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Case Report

Effect of Cellular Therapy Monitored on Positron Emission Tomography - Computer Tomography Scan in Chronic Hemorrhagic Stroke: A Case Report

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Abstract

Stroke is a major cause of complex disability and death worldwide. Stem cell therapy has improved functional status in many of the neurological disorders, including stroke, cerebral palsy, head injury, spinal cord injury, etc. We present a case of 58 years old male with chronic hemorrhagic stroke having right hemiplegia, who underwent intrathecal, autologous bone marrow derived mononuclear cell (BMMNC) transplantation along with neurorehabilitation. This case study is aimed at observing the efficacy of cellular therapy using Positron emission tomography computerised tomography (PET-CT) imaging as a monitoring tool in patient with chronic hemorrhagic stroke. The effects were measured on clinical and functional changes. Over 6 months, gradual improvement was noticed in the voluntary control of upper and lower limb, balance and gait. Functional Independence Measure improved from 94 to 96 and Berg Balance scale score from 44 to 48. PET-CT scan of brain was performed pre intervention and 6 months post intervention. On comparison, the scan reported improvement in the metabolism of left hemisphere of the brain in areas such as parietal and frontal lobe, basal ganglia, supplementary motor area, pre and post central gyrus. These radiological improvements correlated with the functional improvements recorded in the patient on follow up. The outcomes suggest that intrathecal autologous transplantation of BMMNCs in chronic hemorrhagic stroke along with continued neurorehabilitation may lead to significant clinical and functional improvements. There were no adverse events. This case also demonstrates the effective use of PET-CT scan brain as a radiological tool to monitor the effects of intervention at a cellular level.

Keywords: *autologous, bone marrow, mononuclear cell transplantation, cellular therapy, chronic hemorrhagic stroke, Positron emission tomography computerised tomography.*

Introduction

Stroke is characterized as neurological deficit attributed to an acute focal injury by a vascular cause, including cerebral infarction, intracerebral hemorrhage (ICH), and subarachnoid hemorrhage (SAH) of the central nervous system (CNS). It is a major cause of complex disability and death worldwide [1]. Recovery after stroke is determined by the site and extent of lesion. The overall outcome of stroke can be improved with rapid diagnosis, early preventive treatment and early recognition of complications. There is no available curative treatment for chronic neuro deficits of stroke [2,3]. The presently available therapies such as vessel recanalization concentrate on alleviating acute stroke [4]. However, treatment strategy for chronic hemorrhagic stroke is still not definite. Research activities focusing on preclinical and clinical stem cell transplantation represents a promising source for functional recovery after stroke. Stem cells have the capacity for self-renewal and differentiation into organ specific lineages [4]. Numerous studies have indicated their positive role in improving the functional status in many of the neurological disorders, including stroke, cerebral palsy, head injury, etc. [5-7] The functional recovery in stroke could be contributed by the process of neurorestoration which leads to neurogenesis, angiogenesis, and cell plasticity [4].

We present a case of 58-year-old male patient with chronic hemorrhagic stroke, who underwent intrathecal, autologous bone marrow derived mononuclear cell (BMMNC) transplantation. This case study aims at observing the efficacy of cellular therapy using Positron emission tomography computerised tomography (PET-CT) imaging as a monitoring tool in patient with chronic hemorrhagic stroke.

Case Report

A 58-year-old male patient with right hemiplegia had a history of sudden onset of weakness on right side of body and face 3 years 8 months back for which he underwent medical treatment and rehabilitation. Post rehabilitation there was very minimal improvement in the walking balance and voluntary control of right lower limb. However, after 2 years there was no improvement inspite of regular rehabilitation. He has history of hypertension since 4 years and diabetic mellitus since 12 years. On assessment, he was unable to move right upper limb and hold objects with hand. There was imbalance while walking and hemiplegic gait pattern was observed. He had hemineglect of right side of the body perception. His behavior,

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perception and cognition were impaired along with slurred speech. He was less socially interactive and had confusion in remembering names. There was also presence of uncontrolled emotional outbursts, like crying, anger and irritability. On neurological evaluation, patient was hypertonic and hyperreflexic on the right side. According to Modified Ashworth Scale (MAS) he had grade 2 spasticity in right upper and lower limb. For ADL, he required minimum assistance for grooming and bathing. His Functional Independence Measure (FIM) score was 94, Berg Balance Scale (BBS) score was 44 and Mini Mental State Examination (MMSE) score was 25.

On investigation, MRI of brain revealed slit-like gliotic area with hemosiderin staining in the left lentiform nucleus represents sequel of an old hemorrhage. Also mild, microangiopathic, chronic ischemic changes in bilateral frontoparietal white matter with few gliotic lacunes in bilateral deep gray nuclei was seen. EEG was normal. PET-CT imaging of brain suggested moderate hypometabolism involving left fronto-temporo-parietal cortex, left hippocampus, left thalamus and right cerebellum and mild hypometabolism involving left basal ganglia, left amygdala, left parahippocampal gyrus (Figure 1a).

Materials and Methods

The patient was selected for the therapy, based on the inclusion criterion of the World Medical Associations Helsinki Declaration [8]. The intervention was approved by the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT). Before therapy a signed informed consent was obtained from the patient and his relatives. 72 hours prior to the bone marrow aspiration, Granulocyte colony-stimulating factor (G-CSF) (300 mg) injections were administrated subcutaneously. This helped in stimulation of CD34+ cells and also in survival and multiplication of the stem cells [9].

Bone marrow (100 ml) was aspirated from the iliac bone and mononucleocytes (MNC) were obtained from them after density gradient separation. Viable count of the isolated MNCs was taken and checked for CD34+ by fluorescence-activated cell sorting (FACS) analysis. The viability of cells was 98%. A total of 1.2 x 10^8 MNCs were injected intrathecally in L4-L5 using a lumbar puncture needle. Methyl prednisolone (1 gm) in 500 ml of Ringer lactate solution was administered intravenously.

Post stem cell therapy patient underwent neurorehabilitation including physiotherapy, occupational therapy, psychological intervention and dietary advice. Physiotherapy aimed at normalizing muscle tone and to improve voluntary control of extremities and gait pattern, increasing static and dynamic balance. Whereas, occupational therapy aimed at functional and coordination training and hand rehabilitation. Psychotherapy included activities like recall, crossword puzzles, word search and sequencing activities. Patient was advised regular therapy at home.

Results

The patient was followed up at 3 and 6 months after the cellular therapy. After 3 months, improvements were reported in his upper limb function, balance and gait. He was able to perform overhead activities and hand opening and closing with ease. Standing and sitting posture and balance was improved and he was able to reach out in multi direction. He was able to walk more confidently. Memory and problem solving skills also improved. There was marked improvement in BBS and FIM. (Table 1)

After 6 month there was significant improvement in the gait as he was able to walk with less circumduction and better foot clearance. Climbing stair up and down and crossing obstacles was easier. Voluntary control of upper and lower limb improved. Bed mobility like rolling, supine to sitting was better. Perception, joint proprioception was improved and the hemineglect of right side of the body was reduced. Speech also improved and the words were clearer as compared to before. Uncontrolled emotional outburst had reduced and he socialized well with people. The FIM and BBS scores remained improved. (Table 1) On comparing the PET CT scan brain, improvement was observed in the metabolism of left parietal and frontal lobe, left basal ganglia, left supplementary motor area, left pre- and post-central gyrus. (Figure 1a & b) (Table 2)

Table 1: Change in the outcome measures over 6 months.

Outcome measures	Pre-intervention	Post Intervention (3 months)	Post Intervention (6 months)
Functional Independence Measure (FIM)	94	96	96
Berg Balance Scale (BBS)	44	48	48

Discussion

Stroke is the second commonest cause of death and fourth leading cause of disability worldwide [10]. After stroke the permanent change in the structure of CNS leads to long lasting physical impairments, observed as residual problems, which leads to activity limitation and restricts community participation. Damage to the brain tissue is inevitable and no efficient treatment for functional improvement is presently available except neurorehabilitation. Cellular therapy is a developing field and has recently opened new avenues for brain repair strategies [7].

In this case, autologous BMMNCs were chosen as the mode of intervention because of the safety profile, easy availability in abundance and absence of ethical issues. The intrathecal route of administration is a focused route which directly inserts the cells into the CSF. Thus, the accessibility of cells to the brain is enhanced. Intrathecal route is devoid of any major side effects and is easy to administer [11]. Post transplantation neurorehabilitation program was included as it has the ability of promoting recovery post stem cells transplantation in stroke [12-13]. BMMNCs are multipotent and can differentiate into different tissue types; including astrocytes, neurons, and endothelial cells in the brain [3]. The mobilization of stem cells to the injured areas of the brain initiates the process of neurorestoration. At the site of injury, these cells either, multiply and replace the damaged or dead cells and carry out the repair directly or indirectly through paracrine activity, encourage tissue remodeling, prevent apoptosis, decrease inflammation, and release growth factors [12]. They secrete growth factors like vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF) which support and amplify angiogenesis, neurogenesis and cell plasticity at the penumbral region [3]. A preclinical study has also demonstrated the role of these growth factors secreted by human MSCs in improving the functions in stroke brain [14]. In stroke, along with neuroreparative processes, cellular therapy also decrease the glial scar formation and promote glial-axonal remodeling [4].

Clinical studies have shown that autologous BMMNCs intrathecal transplantation, followed by multidisciplinary neurorehabilitation in chronic stage of stroke improves the prognosis of functional recovery based on standing and walking balance, ambulation, hand functions and FIM objectively [3]. A non-randomized clinical trial has also confirmed the feasibility and safety of intravenous BMMNCs in patients with sub-acute ischemic stroke and the results demonstrated favorable clinical outcomes on National Institute of Health Stroke Scale (NIHSS), Barthel Index, modified Rankin Scale, MRI, EEG and PET [15]. In another study increased number of cluster activation in Brodmann areas BA 4, BA 6 was observed on functional imaging indicating neural plasticity along with statistical significant improvement on modified Barthel Index after autologous

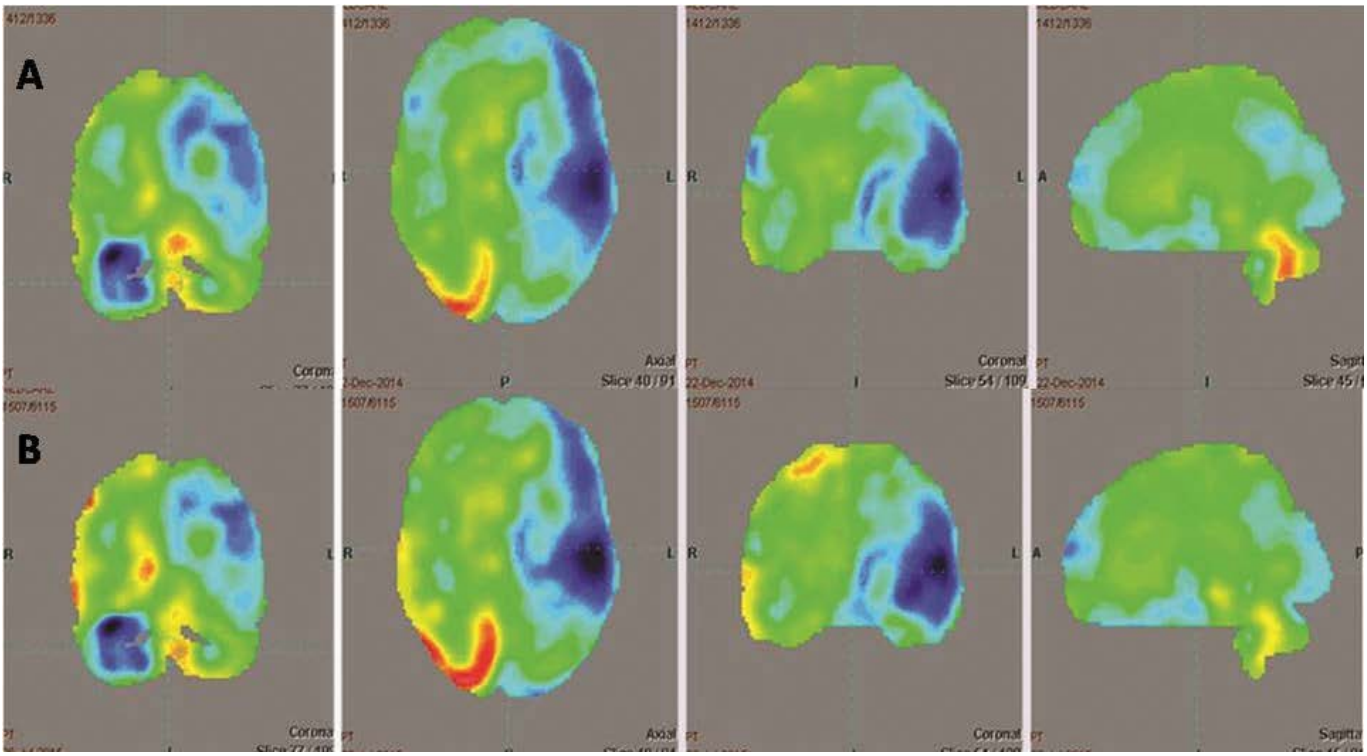

Figure 1: (A). Pre-intervention, PET-CT scan brain showing areas of hypometabolism (blue and black areas) in the left cerebral hemisphere. **(B)** Change in the PET-CT scan brain imaging over 6 months showing areas of improved FDG uptake (reduced blue and black areas) in fronto-parietal areas of left cerebral hemisphere.

Table 2: Change in the FDG uptake on PET-CT Brain imaging, 6 months after cellular therapy.

Region	Pre intervention		Post intervention		Clinical correlation
	Mean SUV	Mean SD	Mean SUV	Mean SD	
Left Basal Ganglia	3.51	-4.1	3.77	-3.6	voluntary control of right upper and lower extremities
Left Caudate Nucleus	2.38	-3.6	2.6	-3.3	voluntary control of right upper and lower extremities, memory and social behavior.
Left Lenticualar Nucleus, Pallidum	3.56	-3.3	3.78	-3	voluntary control of right upper and lower extremities.
Left Lenticualar Nucleus Putamen	4.58	-2.9	4.89	-2.5	voluntary control of right upper and lower extremities, cognitive function and emotions
Left Supplementary Motor Areas	4.23	-2	4.12	-1.7	postural stabilization, voluntary control of right upper and lower extremities.
Left Frontal Lobe	4.05	-5	4.3	-4.4	cognitive function and voluntary control of right upper and lower extremities.
Left Paracentral Lobule	4.03	-1.8	4.36	-1.1	right lower extremity function
Left Post Central Gyrus	3.69	-5.3	3.83	-5.2	perception, joint proprioception, improved and hemineglect of right side of the body reduced.
Left Pre Central Gyrus	3.91	-4	4.12	-3.7	motor function of right upper and lower extremities.
Left Precuneus	4.31	-4.5	4.54	-4.2	memory, judgment, motor coordination
Left Superior Parietal Gyrus	3.63	-4.2	3.86	-3.8	Memory
Left Cerebellum	4.56	-2	4.78	-1.1	posture, balance, coordination, and speech
Vermis 8	6.1	5.4	5.88	3.5	coordination of movements.

* SUV: Standardized Uptake Value; SD: Standard Deviation

mononuclear and mesenchymal cell transplantation in stroke patients [16].

In the present case study after the cellular therapy improvement were reported in upper limb function, balance and gait. Qualitative improvement in cognitive function like memory, problem solving skills and social activity were noted. Uncontrolled emotional outburst was reduced. There was improvement in BBS and FIM. Functional improvement of 2 points on the FIM scale was measured but the change was not Minimal Clinically Important Difference (MCID). However, it has also been observed that lower the FIM score at the time of admission the higher is the change in score [17]. The patient we treated had a significantly independent functional status. The FIM score did not show a greater variation at the end of follow up.

The PET-CT scan used in the study is based on the principle of change in the blood flow and the energy metabolism which is associated with the activity of the nervous tissue [18]. Along with evaluating the function of the brain with respect to cellular and molecular events like apoptosis, inflammation, infection, change in pH, and metabolism, it also produces functional images of metabolic processes of the brain [19]. Fluorodeoxyglucose (FDG) measures regional cerebral glucose consumption with PET as glucose metabolism is strongly associated to neuronal activity. Therefore, the alteration in neuronal activity caused by disease is reflected in change of glucose metabolism. PET is amongst the most accurate method for the examination of regional brain metabolism in health and disease states [20]. The standardized uptake value (SUV) denotes the mean uptake of FDG dye in that particular area which is directly proportional to the metabolism occurring in the brain. The SUV is then compared to the normative data and standard deviation (SD) value is determined. If there is hypometabolism of glucose the PET-CT scan shows blue areas and the SUV is less as compared to the normative data. In this case,

favorable changes were observed in the PET-CT scan following the cellular therapy, in form of improved FDG uptake shown by mean SUV and SD values in various areas of brain when compared to pre-intervention PET-CT scan. Improvement was recorded in the metabolism of left hemisphere of the brain in areas such as parietal and frontal lobe, basal ganglia, supplementary motor area, pre-and post-central gyrus (Figure 1a & b). These improvements can be correlated with the clinical outcome as the cognitive function like memory, problem solving, social interaction and speech; voluntary control of movement, balance was also improved (Table 2).

One of the limitations of the study is that it has no control case to compare but since the patient was not recovering with rehabilitation but only after cellular therapy and neurorehabilitation there was improvement in the symptoms we may postulate that the cell transplantation played a vital role. The other limitation was no molecular biomarker was used for the investigation. Although the case report is an observation of a single patient, it may support undertaking further research.

Conclusion

Autologous intrathecal transplantation of BMMNCs along with continued neurorehabilitation demonstrated improved functions in patient with chronic hemorrhagic stroke. This case also displays the effective use of PET CT scan brain as a radiological tool to monitor the effects of intervention at a cellular level. The functional improvements correlated with the areas of the brain showing improved metabolism further substantiating the effect of cellular therapy. Further robust analysis and large randomized clinical trials with superior methodology are required to establish the optimum frequency, source, and dosage of transplantation.

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