which showed significant improvement in periventricular areas which are typically damaged in cerebral palsy. This further led to functional improvements in oromotor skills, speech, balance, fine and gross motor activities and cognition. In the duration of 3 years, her GMFCS score improved from Level 5 to Level 4 and GMFM score improved from 3.92% to 9.24%. No adverse events were recorded in this period of follow up. The outcome of this case indicates that cellular therapy along with neurorehabilitation improves neuronal function and enhances functional recovery in CP patients. PET CT scan can also be used effectively to map the changes occurring after intervention.

Keywords: PET CT scan, cerebral palsy, autologous bone marrow mononuclear cells, stem cells, cellular transplantation

Introduction:

PET CT scan is a functional neuroimaging tool which can be used effectively to detect changes in blood flow and metabolism of the brain. This is achieved by using tracers which are labeled with radioactive substances such as 18F-fluorodeoxyglucose.

In this study, we have used 18 FDG PET CT scan

brain to map the changes occurring in the brain metabolism of a one and half year old female child diagnosed as dystonic CP; who underwent intrathecal transplantation of autologous BMMNCs.

Cerebral palsy (CP) is a non-progressive heterogeneous group of neurological disorders resulting in irreversible damage to the brain at or around birth which further causes motor impairments and neurological deficits. (1) The prevalence of CP is 3-4 per 1000 children and it is one of the most common motor disabilities in children (2). Various antenatal, perinatal and postnatal factors contribute to the etiology of CP (3). CP is often characterized by motor disorders, involuntary movements, spastic syndrome, cognitive impairments, perception and communication disorders and epilepsy (4-5). Currently, the standard treatment strategies available for CP comprises of medications, physical therapy, occupational therapy, speech therapy, use of orthotic devices; but these strategies do not address the neural tissue damage and are seldom effective in the reparative process of the damage (6). Due to the heterogeneity of CP, a multidisciplinary approach may help reverse the damage.

Cellular transplantation has gained attention recently due to its neurorestorative properties, as stem cells have a unique potential to self regenerate and multiply into multi-potent cells (7). Various clinical studies propagate the use of cellular transplantation in neurological disorders such as autism, spinal cord injuries, stroke, head injury, etc (8-12). Preclinical studies suggest that these cells improve the functional deficits in the animal models of CP (13-14). Their mechanism primarily includes neuroprotection, neuroangiogenesis, neurorestoration and neuroregeneration. Administration of these cells may enhance the cerebral developmental process, improve lost functions and the quality of life in these children (15-16)

Case Report: One and half year old female, a known case of dystonic CP, was administered autologous BMMNCs intrathecally. She was born as a full term baby with normal delivery. There was history of asphyxia at birth. At the age of 6 months, her parents noticed delay in the developmental milestones such as head holding and rolling. She was then diagnosed as CP on the basis of her clinical presentation and magnetic resonance imaging (MRI) which suggested complete prenatal hypoxic-ischemic encephalopathy (HIE) with perirolandic and subcortical injury.

At the time of intervention, she presented with dystonia. On examination, she was hypertonic. She also presented with trunkal hypotonia. Deep tendon reflexes such as biceps, supinator, triceps, knee and ankle were exaggerated. Primitive reflexes such as tonic labyrinthine reflex (TLR) stepping and startle reaction were not integrated in her. Voluntary control in the upper and lower extremity bilaterally was fair. She exhibited partial head control and poor oromotor skills. She had not achieved motor milestones such as rolling, supported sitting and supported standing. Speech comprised of babbling sounds. She could recognize her family members and a social smile was present. Hand function of grip, grasp, opposition, pinch and release was affected. She could grasp sizeable objects but was unable to release them. Midline activities were present. She was dependent for all her activities of daily living. She had not developed any bowel and bladder sensations. No contractures or deformities in the extremities were present. No muscle wasting was noted. On Wee functional independence measure (WeeFIM) she scored 70. On gross motor function classification system (GMFCS) was level 5. On Gross Motor Function Measure (GMFM) she scored 3.92% indicating very minimal motor functions.

MRI brain with DTI showed gliotic changes in bilateral perirolandic regions with T2 hyperintensity and paucity of bilateral frontoparietal white matter. Bilaterally symmetric T2 hyperintense signal abnormality was also seen in the posterior putamina and thalami. The fea-

tures represented sequalae of severe prenatal hypoxic-ischemic insult.

Electroencephalogram (EEG) was suggestive of repetitive epileptiform discharges over the right frontotemporal and right temporal regions and at times extending into the right central region in sleep>>wake. Few independent epileptiform discharges were recorded over the left frontocentral region. No generalized epileptiform discharges were seen. No focal slow wave abnormality was recorded. Background in wake and sleep was normal.

Positron Emission Tomography Computed Tomography (PET CT) images revealed evidence of minimally increased fluorodeoxyglucose (FDG) uptake in the frontal and parietal lobes. Reduced FDG uptake was seen in the mesial temporal structures and bilateral basal ganglia. In the mesial temporal structures reduced FDG uptake was seen in the hippocampus, amygdale and also increased uptake in the cingulated gyrus was noted.

Intervention:

The ethical basis for using BMMNCs for the treatment was according to the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The ethical approval for the intervention was obtained from Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). The patient's parents were explained about the procedure and a written informed consent was obtained from them. She was thoroughly examined by a team of expert doctors. Pre-surgery routine blood tests, urinalysis and chest x-ray were carried out for anesthetic and surgical fitness. 150 mcg of Granulocyte colony-stimulating factor (G-CSF) injections were administrated 72 hours and 24 hours prior to BMMNCs transplantation as it enhances the mobility of BMMNCs and stimulates CD34+ cells (17). 80 ml bone marrow was aspirated from the iliac bone under local anesthesia with the help of a bone marrow aspiration needle and collected in heparinized tubes. Separation of mononuclear cells (MNCs) was achieved by the density gradient method and the viability of these cells was calculated. Cell viability was found to be 96% and 1 x 108 cells were administered intrathecally at the level of L4-L5 using 25 G spinal needle. These MNCs were then examined for CD34+ markers by FACS analysis and were found to be 1.08%. 150 mg methyl prednisolone in 500 ml Isolyte P was simultaneously injected intravenously to reduce the local inflammatory response and improve the stem cell survival. The patient was prescribed upper and lower extremity strengthening exercises, trunk strengthening exercises, bed mobility exercises, balance exercises, gait training and exercises to improve gross and fine motor function. She was put on a home exercise program to enhance the effectiveness of stem cell transplantation. She was closely monitored for any adverse events. Comparative PET CT scan brain was performed after one year to study the changes in brain metabolism after intervention.

One year after cell transplantation, she was administered autologous BMMNCs intrathecally for the second time, the protocol for which remained the same.

Results:

3 months after cell transplantation, her oromotor skills improved. Drooling reduced significantly and she showed improvement in speech. Neck holding improved along with static and dynamic sitting balance and could sit with support and balance herself, which she was unable to do prior to cell transplantation. Movements in the lower extremity, bed mobility, overhead, gross and fine motor movements also showed improvements. She could roll from prone to supine with ease. Her cognitive function was better as compared to before.

In the duration of 1 year after cell transplantation,

she continued to demonstrate improvements. Her oromotor skills had further improved. Drooling had completely stopped and her speech was better as she could speak few words. Improvements were also observed in neck holding, static and dynamic sitting and standing balance, leg movements, overhead, gross and fine motor movements. The dystonic component in her upper and lower extremities reduced bilaterally. Her cognitive function also improved.

On comparing the PET-CT scan performed before transplantation and one year after transplantation, improvement was found in the periventricular areas. (Figure 1) In the CT scan, which showed abnormality in ventricular dilation before intervention, there was significant improvement observed in the periventricular regions and supraventricular white matter tracts indicating improved myelination. (Figure 2,3)

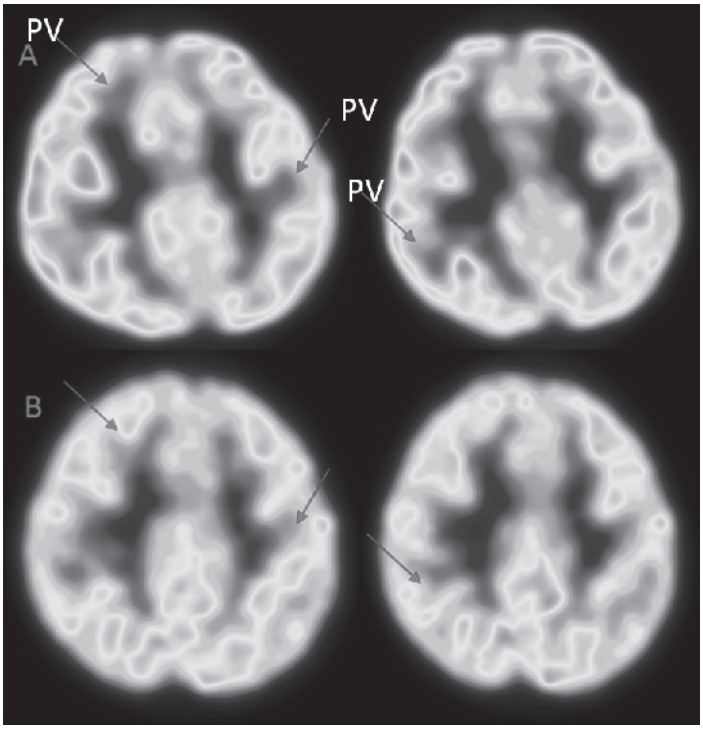

Figure 1: Comparative FDG-PET CT Brain demonstrates (A) Dilation in the lateral ventricle before cellular transplantation (b) Significant improvement observed in periventricular areas -PV after cellular transplantation indicated by arrows.

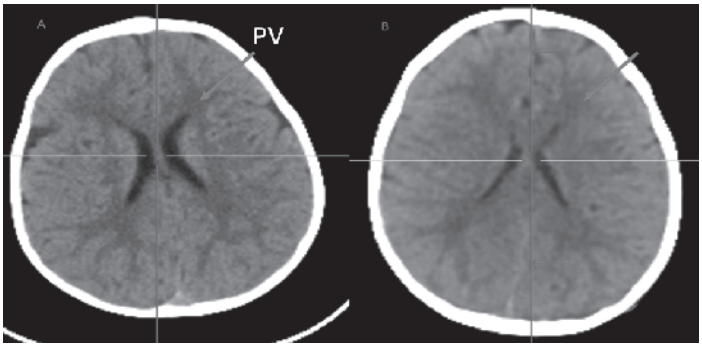

Figure 2: CT Brain demonstrates (A) abnormality in lateral ventricles before cellular transplantation (b) Significant improvement observed in periventricular areas after cellular transplantation

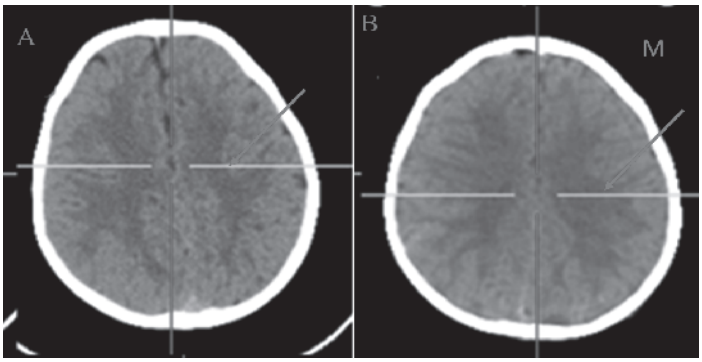

Figure 3: Multimodality imaging in dystonic cerebral palsy. (A) represents the region of white matter tracts before the intervention of cell therapy and (B) demonstrates improvement in supraventricular white matter tracts indicating improved myelination (M)

One year after the transplantation, she underwent a second dose of autologous BMMNCs. She was regularly followed up and all her improvements were maintained for 2 years.

Two years after the 2nd transplantation, in addition to all the above improvements, her trunk control and sustainance had improved. She initiated creeping now. Voluntary control of bilateral upper extremities had improved and she could initiate reach outs along with balance and equilibrium reaction. Cognitively, she further improved and could convey her needs by actions, words or by pointing out. Overall dystonia had reduced and full arm weight bearing of upper extremity had improved. She had 3 episodes of

seizures, two years after the intervention. However, they were managed using anticonvulsants.

In the duration of total 3 years, The GMFCS score improved from Level 5 to Level 4. GMFM score improved from 3.92% to 9.24%.

Discussion:

18 FDG PET-CT scan is one of the most sensitive and non invasive neuroimaging techniques. In CP, it measures the brain glucose metabolism using 18F-FDG. FDG is a glucose analog with physiological aspects almost identical to glucose. Any change in the neuronal activity of the brain is usually accompanied by altered perfusion and glucose metabolism. Damage or loss of neurons may result in decreased glucose metabolism while increased activation of neurons may result in increased metabolism. (29-30). In this study, we compared the PET CT scan brain performed before and after cellular transplantation in a case of CP, to map the changes occurring at cellular level after intervention. It was found that, post intervention significant metabolic improvements occurred in periventricular areas which are usually damaged in case of cerebral palsy. Similar changes were also observed on the CT scan alongwith improved myelination, increased white matter, and reduced necrotic areas and cortical atrophy.

In CP, the pathophysiology is predominantly attributed to periventricular leukomalacia (PVL). PVL is a diffuse injury of deep cerebral white matter, with or without a focal necrosis (18). It may lead to and/or loss of premyelinating oligodendrocytes (pre-OLs), astrogliosis, and microglial infiltration (19), which may further result in neuronal dysfunctions (20-21). Another contributing factor to the pathophysiology of CP is the microglial activation after hypoxic ischemic injury which secretes various neurodegenerative cytokines; exerting a neuro-toxic effect on oligodendrocytes, thus leading to neuronal dysfunction. (22).

Since, no standard therapeutic intervention delivers favorable outcome measures, cellular therapy holds a promising future in the management of CP. Preclinical studies have also suggested safe and effective use of cellular therapy in the animal models of CP (13-14). During childhood, the neuroplasticity of the brain is at zenith, hence rendering cell transplantation as a potential treatment modality in CP children. In this case study, autologous BMMNCs were used as they can be easily isolated and do not have any ethical issues. To reduce the complications of cell rejection, graft vs. host disease, an autologous approach was preferred. The targeted route of transplantation of stem cells is preferred to maximize beneficial effects of cellular transplantation. (23) Hence, we chose intrathecal route of transplantation as it is minimally invasive and ensures a targeted delivery of stem cells (23).

Different types of cells have been used as a treatment approach to address the multifactorial pathology of CP (24-25). Our previously published studies evaluated the benefits of cellular therapy along with neurorehabilitation suggesting that cellular therapy may accelerate the development and improve the quality of life of patients with CP. (26-28).

The mechanism of action of autologous BMMNCs is twofold; to protect the viable neurons and replace the damaged ones. These cells home onto the injured site through various neurochemical pathways. Neurotrophic factors such as brain derived neurotrophic factor (BDNF), interleukins tumour necrosis factor, etc bring about cell proliferation. Secretion of growth factors like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), brain fibroblast growth factor (bFGF) leads to neoangiogenesis. Improving microcirculation helps to regain tissue function. (32-33). Hence, BMMNCs may play a pivotal role in the reparative process of the neural tissue and improve function through the above

mentioned mechanisms. In this case study, the improvements may be attributed to cellular transplantation along with neurorehabilitation. It is postulated that exercises increase the number of stem cells and enhance the paracrine effects of cellular therapy (31).

This case of CP, demonstrated significant functional improvements which clinically correlated to the improvements observed on PET CT scan. The improved myelination seen in CT scan may have led to improved neuronal function of the child.

Two years after the second intervention, the child had 3 episodes of seizures. No history of seizures was reported before transplantation. However, the EEG was abnormal which increased the risk of occurrence of seizure in her. Since, these seizures did not occur shortly after the intervention, their cause may or not be attributed to cellular therapy.

Conclusion:

The outcome of this case report demonstrates the ability of autologous BMMNC transplantation to reduce the degree of impairment and improve neuronal function and the quality of life in cerebral palsy. It is a feasible, safe and effective treatment alternative for CP when combined with neurorehabilitation. This case report also establishes PET CT scan brain as an effective neuroimaging modality which maps the changes in brain metabolism occurring after cell transplantation.

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