Cellular therapy in Neurodevelopmental disorders

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ABSTRACT

Neurodevelopmental disorders (NDD) are characterized by developmental deficits that usually appear in the early course of development and maturation, and are caused by a variety of genetic and environmental conditions. They involve 1-2% of the population, and because of their typically life-long course, the treatment cost is very high thus increasing the burden of care-givers. Establishing a definitive treatment for NDD is hence, the need of the hour. Through this review, we have explored the prospects of cellular therapy as a standard approach for NDD. We have reviewed the benefits of intrathecal administration of autologous bone marrow mononuclear cells in autism, cerebral palsy and intellectual disability. Various theories have been put forward to explain the mechanism of these cells. We have justified them by enumerating in detail, our published clinical data in autism, cerebral palsy and intellectual disability. Our studies have collectively demonstrated the positive outcome of cellular therapy. They have shown to improve the quality of life of the affected by improving functional independence alongwith showcasing positive clinical and objective changes. Autologous bone marrow mononuclear cells are safe, feasible, and efficacious and have an immense therapeutic potential.

Keywords: *Neurodevelopmental disorders, autism, cerebral palsy, intellectual disability, autologous, bone marrow, mononuclear cells*

INTRODUCTION

Neurodevelopmental disorders (NDD) are characterized by developmental deficits that usually appear in the early course of development and maturation, and are caused by a variety of genetic and environmental conditions. (1) They are relatively common conditions ranging from mild to severe and producing life-long disability and behavioral abnormalities that can be a burden to families.(1) They can be collectively expressed as disorders with severely impaired neural plasticity. NDDs are apparently caused by mechanisms that affect molecular plasticity, cellular plasticity and tissue plasticity. They are associated with a wide variation of physical, mental, emotional and behavioral features. Commonly known NDDs include autism spectrum disorders, cerebral palsy, attention deficit/ hyperactivity disorder, intellectual and other genetic disorders.(2,3) NDDs are amongst the most diverse and complex conditions, in terms of both pathophysiological mechanisms and possible treatments.

Available treatments can be divided into behavioral, rehabilitation, nutritional, and medical approaches, although no defined standard approach exists which can cure the disease for the NDDs. NDDs involve 1-2% of the population, and because of their typically life-long course the treatment cost is very high. As a result, even a slight improvement in the performance of these patients would be of great significance to the patients themselves, along with their families and society.(4)

Reduction in synaptic plasticity and subsequent generation of incorrect synaptic connections between neurons are the major concern in NDDs.(5) Earlier, it was considered that damaged or incorrect neural connections in the central nervous system (CNS) could not be easily

regrown.1 However; cellular therapy has proved this long standing belief wrong. It has emerged as a powerful therapeutics in the sector of neurological disorders like cerebral palsy, autism, intellectual disabilities, stroke, multiple sclerosis, brain injury.(6-11)

This article discusses the rationale of use of autologous bone marrow derived mononuclear cells as a therapeutic approach in neurodevelopmental disorders, which has been strongly supported by the published work of the authors.

MECHANISM OF ACTION OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS IN NEURODEVELOPMENTAL DISORDERS

Autologous bone marrow mononuclear cells (BMMNCs) are amongst the safe cell types and are available in abundance.(12) They are easily accessible, they provide a renewable population and involve no ethical concern unlike other cell types. Autologous transplantation does not involve the risk of immune rejection. The underlying mechanism of action of bone marrow cells involves neuromodulation, neuroprotection, axonal sprouting, neural circuit reconstruction, neurogenesis, and neurorepair.(6,13,14). The BMMNCs constitute of combination of cells like hematopoietic stem cells, tissue-specific progenitor cells, stromal cells. Various studies have demonstrated the benefits of using a mixture of cells over using subfractionated cell types. (15,16) These cells migrate to the site of injury, inhibit the release of pro inflammatory cytokines and have carry out strong immunosuppressive activities.(17). They act through various mechanisms possible which stimulates the plastic response in the injured tissue of the host.(14) They secrete survival promoting growth factors and restore the synaptic transmitters released and provide local reinnervations. They also integrate into existing neural and synaptic network, and reestablish connections of functional afferent and efferent cells. (13,17) Additionally, these cells have the potential to differentiate into neural cells. (18-20) The growth factors, cytokines, chemokines, bioactive lipids and microvesicles released by the BMMNCs are the major paracrine effects of cellular intervention. The surface of the cells release microvesicles which may deliver RNA and micro RNA into the injured structure (21) and express numerous neural related genes which influence trans differentiation of the cells. (22) These trophic factors altogether modulate the molecular composition of the environment therefore, evoking responses from the resident cells.(23)

We studied the effect of these cells in neurodevelopmental disorders such as autism, cerebral palsy and intellectual disability (ID).

Cellular therapy in autism

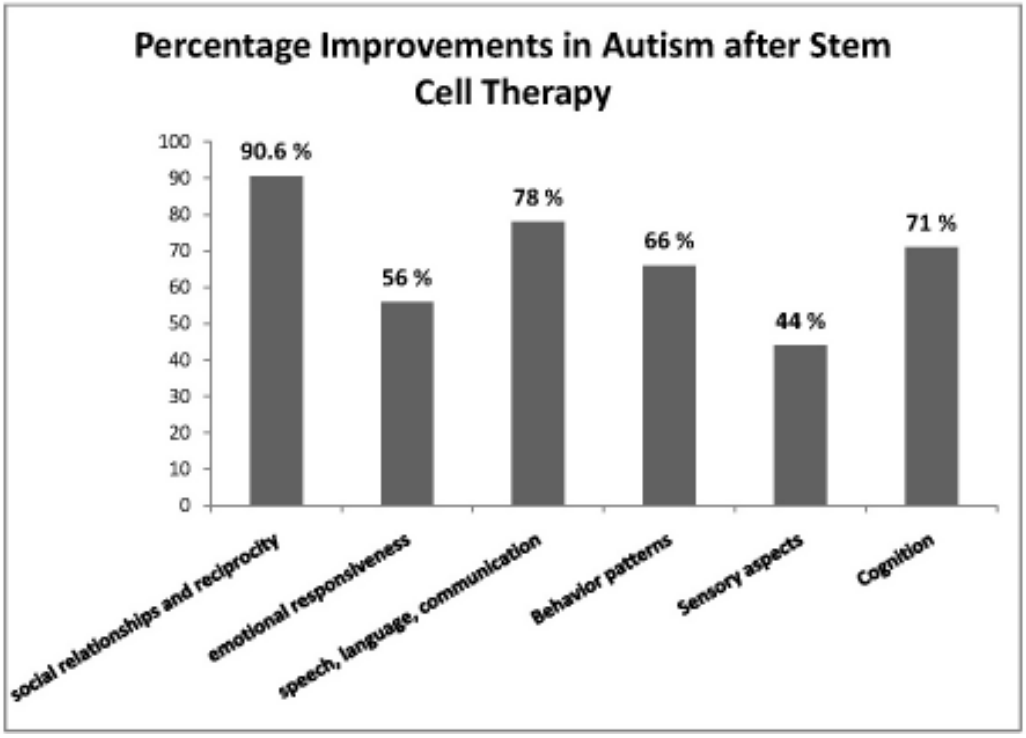
In autism, the abnormal functioning of brain may be due to decreased number of Purkinje cells (PCs), cerebellum alterations, defective cortical organization, and altered plasticity of dendritic spine morphology.(24,25) Cellular therapy has the potential to ameliorate altered brain by integrating and restoring the damaged functions. These cells can reinforce cortical plasticity, promote synaptic plasticity and restore cerebellar PCs. They also have immunomodulatory capacities and can reverse hypoperfusion. (17,26) BMMNCs carry out immunomodulation by increasing anti-inflammatory TGF-? and IL-10; along with an inhibiting pro-inflammatory cytokine production of TNF-?, IL-1? and INF-?. They also show paracrine effects and induce angiogenesis by the production of vital growth factors like fibroblast growth factor (FGF2), vascular endothelial growth factor and ciliary neurotropic factor (CNTF). Immunomodulation counterbalances the dysregulated immune system and restores normal functioning by reducing neural damage.(17) Hypoperfusion causes abnormal neurotransmitter or metabolite accumulation and hypoxia, further causing neural tissue damage. The severity of symptoms observed in autism is believed to be directly proportional to the degree of hypoperfusion. One of the important effects of BMMNCs is neoangiogenesis. This reduces hypoxia by improving clearance of toxic metabolites and perfusion. (27,28)

Clinical Evidence Supporting The Use Of Cellular Therapy In Autism

The above theories are further substantiated by our published results. In 2013, we published an open label proof of concept study in 32 patients of autism. (29) (Figure 1) All patients were

followed up for 26 months (mean 12.7). The outcome measures used were Childhood Autism Rating Scale (CARS), Indian Scale for Autism Assessment (ISAA), Clinical Global Impression (CGI), and Functional Independence Measure (FIM/Wee-FIM) scales. It was found that out of 32 patients, a total of 29 (91%) patients improved on total ISAA scores and 20 patients (62%) showed decreased severity on CGI-I. On CGI-II 96% of patients showed global improvement. Positron Emission Tomography-Computed Tomography (PET-CT) scan recorded objective changes. Few adverse events were reported, including seizures in three patients, but these were reversible and easily controlled with medications.

Figure 1: Graph representing symptomatic improvements in autism after stem cell therapy (N=32)



In addition to the above case series, 6 separate case reports have also been published documenting the safety, efficacy and objective radiological improvements in patients of Autism following cell therapy. (30-35)

A 14 yr old boy with severe autism was administered with autologous BMMNCs, intrathecally and followed up after 6 months and 1 year. He showed significant functional improvements in his behavior, social interaction and emotions. Aggression and hyperactivity had reduced. Improvement in impulse control, reading skills, tracing, recognition of all shapes, learning new tasks and command following was also noted. His score on CARS reduced from 42.5 (Severely autistic) to 23.5 (Non-Autistic) but the general impression on clinical assessment showed mild autism. On repeating brain PET scan after 6 months, there was marked increased uptake in bilateral temporal lobes and bilateral calcarine cortices with mild increased uptake in left medial pre-frontal cortex.

We also reported a case of adult autism (33 year old), who was administered with autologous BMMNCs twice at an interval of 6 months. Over a period of 9 months, his ISAA scores reduced from 94 (Mild autism with 60% disability) to 64 (no autism). The CGI showed improvement by change in severity of illness from 3 (Mildly ill) to 1 (Borderline mentally ill). Global improvement on CGI was scored 2 (much improved) with an efficacy index of 5 (moderate therapeutic effect). PET CT scan was repeated at 6 months which showed a balancing effect in the metabolism of frontal, temporal, mesial temporal, amygdale, hippocampus, para hippocampus, parietal, para hippocampus, basal ganglia, cerebellum amongst others. Functionally, improvements were

observed in his attention span, tongue movements,eye contact, eye-hand co-ordination, behavior pattern, language and communication, sensory aspects, problem solving. There was significant decrease in aggressive behavior and hyperactivity.

In another case of autism with comorbid mental retardation treated with intrathecal administration of autologous BMMNCs, there was significant clinical improvement recorded in social relationship, communication and behavior. On ISAA, score improved from 111 (Moderate autism) to 73 (Mild Autism). PET CT scans comparison of pre and post therapy showed improved metabolism in Brocas, external frontal, medial temporal pole, precentral, sensory motor, prefrontal and insula in the right hemisphere and medial prefrontal and thalamus in the left hemisphere. Intelligent Quotient (IQ) on The Malin's Intelligence Scale for Indian Children increased from 44 to 49.3 (Moderate Mental Retardation). Functional improvements were recorded in social relationship and reciprocity, emotional responsiveness, speech-language and communication, behavioral patterns, sensory aspects and cognitive component.

In a case of an 11 year old boy with autism who underwent intrathecal autologous BMMNC transplantation, improvements were recorded in his speech, awareness of the surroundings, eye contact, attention and concentration, logical thinking, command following, emotional responses and writing speed. His stereotypical and self stimulatory behavior along with aggressive behavior and hyperactivity had reduced. He could now eat on his own which he could not do before. His scores on CARS improved from 31 to 25, on ISAA from 130 to 98, on CGI-I from 6 to 5 and on FIM from 104 to 110.

In a case of a 7 year old boy with autism treated with intrathecal autologous BMMNCs clinically significant improvements were observed in behavior, social interaction, speech, communication, cognition and command following. The ISAA score improved from 131 to 112, CARS from 40.5 (severely autistic) to 32 (mild to moderate autism), and WeeFIM score from 31 to 36. Severity of illness on CGI (CGI I) changed from 4 (moderately ill) to 3 (mildly ill). Global improvement on CGI (CGI II) was measured at a score of 2 (much improved), along with an efficacy index (CGI III) of 5 showing moderate therapeutic effect.

In a 9-year-old boy with autism, 2 doses of autologous BMMNCs were administered intrathecally. On follow up, significant clinical improvements were noted in social relationship, communication and behavior. His ISAA score improved from 132 (moderate autism) to 103 (mild autism) and CARS improved from 31 (moderately autistic) to 26 (non-autistic). On comparison of the PET-CT scan, changes in metabolism were recorded in parahipppocampal and hippocampal regions, the cingulate and the paracingulate gyrus, the mesial temporal structures and the temporal lobe which further correlated with the clinical improvements.

Cellular therapy in cerebral palsy

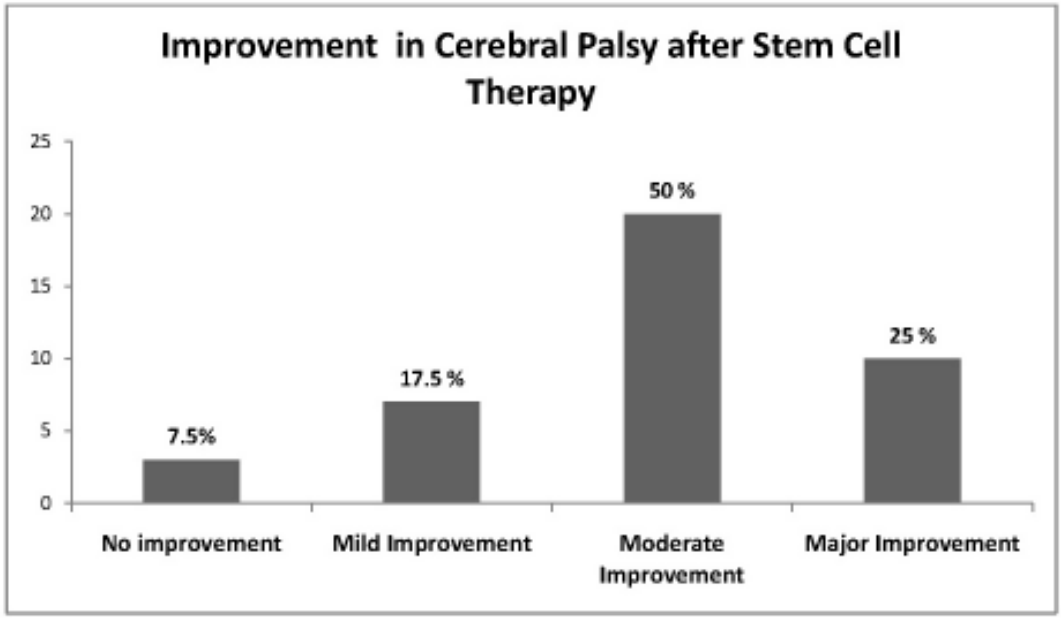
In cerebral palsy, events due to multiple factors like inflammation with excessive cytokine production, oxidative stress, excess release of glutamate triggering the excitotoxic cascade result in permanent lesions of the brain. This also leads to hypoxia, disturbances in axonal and dendrite growth, synapse formation and myelination.(36) The core neuropathology behind cerebral palsy comprises white-matter injury, called as periventricular leukomalacia, as well as germinal matrix hemorrhage with intraventricular extension, and damage to the cortex, basal ganglia, and thalamus.(37) Cell transplantation in CP could have lead to the survival, homing, and differentiation of cells into neurons, oligodendrocytes, and astrocytes in the injured structure to carry out regeneration. Bone marrow cells may also secrete neurotrophic factors and growth factors such as connective tissue growth factor, fibroblast growth factors 2 and 7, interleukins, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) which are accountable for cell proliferation, cytoprotection, and angiogenesis, retrieving the lost tissue functions. These processes will help to overcome the injury occurred due to hypoxia.(38)

Clinical Evidence supporting the use of cellular therapy in cerebral palsy

In 2015, we published a nonrandomized study

on 40 cases of all types of cerebral palsy treated with autologous bone marrow mononuclear cells intrathecal administration. (39) Three months after intervention, 14 patients showed improvement in oromotor activities, 11 in neck control, 17 in sitting balance, 15 in standing balance, 9 in walking balance, and 12 in speech. At six months, 38 out of 40 (95%) patients showed improvements and 2 did not show any improvement but remained stable without any deterioration. No major adverse events were noted except for seizures in 2 patients which were self limiting and were controlled by medications. The study, thus demonstrated the safety, feasibility, and efficacy of the intervention.

Figure 2: Graph representing improvement in cerebral palsy after stem cell therapy



Apart from the above case series, 3 separate case reports by Sharma el al, have also been published documenting the safety, efficacy and objective radiological improvements in patients of Cerebral Palsy following cell therapy. (40-42)

A 20 year old male with CP and Mental Retardation (MR), administered with autologous BMMNCs, showed significant increase in metabolic activity in frontal, temporal, parietal, and occipital lobes, mesial temporal structures and cerebellar hemisphere on comparative PET CT imaging of brain. These changes were also supported by clinical improvement in cognition, attention, concentration, eye contact, motor activities and co-ordination, social behavior, speech, balance, command following, and daily functioning. He could walk independently without support and could perform tandem staircase climbing with minimal support.FIM score improved from 89 to 93. IQ score improved from 44 to 53.

A 2 year old girl with spastic cerebral palsy, administered with autologous BMMNCs intrathecally, showed significant functional improvements along with correlating dramatic changes in PET-CT scan brain, 6 months after the therapy. Improvements were recorded in neck control, standing and sitting balance, spasticity of all limbs and speech. On comparing the PET scan of brain, increased FDG uptake was seen in the mesial temporal lobe, right basal ganglia, frontal, temporal, parietal and occipital lobes

In a 12 year old CP case, positive clinical and functional outcomes were noted 6 months after intrathecal administration of autologous BMMNCs. His trunk strength, upper limb control, hand functions, walking stability, balance, posture and coordination improved along with ability to perform activities of daily living. FIM score increased from 90 to 113. On repeating the PET-CT scan of the brain six months after the intervention, the mean standard deviation values of the central region, cerebellum,

vermis, supplementary motor areas and paracentral lobule progressed towards normalization.

Cellular therapy in Intellectual disability

The primary pathology behind IDs is the deficiencies in neuronal network connectivity in the major cognitive areas of the brain, which secondarily results in impaired information processing. (43) Bone marrow cells have the property to restore the synaptic transmitters released and provide local reinnervations to the area affected. It also integrates existing neural and synaptic network, and reestablishes connections of functional afferent and efferent cells which may have contributed in mending the cognitive and functional deficit in IDs.(13-14)

Clinical Evidence supporting the use of cellular therapy in Intellectual disability

Our published case report in 2015, of a 13 year old boy with intellectual disability, verified the above premise. He was administered with autologous BMMNCs and followed up after 3 months and 6 months of intervention. No major adverse events were recorded post intervention. Over a period of 6 months, he showed improved eye contact, cognition, learning ability, behavior and ability to perform activities of daily living. His score on Functional Independence Measure (FIM) increased from 67 to 76. On comparing the pre and post PET CT scan, improvement in metabolic activity of hippocampus, left amygdala and cerebellum was recorded. These changes correlated to the functional outcome.(44)

The route of delivery of cells plays an important role in maximizing the clinical output of cellular therapy. In all the above studies, the autologous BMMNCs were administered intrathecally which enhanced the accessibility of the injected cells into the brain.(45) Intrathecal route of administration is easy, relatively minimally invasive and is devoid of any major side effects.(46) Studies have shown that on administering cells intravenously, only few cells reach the damaged site while a majority of cells get trapped in pulmonary passage. (47) An alternate route of administration is via intracerebral route. However, it is an invasive technique and might result in secondary complications such as bleeding and neural tissue injury. (48) Hence, as compared to all the delivery routes, intrathecal administration is most efficacious.

Role of Rehabilitation in combination with cellular therapy

In all our studies, the children were advised to follow personalized neurorehabilitation program post transplantation. Exercise augments the effect of stem cells by mobilization of local stem cells, encouraging angiogenesis and promoting recovery after stem cells transplantation.(49) Regular exercise decreases the proinflammatory cytokines and up regulates anti-inflammatory cytokines in various tissues of the body including brain. Exercise also up regulates expression of CXCR4+ receptors, key factors responsible for homing, in ischemic tissue ensuring enhanced homing of stem cells. (50-52) Hence, the therapeutic potential of cellular therapy manifolds with rehabilitation.

Positron emission tomography-computed tomography brain (PET-CT) as a monitoring tool

PET-CT brain can be used effectively as a monitoring tool to study the outcome of cellular therapy. It 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. (53) It discriminates tissues that normally show high metabolic activity from those with abnormally increased activity. Fluorodeoxy-glucose (FDG) measures the regional cerebral glucose expenditure as neuronal activity is strongly associated to glucose metabolism. So the alteration in neuronal activity caused by disease is reflected in change of glucose metabolism and can be revealed in the PET-CT brain scan.(54)

In our above mentioned studies, we have recorded changes in the brain metabolism of patients with cerebral palsy, autism and ID. (Figure 4-6) These changes correlated with the clinical improvement indicating that PET-CT brain scan identify alteration occurring at the tissue levels.

Figure 3 : PET CT scan showing improved metabolism of brain in autism. The hypermetabolic areas (Red) and hypometabolic areas (Blue) reduced significantly demonstrating improvement.

indeed

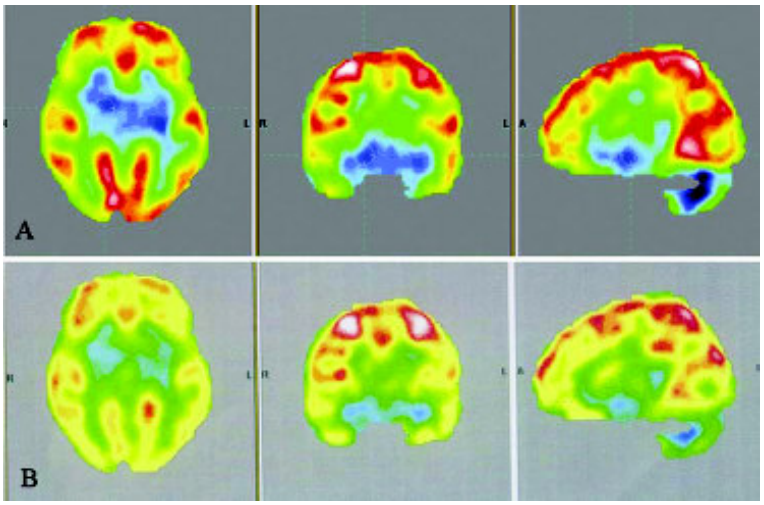


Figure 4 : PET CT brain showing improvements in cerebral palsy. Hypometabolic areas (Blue) have reduced significantly after stem cell therapy

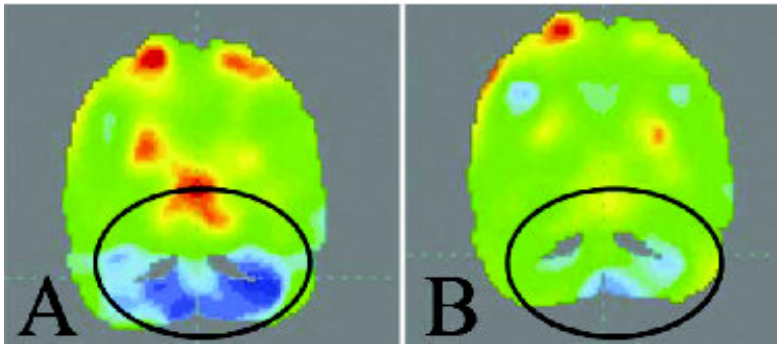
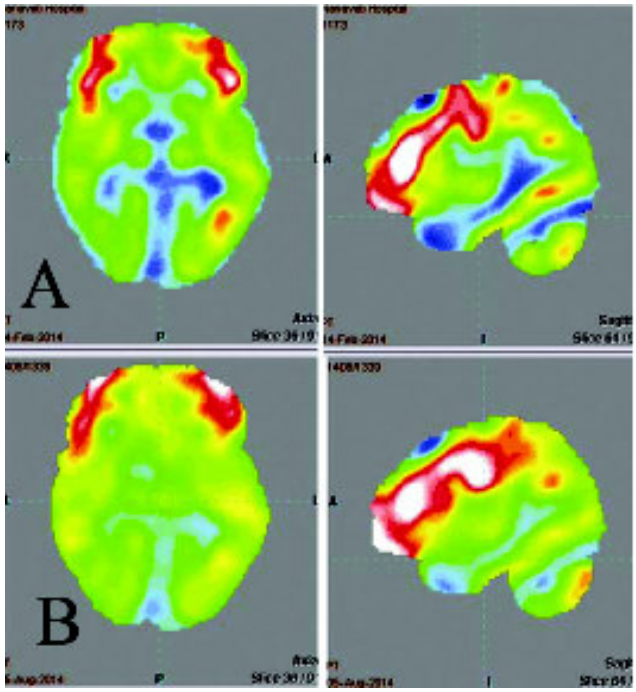


Figure 5: PET CT Brain showing improvements in intellectual disability . The hypometabolic areas (blue and black areas) have reduced significantly indicating improvement post intervention.



CONCLUSION

Human brain is not fully matured and developed in the early life hence the neural plasticity is maximal during this period (55) so the intervention during this period might be of more value than at any other phase of life. Cellular therapy combined with neurorehabilitation plays a major role in improving the overall symptoms in children with NDDs. It has the potential for neuroprotection and neuroregeneration of the CNS. It can address the core pathology in NDDs and can lead to neural plasticity, improved functions, quality of life and independence levels of these children. This review summarizes the effect of cellular therapy on the functional deficits with minimal risks of rejection and side effects in different NDDs like autism, cerebral palsy and intellectual disabilities further strengthening the rationale underlying the use of autologous BMMNC transplantation in NDDs.

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