FUNCTIONAL IMPROVEMENTS MONITORED BY POSITRON EMISSION TOMOGRAPHY IMAGING AFTER CELL TRANSPLANTATIONS IN SEVERE CHRONIC TRAUMATIC BRAIN INJURY

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ABSTRACT Introduction: Positron Emission Tomography (PET) imaging provides excellent sensitivity information regarding changes in brain metabolism after traumatic brain injury (TBI). TBI is a significant cause of morbidity and mortality resulting in permanent neurobiological damage and permanent deficits. Cell transplantation in combination with neurorehabilitation has a potential to cease the degeneration and replace the damaged neurons, which current standard intervention may not tackle. **Methods:** Herein, we present a case of a 7-year-old female who suffered from TBI 15 months before she underwent first cell transplantation. She underwent two cell transplantations at an interval of 4 months with intrathecal autologous bone marrow-derived mononuclear cells. **Results:** Throughout nine months, improvements in posture, balance, bed mobility, voluntary control of the upper extremity, above activities, cognition, speech, understanding and attention was observed. Percutaneous endoscopic gastrostomy (PEG) tube was removed as her swallowing capacity improved. The Functional independence measure (FIM) score improved from 22 to 35, and Disability rating scale (DRS) improved from 15 to 12. There was an improvement in the Glasgow coma scale from 8 to 15. A repeat PET CT scan of the brain at four months' post first cellular transplantation revealed improved metabolism in the cerebellum, cingulate regions, vermis and parietal gyrus. These changes corresponded to the clinical improvements seen in the patient. No adverse events related to the procedure was observed. **Conclusion:** Cell transplantation is a safe and productive treatment for chronic TBI. However, we recommend future controlled clinical trials with objective imaging like PET CT to further establish cell transplantation as a therapeutic modality in severe chronic TBI.

KEYWORDS traumatic brain injury, autologous bone marrow mononuclear cells, cell transplantation, stem cells, PET CT, neurorehabilitation

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Introduction

Neuroimaging after traumatic brain injury (TBI) serves as a crucial tool in the identification of acute and chronic consequences of injury, such as intracranial hematomas, brain contusions, and posttraumatic complications.[1] Positron emission tomography-computed tomography (PET-CT) imaging provides exquisite sensitivity for small molecular changes and can provide relevant information regarding changes in brain metabolism after TBI. [2] PET imaging was done in this case to understand the tomo-

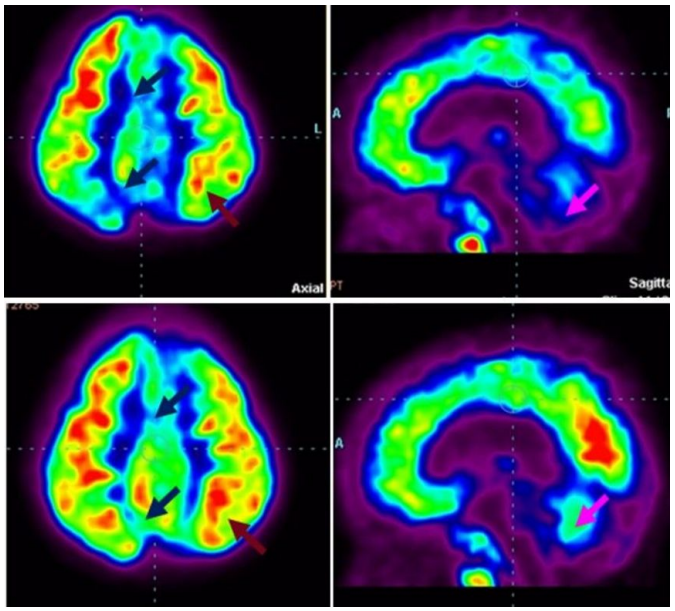

Fig.1 PET-CT scan images pre-stem cell therapy and post stem cell therapy. (Colour code: black- severe hypometabolism, blue-hypometabolism, green-normal metabolism): The areas marked with the arrows depict the areas of the brain which have improved post intervention. Improvement in metabolic activity of cerebellum, cingulate regions, vermis and parietal gyrus was seen.

graphic, volumetric and functional changes in the brain and find objective evidence regarding the effect of the cell transplantation.

TBI is caused when sudden trauma disrupts the function of the brain. Brain injuries vary in severity from mild (brief change in mental status) to severe (period of unconsciousness). [3,4] It may lead to long-term impairment of cognitive, psychosocial functioning depending on the part of the brain that is damaged. [5] Along with this, patients could also develop functional changes and medical conditions such as epilepsy, which requires long-term or lifelong supportive and medical care. [6] The standard treatment modalities for brain injury involves medications, physical and behavioural therapy, hyperbaric oxygen therapy (HBOT), and medical management of associated conditions that aims at improving the functional abilities and restoring the patient to daily life. However, these strategies have not been able to translate into a successful treatment strategy for brain damage at the cellular level.[7] Cell transplantation can address the core pathology occurring in TBI through its neuro-regenerative, neurorestorative and neuroprotective mechanisms. This novel intervention aims to cease the degeneration and replace the damaged neurons. This case explores the importance of PET CT as a monitoring tool to objectify the clinical effects of intrathecal administration of autologous bone marrow-derived mononuclear cell (BMMNC) in severe chronic TBI.

Materials and Method

Case report:

Herein, we present a case of a 7-year-old female who suffered from TBI 15 months before she underwent first cell transplantation. The injury occurred as a result of an accident while crossing the road. Intraventricular haemorrhage was detected with frontal bone fracture as a consequence of the injury. She also sustained multiple abrasions on her forehead and fracture of the femur which were managed immediately. Left pneumothorax was managed with intercostal drainage. On the Glasgow Coma Scale, she scored 8/15, indicating severe TBI. The child had seizure episode with decerebrate posturing after which she was put on anti-epileptics. Percutaneous endoscopic gastrostomy (PEG) tube was used for feeding after the incident. She underwent physiotherapy and occupational therapy after which minimal improvements were observed in posture and oral-motor skills.

Before cell transplantation, on neurological examination, she was hypertonic (left more than right side) and hyper-reflexes with grade 2 spasticity in bilateral plantar flexors, hip adductors, hip extensors, and knee flexors. Severe motor weakness was present in all the limbs. The sitting and standing balance was poor. The postural alignment in the sitting position was kyphotic with the left arm abducted at an angle of 90. She could not move or hold anything in her left arm. There was no bladder or bowel sensation and control. The speech was non-coherent. Attention span was short, and memory was affected. Functionally, she was dependent on all her daily activities of living and was wheelchair bound for mobility. The score on the Functional independence measure (FIM) score was 22. On the Disability rating scale (DRS), she scored 15 indicating the severe state of disability.

The Magnetic resonance imaging (MRI) of the brain with Diffusion Tensor Imaging (DTI) showed a gliotic scar in the left thalamo-capsular region, left frontal lobe, left temporal lobe with multiple chronic hemosiderin straining in both cerebral hemispheres and left cerebellar hemisphere. The brain PET CT scan showed severe hypo-metabolism in the left hippocampus, left parahippocampal gyrus, left thalamus and left cerebellum; moderate hypo-metabolism in left superior frontal gyrus, right hippocampus and left precuneus; mild hypo-metabolism in the left posterior cingulate gyrus, right thalamus, bilateral amygdala and right cerebellum. Electroencephalogram (EEG) recorded in the sleep state was normal.

Intervention:

The selection of the patient was based on the World Medical Associations Helsinki declaration.[8] The protocol was reviewed, and ethics approval was obtained from the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). The procedure of cell transplantation was explained in detail to the parents, and a duly filled informed consent was obtained from them before the therapy. Consent was also video recorded.

Before the intervention, the patient underwent a complete evaluation consisting of neurological, psychological and preoperative investigations to assess the anaesthesia fitness. Granulocyte-Colony Stimulating Factor (G-CSF) (300 mcg) injections were administered subcutaneously, 72 hours and 24 hours before bone marrow aspiration. On the day of transplantation, 90 ml bone marrow was aspirated from the right anterior superior iliac spine under local anaesthesia with sedation, using bone marrow aspiration needle and was collected in heparinised tubes. The BMMNCs were separated from the aspirate using density gradient method. The purified MNCs were tested for total cell count, viability and CD34+ cell content by Fluorescence Activated Cell sorting (FACS). The separated cells were then injected intrathecally at the level between L4 and L5. Simultaneous intravenous administration of 500 mg methylprednisolone in 500 ml of Isolyte P solution was done to decrease immediate

Table 1 Timeline:

February 2015	Met with Road Traffic Accident and sustained head injury Also sustained fracture femur L/L pneumothorax
February -May 2015	Hospitalized Was unconscious and on a mechanical ventilator The child had seizure episode Was on PEG feeding Underwent physiotherapy and occupational therapy
August 2016	Underwent stem cell therapy
December 2016	Follow up after four months. She was reassessed, and comparative PET CT was performed. Showed functional improvements and objective improvements. PET CT showed improvement in brain metabolism.
December 2016	2nd dose of stem cell therapy was administered
May 2017	Follow up after five months. The patient continued to improve and showed further objective improvements.

inflammation and to enhance the survival of the injected cells. Total numbers of cells injected were 36×108 with 96% viability consisting of 2.68% of CD34+ cells.

Following the transplantation, she underwent customised neurorehabilitation which included physiotherapy, occupational therapy, speech therapy and psychological counselling. These therapies included effective motor learning strategies with task-oriented training for successful attainment of functional outcomes.

Given the improvements observed after the treatment, the patient underwent similar second cellular transplantation four months after the first intervention. The transplantation procedure was replicated. Total numbers of cells injected were 30×108 with 96% viability consisting of 3% of CD34+ cells.

Result

The child was followed up for four months after the first transplantation. No procedure-related adverse events were recorded in this duration. Within four months of transplantation, the upper limb gross motor activity and the overhead activities improved. She could hold and move her left hand by herself which was not possible before. The muscle tone and voluntary control of the upper extremity improved from poor to fair. The child would vocalise with improved understanding and cognition. She was attentive and followed commands better than before. PEG tube was removed as her swallowing capacity had improved and she was tolerating oral feeds very well. Walking for short distances was possible with the support of splints. She developed bowel sensations and could now indicate. The posture was more erect with improved sitting balance. On FIM, the score improved from 22 to 29. On the DRS scale, she improved from 15 to 12. The repeat PET CT scan of the brain showed improved metabolism in the bilateral cerebellum, vermis, cingulate regions, right superior parietal gyrus. Based on the improvements seen, the child underwent a second dose of autologous BMMNC transplantation.

At five months follow up after second transplantation, there were improvements seen in the bed mobility like supine to sit and side lying to sit transitions. She would try to learn and speak new words. Expression of emotions was evident. On FIM, the score further improved from 29 to 35. The GCS score improved from 8 to 15. All the other improvements were maintained.

Discussion

Modern brain imaging provides quantitative information about the severity and distribution of damage, which allows more specific correlation between disability and damage to specific parts of the brain. [9,10] PET-CT scan of brain is a noninvasive, relatively safe, and feasible modality to record the functional activity of brain. PET imaging with the tracer [18F] Fluoro-2-deoxy-D-glucose ([18F] FDG) provides vital information regarding the correlations between cerebral glucose utilisation at the cellular level and behavioural maturation, synaptogenesis and plasticity in the normal and abnormal brain. Decreased metabolism is suggestive of decreased function of the neurons. Increased uptake of FDG indicates increased metabolism which is implied as the improved functioning of the neurons.[11] Few studies have explored FDG-PET in TBI with varying degrees of sensitivity to detection at acute, subacute, and chronic phases of injury. These studies have shown reduced metabolism in the frontal, temporal, thalamic and cerebellar regions after the trauma. [12-14]

At the time of head trauma, a series of destructive intracellular and extracellular pathologic processes occur. Intracranial secondary brain insults include severe intracranial hypertension, extra-axial lesions, seizures, and cerebral oedema. [15,16] Neuronal and glial cell death, degeneration of oligodendrocytes and traumatic axonal injury contribute majorly to the pathology of TBI. [17] Intraventricular haemorrhage, as well as injuries of the inner cerebral structures, is seen mostly in severe blunt head injury. [18] The motor weakness in patients with TBI could result from a deep cerebral haemorrhage which was observed in this case. [19] An understanding of the underlying mechanisms of brain injury of motor weakness and cognitive disabilities in patients with TBI is essential to set treatment strategies and predict their outcomes.

Cell transplantation, in recent years, has gained recognition as a therapeutic modality in TBI, as it addresses the damage of the brain at the cellular level. Numerous preclinical and clinical studies have proved the safety and efficacy of cell transplantation in TBI. These studies have shown to promote recovery process by modulating the inflammatory cascade and remodelling the damaged brain through different mechanisms. [20-33] Potential mechanisms of action of these cells include engraftment and

trans-differentiation, modulation of the inflammatory cascade and the systemic immunologic response. They proliferate and differentiate into various cells including neural cells and oligodendrocytes. [34] The oligodendrocytes help in re-myelination of the damaged axons in the injured brain and repair the neural connections. [35] The neuro-restorative effects exerted by the BMMNCs like angiogenesis, neovascularization, production of growth factors, and paracrine effects lead to improved synaptic connectivity and information processing in the damaged brain areas.[36] Bone marrow cells also secrete various growth factors and neurotrophic factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), etc. which stimulate the endogenous neuroprotection and repair [37] This suggests that cell transplantation has the potential to repair the damage to the neural circuits at the molecular, structural and functional levels. In this case, we intrathecally administered autologous bone marrow mononuclear cells as cells through this path are well tolerated and migrate efficiently to the injured brain tissue and exert a significant beneficial effect.

A comparative PET CT scan performed before and after cell transplantation demonstrated improved metabolic activity in the bilateral cerebellum, vermis, cingulate regions, right superior parietal gyrus in the brain. [Figure 1] The functional improvements shown by the patient corresponded to the areas of the brain that showed evidence of improvements in metabolism. [Table I] This indicates restoration of neuronal func¬tions in the affected areas post cell transplantation.

The patient was on continuous rehabilitation regime post cell transplantations. Exercise induces cortical reorganisation of the neural network, increases the mobilisation of local stem cells, enhances neuronal proliferation and differentiation by the asymmetric cell division.[38] It also enriches the micro-environment by improving angiogenesis and release of cytokines and nerve growth factors. [38,39] This suggests that a combination of multiple doses of cell transplantation and neurorehabilitation leads to functional restoration which reduces disabilities in severe chronic TBI, thereby improving the quality of life of the patient.

This is a single case of severe chronic TBI, and hence the outcome of the intervention cannot be generalised.

Conclusion

PET imaging showed improvements in the metabolism of the affected brain regions providing objective evidence regarding the effect of cell transplantation. Clinical improvements along with improved objective scores suggest that cell transplantation in combination with neurorehabilitation has a potential to reverse the damage occurring in the brain after chronic TBI. Future controlled clinical trials with a larger population are warranted to establish the therapeutic effect of cell transplantation in severe chronic TBI with PET CT as a monitoring tool.

Conflict of interest

The authors have no conflicts of interest.

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