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Improvements in a Case of Autism Spectrum Disorder after Cell Therapy As Noted On PET CT Brain Scan

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Abstract

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder affecting communication, behavior and socialization. The exact etiology is unknown, but combination of genetic, environmental, and immunological factors is likely to be responsible for ASD. Autologous bone marrow mononuclear cells (BMMNCs) have been studied in great detail and have a safe, feasible and ethical profile. In this study, we present the case of a 4 year old boy with ASD who underwent intrathecal administration of autologous BMMNCs. After 8 months follow up, significant social, behavioral and communication improvements were observed. A comparative PET CT scan performed before and 8 months after the intervention revealed improvement in metabolism of frontal lobe, parietal lobe, medial temporal lobe (bilateral hippocampi and amygdale), bilateral cerebellar hemispheres when compared to previous brain scan. The score on Indian Scale for Assessment of Autism (ISAA) reduced from 114 (moderate autism) to 99 (mild autism), Indian Scale for Assessment of Autism (CARS) score improved from 35.9 to 28.5, Wee Functional Independence Measures (WeeFIM) score improved from 51 to 56. Severity of illness score on Clinical Global Impression CGI reduced from 4 (moderately ill) to 3 (mildly ill), Global improvement on (CGI) scale graded him with a score of 2 (much improved) and efficacy index on CGI showed moderate therapeutic effect (score 5).

Keywords: Autism spectrum disorder (ASD); Cell therapy; Stem cell therapy; Autologous bone marrow mononuclear cells (BMMNCs); PET CT scan.

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition associated with impairments in socialization, verbal and nonverbal communication, and restricted and repetitive patterns of behavior [1]. Symptoms begin around 12-18 months of age [2] with mean age of diagnosis ranged from 38 to 120 months for ASD [3]. According to Centers for Disease Control and Prevention (CDC), about 1 in

every 68 children has been documented with ASD in United States [4]. The causes of ASD include genetic and environmental risk factors or a toxic environment, and intracellular pathogens that could induce an immune response, resulting in neuro-inflammation, autoimmune reactions, brain injury, and ASD [5].

Current therapeutic options for ASD includes long term management by behavior modification and applied behavior analysis, occupational therapies, speech therapies, sensory integration, nutritional guidance and pharmacological treatment. Most of the children with ASD experience difficulty with independent living, employment, social relationships and mental health. Recent studies signify that cellular therapy has become a promising and novel therapeutic modality which aims at restoring lost neural connections in the brain due to different properties like capacity to regenerate into identical cells, capacity to give rise to more differentiated cells and paracrine mechanism [6,7].

Positron emission tomography (PET) scanning is a relatively non-invasive functional neuroimaging technique that demonstrates physiological functioning of the brain after administration of chemical agents. PET scanning along with [^{18}F]fluoro-2-deoxyglucose (FDG) can measure regional cerebral glucose metabolism [8] and help to detect abnormalities in the metabolic functions at the cellular level in patients with ASD [9]. Characteristic imaging patterns on PET scan can provide valuable information and increase the diagnostic confidence for the same. Therefore PET/computed tomography (CT) scan can be a valuable tool to confirm the clinical findings as well as to rule out any other cause. PET CT scan has previously been used to record significant improvements with autologous BMMNCs in various pediatric neurological disorders [10-13].

In this report we present a case of ASD, who underwent intrathecal autologous bone marrow mononuclear cells (BMMNCs) transplantation along with neurorehabilitation. The primary goal of the treatment is to improve the quality of life of the patient to assess, whether PET CT scan can be used as valuable tool for the evaluation and diagnosis of ASD.

Materials and Methods

Case Presentation

Here in we present a case of 4 year old boy with autism spectrum disorder (ASD) born of non consanguineous marriage with maternal age 32 years. He had birth history of full term C-section delivery with nuchal cord, cried immediately after birth with normal weight. He had normal motor milestones with delayed speech. By two and half years of age parents noticed poor eye contact, poor social smile and hyperactivity. Child psychiatrist diagnosed him as a case of ASD. There was no history of seizure. His maternal uncle was suffered from mental retardation. He was on regular occupational therapy, speech therapy, sensory and behavioral therapy since two years and eight months of age. On evaluation, he was hyperactive and restless with poor sitting tolerance. Attention span was fair. Social interaction and peer relations were affected and engaged in repetitive and solitary play activities. He was attached to inanimate objects and there was also presence of motor mannerisms such as swinging things. Sensory issues such as not liking hair cut, avoiding touching liquids or soft food and preferring hard textures were present. He was hyposensitive to vestibular and proprioceptive sensory input and exhibited seeking behavior such as swinging, running around, jumping etc. He was more hypersensitive in oromotor areas compared to other body parts. Neurologically, he had normal muscle tone and power in trunk and limb muscles. Functionally, he was dependent for activities of daily living and was not toilet trained.

On Indian Scale for Assessment of Autism (ISAA), he scored 114, and on Childhood Ataxia Rating Scale (CARS), he scored 35.5 suggestive of moderate autism and on WeeFIM, he scored 51. Severity of illness on Clinical Global Impression (CGI) scale i.e. CGI I was 4. Magnetic resonance imaging (MRI) of brain showed no significant intracranial abnormality and electro encephalogram (EEG) was normal in the sleep state. The brain positron emission tomography computed tomography (PET CT) scan was suggestive of severe hypometabolism in bilateral cerebellar hemisphere, moderate hypometabolism in bilateral hippocampi, parahippocampal gyri and amygdale and mild hypometabolism in bilateral paracentral lobules and bilateral thalami.

Procedure

The patient selection was in compliance with the World Medical Associations Helsinki declaration criteria [14]. The protocol of autologous BMMNC intrathecal administration was reviewed and approved by the Institutional Committee for Stem Cell Research and Therapy (IC-SCRT).The procedure was explained to patient's parents and a duly filled informed consent was obtained. Pre-intervention routine blood tests, urinalysis and chest x-ray was performed to rule out active infection and assess fitness for anesthesia. Granulocyte colony-stimulating factor (G-CSF) injections were administrated 72 hours and 24 hours prior to BMMNCs transplantation, to stimulate CD34+ cells and increase their survival and multiplication. 80-100ml of Bone marrow was aspirated from the iliac bone. 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 98%. Approximately $96x10^6$cells were administered immediately post separation, in L4-L5 using a lumbar puncture needle. These cells consisted of 9.76 CD34+ cells/µl. 300 gm methyl prednisolone (solumedrol) in 500 ml Ringer's Lactate (RL) was simultaneously injected intravenously to increase the survival and multiplication of cells. Patient underwent extensive neurorehabilitation which included occupational therapy, applied behavior analysis, sensory integration, speech therapy and psychological therapy. Follow up was conducted after 8 months and he was reevaluated on ISAA, CARS, WeeFIM and CGI scales and a repeat PET CT scan was performed.

Results

After the procedure the patient had no adverse effect and he showed significant improvements over a period of 8 months. His attention and concentration was improved and started following single step commands. Improvements were noted in awareness and judgment about surrounding, imitation skills, emotional response, sitting tolerance and eye contact. He started cycling and playing with ball which he could not do earlier. Parents noted reduction in hyperactivity, motor mannerisms and sensory issues. Now, he had started speaking few words and his memory had also improved. Academically, he showed improvements with respect to identifying fruits, few colors like red, green, blue and yellow, and write alphabets from A to Z, numbers from 1 to 15, and solving puzzles. So there were improvements seen in expression, social interaction, problem solving and memory.

Significant objective improvements were noted after on follow up (Table 1). On ISAA, the score reduced from 114 (moderate autism) to 99 (mild autism). On CARS it improved from 35.9 to 28.5. His WeeFIM score remained the same. Severity of illness on CGI reduced from 4 (moderately ill) to 3 (mildly ill). WeeFIM score improved from 51 to 56. Global improvement on CGI scale (CGI-II) graded him with a score of 2 (much improved). The efficacy index on CGI (CGI-III) showed moderate therapeutic effect (score 5). On comparing the PET-CT scan performed before and

after the intervention improvements were observed in metabolism of frontal lobe, parietal lobe, medial temporal lobe bilateral (bilateral hippocampi and amygdale), cerebellar hemispheres (Table 2).

	Pre (on admission)	Post (After 8 months)
ISAA	114	99
CARS	35.9	28.5
CGI (Severity of illness)	4	3
WeeFIM	51	56

Table 1: Objective improvements after 8 months.

Areas improved on PET CT scan	**Clinical functional improvements**
Frontal lobe (Hypermetabolism)	Initiation, planning, anticipation, organization, problem solving, emotions, attention
Parietal lobe (Hypermetabolism)	Integration of sensory information and language
Medial temporal lobe (B/L Hippocampi and amygdale) (Hypometabolism)	Social brain processing
Cerebellum (Hypometabolism)	Coordination, memory, emotions

Table 2: Areas improved on PET CT scan with clinical functional improvements.

Discussion

Autism spectrum disorder (ASD) is a complex disorder that impairs a child's communication and social interaction abilities due to restricted repetitive behaviors, interests and activities [15]. The exact etiology of autism is unknown [5]. An imbalance of various cytokines like IFN-γ and interleukins (ILs), increased activation of pathways involving Th1 and Th2, increased levels of serum proteins with serum IgG, IgG2 and IgG4 have been reported in patients with ASD indicating an underlying autoimmune mechanism [16]. Hypoperfusion causes abnormal neurotransmitter or metabolite accumulation and hypoxia, causing neural tissue damage. The severity of symptoms observed in ASD is believed to be directly proportional to the degree of hypoperfusion [17]. Imbalance in the excitation-inhibition pathways of neurons may also play a crucial role in the neuropathology of ASD [18]. Various treatment options have been explored for children with ASD. However, none of these has been address the core pathophysiology of ASD. Cell therapy has been emerging as potential treatment for ASD. To study the benefits of cell therapy, we administered autologous BMMNCs. These are comprised of a variety of cells which includes mesenchymal stem cells (MSCs), hematopoietic stem cells, tissue specific progenitor cells and stromal cells [7]. Mesenchymal cells have a unique property of homing, wherein the cells migrate to the site of injury and carry out the repair process. They also have the ability to modulate the immune system and restore the altered brain organization [19]. MNC's enhance angiogenesis by producing signaling molecules such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF2) [20]. They also promote tissue remodeling, prevent apoptosis, decrease inflammation and activate the satellite cells. Angiogenesis reduces hypoxia by improving clearance of toxic metabolites and perfusion [17].

Studies done by Chivate et al have assessed autistic children and majority of them demonstrated low 18FDG uptake on PET imaging predominantly in hippocampus and amygdala, followed by mesial temporal lobe and cerebellum, with high uptake in

frontal lobes [21] The findings in our case report corroborated with what they have described. Reduced glucose uptake reflects reduced metabolic activity of those brain cells [22]. Hence, the hyperfunctioning areas of the brain will show increased metabolism and hypofunctioning areas will show reduced metabolism. There is a correlation between hypoperfused brain areas and the regions of the brain responsible for dysfunction in ASD. The specific temporal lobe areas associated with face recognition, social interaction, and language comprehension, have been demonstrated to be hypoperfused in ASD [7]. In this case comparative PET CT study done at 8 months after the cell therapy has demonstrated positive changes in multiple brain areas (Figures 1 and 2) such as reduced metabolism in frontal and parietal cortex along with an increased metabolism metabolism in medial temporal region, cerebellum indicating improved cellular metabolism in brain.

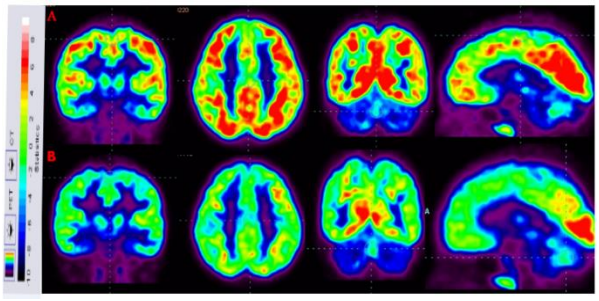

Figure 1: Representative cortical ^{18}F-FDG PET of pre and post cell therapy of autism child. A, represents the ^{18}F-FDG PET of autism child before cell therapy and B, represents the ^{18}F-FDG PET of autism child after cell therapy. Standardized uptake value (SUV) of the images are shown in a scale with values red > yellow > green > blue as indicated on the color chart. Red areas [hypermetabolism] have turned into green areas [normometabolism] in frontoparietal regions of the brain after cell therapy.

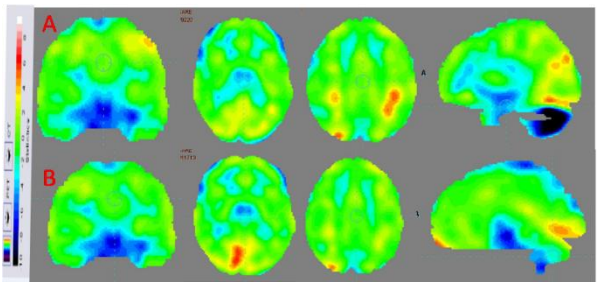

Figure 2 Difference in pre (A) and post (B) cell therapy of normalized PET image with respect to probabilistic population based brain atlas of human brain structure using SCENIUM ratio analysis software. Here we can see the blue and black [hypometabolism] turned into green areas [normometabolism] in medial temporal, and cerebellum after cell therapy.

In this case report improved functions of frontoparietal regions as per PET CT brain scan are correlated with improvements in frontal lobe functions like initiation, problem solving, emotions, attention and parital lobe functions like integration of sensory information and language. Previously published study have also shown improvements after cell therapy [23,24] We observed that the change in the scores on objective measurements of this case report has been well correlated to the clinical improvements noted in social relationship, emotional responses, behavioral patterns, sensory aspect and cognitive components.

There are some limitations of this case study. It is a solitary example of benefits of the intervention in absence of the control group and unblinded assessment. Therefore larger randomized controlled studies with blinded assessments are required to explore the efficacy of the intervention.

Conclusion

We conclude that intrathecal transplantation of autologous BMMNCs is safe and feasible in children with ASD. It has great potential as a novel therapeutic modality when used in conjunction with current rehabilitation and it may improve the quality of life in patients with ASD. The clinical changes can be correlated with the PET CT changes. However, larger population studies with clinical diagnosis of ASD need to be evaluated and followed up, to establish the role of PET imaging in the diagnosis of autism.

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