Clinical Study

Autologous Bone Marrow Mononuclear Cell Therapy for Autism: An Open Label Proof of Concept Study

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Cellular therapy is an emerging therapeutic modality with a great potential for the treatment of autism. Recent findings show that the major underlying pathogenetic mechanisms of autism are hypoperfusion and immune alterations in the brain. So conceptually, cellular therapy which facilitates counteractive processes of improving perfusion by angiogenesis and balancing inflammation by immune regulation would exhibit beneficial clinical effects in patients with autism. This is an open label proof of concept study of autologous bone marrow mononuclear cells (BMMNCs) intrathecal transplantation in 32 patients with autism followed by multidisciplinary therapies. All patients were followed up for 26 months (mean 12.7). Outcome measures used were ISAA, CGI, and FIM/Wee-FIM scales. Positron Emission Tomography-Computed Tomography (PET-CT) scan recorded objective changes. 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. The difference between pre- and postscores was statistically significant ($P < 0.001$) on Wilcoxon matched-pairs signed rank test. On CGI-II 96% of patients showed global improvement. The efficacy was measured on CGI-III efficacy index. Few adverse events including seizures in three patients were controlled with medications. The encouraging results of this leading clinical study provide future directions for application of cellular therapy in autism.

1. Introduction

Autism spectrum disorders (ASD) are a group of heterogeneous neurodevelopmental disorders characterized by deficits in verbal and nonverbal communication, social interaction, and presence of stereotypical repetitive behavior. The genetic, environmental, and immunological factors have been attributed as underlying causes, though its exact etiology is unknown. The incidence of autism has increased to a great extent, which may be due to increased awareness leading to an early and accurate diagnosis or due to perinatal complications, genetic factors, environmental factors, and lifestyle changes. Presently, the worldwide incidence is 12 per 1000 children [1]. Despite its increasing rate, currently autism remains untreatable. The available options of behavioral therapy and pharmacological and supportive nutritional treatments are only palliative. Medical therapy is directed towards the neuropsychiatric disorders associated with ASDs. Commonly prescribed medicines are selective serotonin reuptake inhibitors, antipsychotics, mood stabilizers, and psychostimulants. Methylphenidate may be used to treat attention deficit or hyperactivity. Anticonvulsants are used
for seizures with autism [2]. However, the use of medications is limited by their side effects. There is a desperate need of a medical intervention to tackle the basic pathogenetic mechanisms. Several genes have been found to be associated with ASD. This provides the basis for treatment with gene therapy in future. Currently, for safe human gene therapy to be applied to this population, several areas need further research [3].

The major neurophysiological alterations are immune abnormalities and neural hypoperfusion, and its correlation with symptomatology has been reported [4]. Cellular therapy exerts potent angiogenetic and immunoregulatory effects along with other paracrine effects [5, 6]. Recently, cell transplantation has been shown to be safe and efficacious in several neurological disorders [7–10]. A variety of cellular therapies with different cell types and routes of administration is being explored. The major types are embryonic, umbilical cord, induced pluripotent, and adult stem cells. The use of adult stem cells is devoid of any ethical issues and can be obtained from bone marrow, adipose tissue, skin, dental pulp, and other sources. Bone marrow stem cells have been extensively studied and can be easily procured [8, 11]. The intrathecal route is an easy, safe, and direct approach to provide the cells to the brain without causing any neural tissue damage [12, 13].

Conceptually, cellular therapy should give beneficial clinical effects in patients with autism. The concept is based on its potential to counterbalance the core pathogenetic mechanisms of autism [4]. The aim of this study is to assess the safety, efficacy, and clinical effects of autologous bone marrow mononuclear Cells (BMMNCs) transplantation in patients with autism.

2. Materials and Methods

2.1. Study Design. This is an open label proof of concept study on the use of autologous BMMNCs transplantation in 32 patients with autism. The intervention included cellular therapy with intrathecal transplantation of autologous bone marrow derived mononuclear cells followed by occupational therapy, speech therapy, and psychological intervention. The primary objective was to document any adverse events and establish the safety of the intervention within a period of 26 months (December 2010 to February 2013). The secondary objective of the study was to evaluate the effects of the intervention on symptoms, disease severity, extent of disability, and functional impairment caused by autism.

2.2. Participants Eligibility Criteria and Recruitment. Patient selection was based on World Medical Association Helsinki Declaration for Ethical Principles for medical research involving human subjects [14]. A written informed consent was obtained from the parents of all patients. All the patients included in the study had confirmed diagnosis of autism according to the DSM-IV TR diagnostic criteria for autistic disorder. The exclusion criteria were presence of acute infections such as HIV/HBV/HCV, malignancies, bleeding tendencies, renal failure, severe liver dysfunction and other acute medical conditions such as respiratory infection and pyrexia.

2.3. Intervention

2.3.1. Preintervention Assessment. The Institutional Committee for Stem Cell Research and Therapy (IC-SCRT) granted the ethical approval for the treatment protocol. An informed consent was taken from the parents of all the patients. Prior to intervention, all the patients underwent a thorough clinical examination with serological, biochemical, and hematological tests. Magnetic Resonance Imaging (MRI) of the brain and Electroencephalography (EEG) were also conducted in all patients. In view of the clinical improvements observed, a preintervention PET-CT (Positron Emission Tomography-Computed Tomography) scan was introduced at a later stage of the study.

2.3.2. Procurement of Autologous BMMNCs. Patients were administered Granulocyte Colony Stimulating Factor (GCSF) 48 hours and 24 hours before the harvest and transplantation of BMMNC [15]. On the day of the transplantation, bone marrow was aspirated under general anesthesia in the operation theatre with aseptic precautions. Approximately, 100 mL of bone marrow (varying between 80 mL and 100 mL, based on the age and body weight) was aspirated from the region of anterior superior iliac spine using the bone marrow aspiration needle and collected in the heparinized tubes.

2.3.3. Isolation of BMMNCs. The aspirate was then transferred to the laboratory where the mononuclear cells were separated by the density gradient method. CD34+ counting was done by Fluorescence activated cell sorting (FACS) [16]. The MNCs were checked for viability (average viability count was found to be 97%).

2.3.4. Mode of Cell Transplantation. The separated autologous BMMNCs were immediately injected on the same day, intrathecally using an 18 G Tuohy needle and epidural catheter at the level between fourth and fifth lumbar vertebrae. The average numbers of cells injected were $8.19 \times 10^7$. Simultaneously 20 mg/kg body weight methyl prednisolone in 500 mL Ringer Lactate was given intravenously to enhance survival of the injected cells. Patient was monitored for any adverse events.

2.3.5. Post-BMMNCs Transplantation Therapy. All the patients underwent extensive therapy under the guidance of experts. This includes occupational therapy interventions based on sensory integrative approach, activities of daily living (ADL) training, psychological intervention based on behavior modification techniques, speech therapy, and specific dietary recommendations. The therapy protocol was planned out specifically for individual patients, as per the detailed assessment done before the therapy.

The therapy sessions conducted during the stay in the hospital were recorded and compiled in a CD-ROM which
was given to all the patients at discharge. A therapy plan to be followed at home was designed for all the patients. Patients were advised to continue therapy at home under the supervision of a professional. Follow-up assessment was done at regular intervals over a period of 26 months.

2.4. Outcome Measures. To assess the safety of the intervention, outcome measures were used to monitor any major or minor adverse events through the entire duration of follow-up. Patients were counseled regarding the probable adverse events during informed consent. Recording of adverse events during the hospital stay was done by a health professional whereas after discharge it was recorded, as reported by the parents or primary care givers.

2.4.1. Monitoring Procedure Related Adverse Events. Acute procedural adverse events, associated with cell aspiration and injection via lumbar puncture, were stringently monitored over 5–7 days after intervention. Body temperature, blood pressure, respiratory rate, and heart rate were recorded at regular intervals. Patients were examined thoroughly for signs of spinal headache, motor or sensory loss, incontinent bowel and bladder, damage to brain or spinal cord, respiratory distress, cardiac failure, and allergic reaction. Aspiration and injection sites were examined every day for pain, bleeding, and signs of infection. Signs and symptoms of any anesthesia complications, back pain associated with lumbar puncture, headache, nausea, and vomiting were checked regularly. All the minor acute procedural adverse events were treated medically prior to the discharge of the patients from the hospital. Patients were examined thoroughly at each follow-up for neurological deficits exhibiting clinically as motor or sensory loss that was not present before intervention. A detailed history was also taken to rule out any transient neurological symptoms.

2.4.2. Monitoring Cellular Transplantation Related Adverse Events. During the stay in the hospital, signs and symptoms of any allergic reaction were monitored at regular intervals. Long term major and minor adverse events were monitored to establish the safety of stem cell transplantation. A detailed history was also taken to rule out presence of any seizures. If any seizure was reported, EEG was done for evaluation and managed by a neurologist. Any clinical deterioration of the core symptoms of autism was recorded by Indian Scale for Assessment of Autism (ISAA), Clinical Global Impression (CGI) scale, Functional Independence Measure (FIM), and Wee-FIM scales (see Supplementary Material available online at doi: [http://dx.doi.org/10.1155/2013/623875 (Appendices I, II, III, and IV)]).

2.4.3. Monitoring the Effects After Intervention. Outcome measures used for the effects of intervention were CGI, ISAA, FIM, and Wee-FIM scales. CGI scale was used for measuring the change in the severity of the disease, overall improvement, and the efficacy of the treatment. The efficacy component of CGI takes into consideration the improvements and side effects after the cellular transplantation. CGI-I scale for severity of illness was scored before cellular therapy and at follow-up visit. CGI-II scale for global improvement and CGI-III scale for efficacy index were each measured at the time of follow-up assessment. The scores recorded by experienced clinicians during the latest visit were used for the analysis. The CGI-I scale is an ordinal scale and reflects a clinical comparison of patients’ severity of symptoms with the typical clinical presentation. Although CGI scale is grossly perceived as a subjective scale the psychometric properties of the CGI scale have been calculated earlier for various psychiatric disorders. The scale shows good reliability, validity, and sensitivity for these disorders [17–19]. The CGI scale for autism has been previously used successfully as an outcome measure in various trials [20–22].

The effect of cell transplantation on the extent of disability was measured using the ISAA. Most of the available diagnostic tools and outcome measures have been designed for the western population. In a disorder like autism where communication and social behavior is impaired, an assessment tool that takes into account the social and cultural context is crucial. Although derived from CARS, ISAA has been designed for Indian population. It is divided into six domains. There are forty questions which are comprehensive and specific to the difficulties experienced by children with autism. It grades the symptoms in ascending order of intensity of symptoms on an ordinal scale of 1 to 5. The content, construct and concurrent validity, internal consistency and test-retest reliability, and sensitivity and specificity of ISAA were studied by the members of the expert committee for the development of assessment tool for autism. ISAA was thus found to be a valid tool, with good reliability and high sensitivity and specificity [23]. ISAA scores were marked during the assessment before the stem cell transplantation and at every follow-up assessment after transplantation, however the scores marked at the latest assessment were considered for comparison and analysis.

To measure the functional independence in activities of daily living, Wee-FIM for children below the age of 8 years and FIM for above 8 years were used. FIM has been shown to be a valid and reliable tool [24]. In addition to these outcome measures various symptoms were monitored through structured interviews and assessment by clinicians.

In view of clinical improvements, PET-CT scanning was introduced to observe functional neuroimaging changes in brain. Measurements were taken before and six months after the transplantation. PET studies were performed using the Siemens Biograph mCT with 64-slice high speed scanner, 3D PET True V wide detector (Siemens-CTI, Knoxville, Tenn., USA), which has an intrinsic resolution of 0.6 mm full width at half maximum (FWHM) and the images of 45–50 contiguous transverse planes with a field of view of 21.6 cm axial PET FOV with True V.

Standard conditions were maintained during all of the [18F] FDG PET scans. Time duration between injection of the dye and scanning was constant at 30 minutes for all the patients and at all instances. The scan room was dimly lit and there was minimal auditory stimulation during
injection and scanning period. PET scan was performed with patients lying in supine position with eyes closed to reduce any activity related confounding effect. Imaging data were processed using proprietary Scenium Software before final image reconstruction.

2.5. Statistical Analysis

2.5.1. Description of Sample. The demographic data for all the patients was recorded and analyzed. Mean age in years at the time of intervention, mean age in years at the time of diagnosis, and mean time duration in months at which the patients were followed up were calculated. Median CGI and FIM scores were calculated. Median score was calculated for the total ISAAs core and the individual domains.

2.5.2. Statistical Tests. The pre- and postintervention scores of CGI-I, total ISAAs scores, scores of ISAAs domains, Wee-FIM, and FIM were compared using Wilcoxon signed rank test for matched pairs with the predetermined level of significance at 0.05. Percentage analysis was conducted for the CGI-II and CGI-III scales and for individual symptoms as described in ISAAs scale. Statistical analysis was carried out using SPSS 17.0 software.

3. Results

3.1. Description of the Sample. There were total 32 patients with 24 (75%) males and 8 (25%) females. The age of the population ranged from 3 to 33 years with a mean age of 10.5 (5.6) years. They were diagnosed on an average at 71 (5.2) years before the intervention. The follow-up period ranged between 5 and 26 months with a mean followup of 12.7 (71) months. The baseline ISAAs scores ranged from 148 to 160 with a median of 115.5 (18.33) (due to the even number of patients), CGI-I scores ranged from 3 to 6 with a median of 4.5 (1), FIM scores ranged from 48 to 118 with a median of 77 (18), and Wee-FIM scores ranged from 18 to 110 with a median of 76 (24) (Table 1).

3.2. Statistical Analysis. There was a statistically significant difference between the pre- and post-CGI-I scores ($P = 0.001$) and total ISAAs scores ($P < 0.001$) (Table 2). There was no statistically significant difference between the FIM scores

### Table 1: Demographic data and scores of CGI, ISAA, FIM, and Wee-FIM scales before cell transplantation.

<table>
<thead>
<tr>
<th>Category</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average/median</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow-up duration (months)</td>
<td>5</td>
<td>26</td>
<td>12.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Age at intervention (years)</td>
<td>3</td>
<td>33</td>
<td>10.49</td>
<td>5.59</td>
</tr>
<tr>
<td>Diagnosed since (years)</td>
<td>0</td>
<td>18</td>
<td>7.17</td>
<td>4.20</td>
</tr>
<tr>
<td><strong>Baseline scales scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGI-I scale scores</td>
<td>3</td>
<td>6</td>
<td>4.5</td>
<td>0.97</td>
</tr>
<tr>
<td>ISAAs scale scores</td>
<td>148</td>
<td>160</td>
<td>115.5</td>
<td>24.26</td>
</tr>
<tr>
<td>FIM scale scores</td>
<td>48</td>
<td>118</td>
<td>77</td>
<td>18.32</td>
</tr>
<tr>
<td>Wee-FIM scale scores</td>
<td>18</td>
<td>110</td>
<td>76</td>
<td>24.06</td>
</tr>
</tbody>
</table>

### Table 2: Change in the scores of CGI and ISAA before and after intervention.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Median score before cellular therapy</th>
<th>Median score after the cellular therapy</th>
<th>Test statistics</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGI-I</td>
<td>4.5</td>
<td>3</td>
<td>$Z = -3.509$</td>
<td>$P &lt; 0.001^*$</td>
</tr>
<tr>
<td>ISAAs scale</td>
<td>115.5</td>
<td>97</td>
<td>$Z = -4.670$</td>
<td>$P &lt; 0.001^*$</td>
</tr>
</tbody>
</table>

*Statistically significant (level of significance at $P < 0.05$).

($Z = -1.841, P = 0.066$) and Wee FIM scores ($z = -1.000, P = 0.317$). All the ISAAs domains showed a statistically significant ($P < 0.05$) reduction after intervention (Table 3).

3.3. Percentage Analysis. Overall the ISAAs score reduced in 29 out of 32 (90.6%) patients. On CGI-II scale 96.9% showed global improvement. Out of these 43.7% patients showed much improvement and 34.4% patients showed very much improvement. There were 18.8% with minimal improvement and 3.1% patients with minimal worsening (Figure 1). According to the CGI-III scale 93.8% patients had no side effects and only 6.2% patients had minimal side effects that did not interfere with function. 21.8% patients showed marked improvement with no side effects, 40.6% patients showed moderate improvement with no side effects, 28.1% patients showed moderate improvement with side effects that did not interfere with patients functioning, and 3.1% patients showed moderate improvement with side effects that did not interfere with patients functioning (Figure 2).

Here we describe the number of patients showing improvement in a particular symptom as indicated by the percentage in the parenthesis.

In the domain of social relationships and reciprocity, 29 out of 32 (90.6%) patients showed improvement. Patients showed improved eye contact (70%), social smile (56%), and reaching out to others (42%). They were able to take turns in social interaction (55%), respond to social or environmental cues (46%), and maintain peer relationships (55%). There was a decrease in three symptoms: inability to relate to people (30%), tendency to remain aloof (59%), and engagement in solitary and repetitive play activities (41%).
Table 3: Change in the ISAAs scores of individual domains measured before and after intervention.

<table>
<thead>
<tr>
<th>ISAA scale domain</th>
<th>Median score before cellular transplantation</th>
<th>Median score after cellular transplantation</th>
<th>Test statistics of Wilcoxon signed rank test for matched pairs</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social relationship and reciprocity</td>
<td>35.5</td>
<td>13</td>
<td>−4.118</td>
<td>$P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Emotional responsiveness</td>
<td>23</td>
<td>20</td>
<td>−3.153</td>
<td>$P = 0.002^*$</td>
</tr>
<tr>
<td>Speech, language, and communication</td>
<td>13</td>
<td>11</td>
<td>−3.989</td>
<td>$P &lt; 0.001^*$</td>
</tr>
<tr>
<td>Behavior patterns</td>
<td>29</td>
<td>10</td>
<td>−3.126</td>
<td>$P = 0.002^*$</td>
</tr>
<tr>
<td>Sensory aspects</td>
<td>21</td>
<td>17</td>
<td>−2.409</td>
<td>$P = 0.016^*$</td>
</tr>
<tr>
<td>Cognitive component</td>
<td>11</td>
<td>8</td>
<td>−3.508</td>
<td>$P &lt; 0.001^*$</td>
</tr>
</tbody>
</table>

*Statistically significant (level of significance at $P < 0.05$).

Improved emotional responsiveness was observed in 18 out of 32 (56%) patients. Inappropriate emotional responses (42%), exaggerated emotions (48%), engaging in self-stimulating emotions (55%), and getting excited or agitated for no apparent reason (56%) decreased. Lack of fear of danger (37%) did not reduce significantly; yet, few patients were reported to have shown some decrease.

Under the speech, language, and communication domain there was an improvement observed in 25 patients out of 32 (78%). A significant reduction was seen in echolalic speech (54%), engaging in stereotyped repetitive use of language (53%), production of infantile squeals or unusual noises (52%), inability to initiate or sustain conversation with others (45%), and inability to grasp the pragmatics of the conversation (43%) and speech regression (50%). It is noteworthy that few patients also showed clinical improvement in symptoms of difficulty in using nonverbal language or gestures (32%), using of jargon or meaningless words (28%), using of pronoun reversal (27%).

Behavior patterns of 21 out of 32 patients (66%) improved. Hyperactivity or restlessness (71%) and engaging in stereotyped repetitive motor mannerisms (65%) decreased significantly. Attachment to inanimate objects (50%), throwing temper tantrum (42%), aggressive behavior (48%), self-injurious behavior (53%), and insisting on sameness (43%) also reduced.

Sensory aspects improved in 14 out of 32 patients (44%). Unusual sensitivity to sensory stimuli (50%), staring into space for long periods of time (25%), difficulty in tracking objects (46%), unusual vision (62%), insensitivity to pain (26%), and responses to objects or people unusually by smiling or touching or tasting (39%) reduced significantly.

Cognitively they showed improved consistency in attention and concentration and response time. 71% patients showed better attention and concentration, 45% patients showed reduction in the delay in responding.

Functional neuroimaging in the form of PET-CT scan in eight patients noted the following changes. After cellular therapy changes in the glucose metabolism in the form of FDG uptake were observed in the frontal and parietal lobes of six patients, occipital and temporal lobes, of five patients and cerebellum of four patients. Further analysis of other regions showed changes in medial temporal lobe of four patients; amygdala, hippocampus, and parahippocampus of three patients; and cingulate, paracingulate area, and basal ganglia of five patients.
Table 4: Adverse events monitored over the entire period of follow-up of 26 months.

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Present during the period of follow-up</th>
<th>Absent during the period of follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Procedure related</td>
<td>Cellular transplantation related</td>
</tr>
<tr>
<td>Minor</td>
<td>(i) Spinal headache (3.6%)</td>
<td>(ii) Nausea (10.7%)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Major</td>
<td>(i) Seizures* (9%)</td>
<td>(ii) Transient increase in hyperactivity (18.7%)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>(iii) Parasthesia in lower limb</td>
<td>(iv) Loss of sensation in lower limb</td>
</tr>
<tr>
<td></td>
<td>(vi) Hematoma at the site of injection</td>
<td>(vii) Hematoma at the site of aspiration</td>
</tr>
<tr>
<td></td>
<td>(ix) Meningismus or meningitis</td>
<td>(x) Systemic or brain infection</td>
</tr>
<tr>
<td></td>
<td>(xii) Respiratory distress</td>
<td>(xiii) Cardiac failure</td>
</tr>
</tbody>
</table>

*Seizures were considered to be an adverse event when seizures observed were new onset postintervention with no previous history or there was increased frequency or severity of seizures as compared to preintervention.

3.4. Adverse Events Monitoring

3.4.1. Procedure Related Adverse Events. During the procedure there were no complications in the operation theatre. None of the patients had signs and symptoms of local or systemic infection, meningismus, any neurological deficit, paraesthesia in the lower limb, any nerve root damage, or hematoma at the site of aspiration and allergic reaction. During the hospital stay, few patients showed procedure related minor adverse events like spinal headache (3.6%), nausea (10.7%), vomiting (17.9%), backache and pain at the site of injection (7.1%), and aspiration (7.1%). These adverse events were controlled with medication and resolved within one week. There were no procedure related major adverse events (Table 4).

3.4.2. Cellular Transplantation Related Adverse Events. Adverse events related to cellular transplantation were transient minimal increase in hyperactivity (6 out of 32), persistent increase in hyperactivity (1 out of 32), and seizures (3 out of 32). Six patients had transient increase in hyperactivity at three-month follow-up. One patient showed persistent increase in hyperactivity at six months that did not interfere with the global clinical improvement. One patient with marked symptomatic improvement and one patient with moderate symptomatic improvement developed seizures after therapy, which were controlled with antiepileptic medications. One out of four patients who had a previous history of seizures showed increased episodes of seizures for a few weeks which were controlled with increased dosages of his antiepileptic medications (Table 5). Despite this the patient showed moderate improvements with no symptomatic deterioration. Patients who had normal EEG before cellular transplantation did not have seizures after cellular transplantation.

4. Discussion

An important aim of regenerative medicine is to find a definitive treatment for incurable disorders. In this effort, various conditions such as Parkinson’s disease, spinal cord injuries, muscular dystrophy, myocardial infarction, and stroke are studied and have shown beneficial therapeutic effects [7–9]. Recently, cell-based therapy for autism has been explored by researchers to a great extent [4–6].

Autism is one of the most complicated neurodevelopmental disorders with a very high prevalence rate [1]. Its exact etiology and pathophysiology remains poorly understood. The numerous biochemical abnormalities detected in autism are oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, decreased methylation, underproduction of glutathione, intestinal dysbiosis, and toxic metal burden
Table 5: Details of three patients who had seizures as an adverse event after cellular therapy.

<table>
<thead>
<tr>
<th></th>
<th>Patient I</th>
<th>Patient II</th>
<th>Patient III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of seizures</td>
<td>Pre-GTC</td>
<td>Pre-No seizure</td>
<td>Pre-No seizures</td>
</tr>
<tr>
<td>(before and after</td>
<td>Post-GTC</td>
<td>Post-GTC</td>
<td>Post-GTC</td>
</tr>
<tr>
<td>intervention)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of seizure</td>
<td>One</td>
<td>Multiple</td>
<td>Two</td>
</tr>
<tr>
<td>episodes after</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration in months</td>
<td>Six</td>
<td>Four</td>
<td>Three</td>
</tr>
<tr>
<td>between the cellular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>therapy and first</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seizure episode</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration in months</td>
<td>No recurrence</td>
<td>Sodium Valproate dose was</td>
<td>Sodium Valproate</td>
</tr>
<tr>
<td>over which seizure</td>
<td></td>
<td>doubled. Clobazam was discontinued</td>
<td></td>
</tr>
<tr>
<td>episodes recurred</td>
<td></td>
<td>and Lamotrigine was added</td>
<td></td>
</tr>
<tr>
<td>Medication used for</td>
<td>Midazolam</td>
<td>Sodium Valproate</td>
<td></td>
</tr>
<tr>
<td>seizure control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizure related</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect of seizures on</td>
<td>There was no deterioration in the</td>
<td>There was no deterioration in the</td>
<td>There was no deterioration in the</td>
</tr>
<tr>
<td>clinical</td>
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[25]. The environmental factors like organophosphates and heavy metals are also attributed to the origin of the disease [26]. Genetics involve multiple mutations in various genes resulting in its varied phenotype. These mutations may result in structural or molecular or functional defects in synaptogenesis [27].

A range of findings have suggested autism as a disorder of growth of the neural systems and connections, likely to be responsible for the underdevelopment of functions such as communication, behavior, and socialization [28]. U. Frith and C. Frith (2010) proposed a social brain hypothesis to explain theory of mind deficits in ASD [29]. The social brain concept tries to localize the complex social perception to superior temporal sulcus (STS), amygdala, orbital frontal cortex (OFC), and fusiform gyrus (FFG) [30]. The key roles implicated are STS region in analysis of perception, FFG in face detection and recognition, OFC in social reinforcement and reward processes, and the amygdala in analysis and regulation of emotions [31]. These areas form neural interconnections to establish a pathway from perception to action [32]. Neuroimaging studies have indicated dysfunction in the social brain areas and aberrant neuronal circuitry in autism [33, 34].

Recently, brain hypoperfusion and immune dysfunction have been recognized as two major pathogenetic mechanisms associated with autism. Hypoperfusion results in hypoxia, abnormal metabolite or neurotransmitter accumulation leading to neural tissue damage. The degree of hypoperfusion is proportional to the severity of the symptoms of autism. The extent of hypoxia was shown to be inversely correlated to Intelligent Quotient (IQ) [35]. Immune dysfunction is an imbalance in pro-inflammatory and anti-inflammatory factors. The raised macrophage product neopterin, TNF-alpha, MCP-1, and IFN-gamma indicate an augmented inflammatory response. In addition, deficient levels of anti-inflammatory cytokines such as IL-10 and TGF-beta suggest lack of natural inhibitory feedback processes. Autoimmune mechanism is also thought to be causative due to detection of autoantibodies to myelin basic protein, Purkinje cells and gliadin extracted peptides, neurotrophic factors, and neuron-axon filament and glial fibrillary acidic protein [36, 37]. T cell and B cell abnormalities have been demonstrated with systemic T cell lymphopenia, decreased cell proliferation, and abnormal production of cytokines [38]. During the period of neurodevelopment, the deregulated immune activity may result in the neurological dysfunctions in autism [39, 40]. Ichim et al. (2007), in their review, have proposed the administration of stem cells as a novel treatment to address the core pathologies of autism [4].

Cellular therapy has the therapeutic potential to repair the damaged neural tissue at molecular, structural, and functional levels. The stem cells possess unique properties of self-renewal, transdifferentiation [41], and paracrine effects [15]. The paracrine action changes the local micromilieu by secretion of trophic factors like ciliary neurotrophic factor (CNTF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF). This stimulates the local repair by enhancing proliferation, cell recruitment, and maturation of endogenous stem or progenitor cells [42]. The CD34+ stem cells have the capacity to produce angiogenic factors and differentiate into endothelial cells themselves [43]. Therapeutic angiogenesis improves perfusion and clearance of toxic metabolites and reduces hypoxia.

Another important effect is immunomodulation through inhibition of T lymphocyte pro-inflammatory cytokine production (IL-1β, TNF-α, and INF-γ) and upregulation of anti-inflammatory IL-10 and TGF-beta. This counterbalances the aberrant immune systems and reduces neural damage with restoration of functions [44, 45].

4.1. Source Selection and Route of Administration. Several sources of stem cells have been identified such as fetal or...
embryonic, bone marrow, umbilical cord, adipose tissue and dental pulp [46]. Bone marrow derived stem cells are easily procured by a standard procedure [47] and its potency and safety has been well established without any ethical issues [8, 9]. The bone marrow comprises of a heterogeneous population of stem cells, encompassing hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and very small embryonic-like stem cells (VSELs) [48]. This offers advantage of variety effects of different cell types.

The choice of intrathecal route was guided by efficient delivery of cells to brain with a relatively less invasive and safe procedure. The injected cells are transported by CSF to the affected areas in the brain [49, 50]. Various mechanisms have been believed to lead to altered permeability of blood brain barrier allowing the transplanted cells to reach brain areas with marked inflammation [51, 52]. The intrathecal route enhances the possibility of maximal number of transplanted cells "homing" onto damaged sites.

4.2. Clinical Findings in This Study. Conceptually the cellular therapy mechanisms, as described above, address the core pathogenesis of autism. The novel findings on the molecular, cellular, neuroimmunological, and environmental factors contributing to the pathogenesis of autism provide a rationale for cellular therapy as a unique and potent tool. Therefore, as a proof of concept, we studied 32 cases of autism, which were treated with BMNMCs, intrathecally.

The study sample included a total of 32 participants of which 24 were males and 8 were females, which is a ratio of 3:1. The gender ratio is synonymous to findings in previous studies [53]. The study included children as well as adults with autism. A majority of the patients were undergoing rehabilitation therapy since the time of diagnosis. Severity of autism in the participants ranged from mild to severe, as measured on the ISAA.

The clinical results, stated above as evidence to the concept, are discussed here. With a good number of participants showing clinically significant improvements on the ISAA and its subcomponents, the patterns of symptomatic improvement were analyzed. We gather here a theoretical pattern of the improvements noted after cellular therapy. Immediately after the intervention, within one week, initiation or consistency of eye contact and minimal decrease in hyperactivity were observed. Restlessness, rocking, hand flapping, and jumping, which are motor behavior seen in relation to the sensory issues, were seen to reduce early after intervention and continue to do so even later. This aids in better participation during sessions for behavior management and speech therapy. Only a few patients showed an increase in the levels of hyperactivity which could be attributed to the challenges posed by a new social and physical environment at the hospital and the changes in their routine. Arousal and activity levels continued to normalize progressively over the next three to six months. The immediate effect of the decrease in hyperactivity is seen on the levels of attention and concentration. Attention span increases and patients begin to sit at one place for activities, attend to commands, and follow them. These enhance the quality and duration of therapy sessions attended and aids in learning new concepts. With the ability to follow commands emerging and the improved attention span, facilitation of meaningful communication becomes more effective. They later start communicating their needs and using nonverbal gestures too. Besides the sensory issues, behavior has also been attributed to their inability to communicate or express. It was observed that, as they start communicating and indicating their needs and with the appropriate reinforcement strategies, their abnormal behavior slowly fades away. They also began to initiate social interaction and engage in play, forming peer relationships.

Speech was found to have improved at later stages. Speech is a complex function requiring good listening skills, attention, auditory processing, comprehension, and motor coordination. All of these are affected in many individuals with autism. We believe that it requires longer duration of consistent therapy for significant improvements. Developmentally speech develops as monosyllable, bisyllables, words, phrases and lastly sentences. We have observed that in the patients treated with autologous BMNMCs followed by supportive therapies that individuals who were absolutely nonverbal (or mute) before therapy developed nonverbal communication. Individuals who had monosyllables progressed to bisyllables and so on (Figure 3).

Hence, significant improvements were noted in all the interrelated domains of the ISAA, namely, social relationships and reciprocity, emotional responsiveness, speech, language, and communication, behavior patterns, sensory aspects, and cognition. Sensory aspects continually resolved and formed the basis for behavior, followed by social interaction and responsiveness, cognition, and speech or communication.

In each of the domains, a few symptoms did not improve in a large percentage of patients. The ability to relate to people requires recognition, understanding of relations (e.g., father and son), emotional bonding, and reciprocity. Only a few patients (29.63%) showed improvement in this owing to a shorter duration of followup. On the three symptoms, namely, lack of fear of danger under the domain of emotional responsiveness, insensitivity to pain within the domain of sensory aspects, and staring into space for long periods of time; 36.84%, 26.32%, and 25% of individuals improved, respectively. We hypothesize that these are interrelated symptoms. For emotional responsiveness, one may require to develop a clear concept of self and the need to protect oneself. This may lead to the identification and perception of a threat which requires higher levels of understanding (cognition) and conditioning for building the association between cause and effect.

Fear of danger is dependent on the perception of pain which may be affected due to sensory processing and integration problems. They may be underresponsive to tactile, vestibular, and proprioceptive inputs. Yet another possibility is their increased attention towards details and intricacies due to which they miss out on the gross information (e.g., they may be so engrossed in looking at a car’s wheels or design so that they fail to notice it speeding towards them). This intense observation of minute features or objects could be the underlying cause of what seems to us as staring into space.
Eye contact develops/improves

Hyperactivity decreases

Repetitive motor mannerisms decrease

Attention span improves

Cooperation and active participation in therapy sessions increase

Conveying needs and expressing self improve

Communication improves

Verbal communication improves

Nonverbal communication/ gestures improve

Social interaction improves

Speech and language improve

Command following improves

Behavioral issues decrease

Sensory issues decrease

Improved learning and concept formation

Global life-skills development

Figure 3: Schematic representation of clinical improvements after cellular therapy. This figure shows proposed theoretical outline of observed changes after cellular therapy.

Under the speech, language, and communication domain, few individuals improved on the use of nonverbal language (31.58%), decreased use of jargon or meaningless words (27.78%), and decreased pronoun reversals (27.27%). As stated before speech and communication require longer periods of training for individuals with autism. For reducing the use of jargon, the child must understand that what he or she is saying is meaningless and inappropriate. This may not be handled with behavior strategies alone. A higher level of cognition needs to be developed. Pronoun reversals are linked to the deficit in self-identity and the concept of “I,” “Me,” or “You,” which is affected in them. With a longer duration of followup these areas may get addressed.

Within the domain of cognition, unusual memory and savant ability were unchanged as they are intrinsic to the individual with autism and have a complex underlying mechanism of development.

On FIM and Wee-FIM scales there was no statistically significant change, as the FIM scores were maintained after cellular transplantation suggesting preserved independence level for ADL in all the patients after cellular therapy.

4.3. Theoretical Basis for the Clinical Improvements Seen after the Intervention. Kevin et al. presented a model of ASD that implicates an early failure to develop the specialized functions of the social brain that is involved in social information...
processing. They state that due to this early disruption, “an individual with autism must develop in a highly social world without the specialized neural systems that would ordinarily allow him or her to partake in the fabric of social life, which is woven from the thread of opportunity for social reciprocity and the tools of social engagement [30].” We hypothesize that cellular transplantation causes functional restoration of specialized neural systems by neuroprotection, neural circuit reconstruction, neural plasticity, neurogenesis, and immunomodulation. Individual therapies like occupational therapy, psychological intervention, and speech therapy employ the principles of learning to facilitate neural plasticity. In addition, they also provide the opportunity and tools for social engagement. Enhancement of the neural and functional restoration can be optimized by combining these therapies with cellular transplantation.

4.4. Adverse Events. There were some minor adverse events, such as headache (3.6%), nausea (10.7%), vomiting (17.9%), pain at the site of injection (71%), and pain at the site of aspiration (71%) which were reported in few patients immediately after cell transplant procedure. These mild side effects were controlled with medications and resolved within a week. It is important to consider poor communication skills of children with autism while monitoring the symptomatic adverse events.

One (3.1%) patient was marked as minimally worse on CGI-II, due to persistent increase in hyperactivity at three months which did not subside by six months of followup. During this study, we observed that 6 patients (18.7%) showed a transient phase of minimal increase in hyperactivity in the first three months that subsided by six months. In addition this increase in the hyperactivity did not interfere with the overall improvement. The transient increase in hyperactivity may be a result of increased neuronal activity after cell transplantation, changes in daily routine, and exposure to new tasks during therapy sessions. This needs to be further studied in larger sample and controlled studies.

In few patients (9%) there were seizure episodes after cellular transplantation which were controlled with medications. This may be due to activation of epileptogenic focus which already existed in these patients. This is supported by absence of seizures after intervention in the patients with normal EEG before intervention. It was observed that in patients with no history of seizures but EEG reporting epileptogenic focus, there was increased likelihood of seizures after intervention. It is noteworthy that the seizures did not cause clinical deterioration or hamper beneficial effects of cell therapy.

No other major adverse events were noted over the period of 26 months (mean = 12.7 months).

The overall improvements on ISAA was observed in 29 patients out of 32 (90.6%), and 20 patients out of 32 (62.5%) improved on CGI-I, which was significant. The grading of global improvements on CGI-II showed that 37.5% of patients had very much improvement, 46.8% had much improvement, and 18.8% had minimal improvement.

CGI-III efficacy index provides us with evidence that the intervention may be an effective tool for treatment of autism. 21.9% of patients showed marked therapeutic effect with no side effects, 40.6% patients showed moderate therapeutic effect, and 28% showed minimal therapeutic effect; none of these patients had any major side effects.

4.5. PET-CT Scan Evidence. The complex constellation of symptoms of autism cannot be explained by pinpointing to a specific structure or area of the brain. The hypothesis of social brain and theory of mind tasks emphasizes that the symptoms of autism are routed not only in the structural deviations but also in the deficits in neural connectivity [29]. Various attempts have been made to investigate the areas of dysfunction, using various radiological and nuclear imaging techniques [54, 55]. These investigations, however, do not explain the putative neural connectivity deviations causing the symptoms of autism. Functional neuroimaging is thought to give more lucid information about neural connectivity [56]. PET-CT scan and Functional MRI (FMRI) scan are most widely used functional neuroimaging techniques. PET-CT scan of brain is a noninvasive, relatively safe, and feasible modality to record the functional activity of brain. It measures 18-FDG uptake which is related to the glucose metabolism at the cellular level which correlates with the functioning of the area of the brain. PET-CT scan remains a choice of investigation in children with autism due to relative ease of conduction and measurement. PET-CT scan studies in children with autism have earlier identified reduced metabolic activity in bilateral temporal lobes [57, 58]. Another study showed significant hypoperfusion of superior temporal gyrus and superior temporal sulcus in children with autism compared to the control children [33]. These findings are consistent with the theory of social brain. We used PET-CT scan to observe the metabolic activity of the brain before and after cellular therapy. The scan was done in a standardized manner, maintaining similar conditions before scanning to ameliorate confounding factors and therefore the changes in the 18-FDG uptake may be attributed to the intervention.

A comparative PET-CT scan before and six months after cellular transplantation showed a balancing effect on the metabolism. The areas of hypermetabolism implicated in previous imaging studies [59] showed reduction in metabolism after cellular transplantation, and areas having hypometabolism as suggested by Zilbovicius et al. 2000 [33] showed increased metabolism after cellular transplantation. We hypothesize that immunomodulatory effects and neangiogenesis causes improved oxygenation and functioning of the damaged neurons. This improves their metabolism which leads to increased FDG uptake in the previously hypofunctional neurons. The paracrine effects and the anti-inflammatory effects also lead to inhibition of hyper functional neurons causing decrease in FDG uptake in the previously hyperfunctional neurons. The exact mechanism still remains unknown. These changes could be clinically correlated with statistically significant reduction of scores of the domains of ISAA scale and reduction in the severity of disease as measured on CGI-I scale (Figure 4).
Figure 4: Findings in PET-CT scan before and after cellular therapy. (a) PET-CT scan before intervention showing reduced FDG uptake in the areas of frontal lobe, cerebellum, amygdala, hippocampus, parahippocampus, and mesial temporal lobe. (b) PET-CT scan six months after intervention comparison shows increased FDG uptake in the areas of frontal lobe, cerebellum, amygdala, hippocampus, parahippocampus, and mesial temporal lobe.

4.6. Limitations and Future Directions. The study is an open label proof of concept. A small sample size, the absence of randomization, and the absence of control group were the limitations. Large scale, multicentre, and randomized controlled trials are recommended. A longer period of followup may be required to further establish the safety and efficacy. Few patients had increased episodes of seizures after the intervention, which were controlled with medications. We recommend preintervention EEG assessments to identify patients with high risk for seizures, after cellular therapy. The requirement and effectiveness of prophylactic antiepileptic medications in such patients must be studied. PET-CT scan was used as evidence in a small number of patients as it was introduced at a later stage, in view of marked clinical improvements. Future studies should consider the use of PET-CT scan as a monitoring tool and substantiate the effects of cellular therapy in autism.

This study presents results of autologous BMMNCs intrathecal administration, still the other routes of administration; types of cells, combination of cells, dosage of cells, and frequency of the transplantation must be explored. It is believed that the effect of stem cells is larger in younger age group due to greater plasticity of the brain and increased availability of precursor cells in the bone marrow. It may be helpful to identify the most responsive age group for cellular therapy.

In this study, one of our concerns was that the improvements observed may be due to various therapies other than cell transplantation. But, several studies have been reviewed for evidence of effects of individual therapies including occupational therapy, sensory integration, behavior therapy, and speech therapy in children with autism. Case-Smith and Arbesman in their review [60] show that these individual therapies have some positive findings, but the evidence is weak due to limitations of small sample size, short followup, lack of evaluation instruments, inadequate measures of treatment fidelity, and inappropriate data analysis. So, according to them the interpretation of the findings of the studies of the individual therapies needs to be with caution. Therefore, the significant clinical improvements seen in our study cannot be attributable to the multidisciplinary therapies alone. Our study demonstrates that cellular therapy has synergistic effect and enhances the effects of multidisciplinary therapies. Hence, we postulate that the combination of cell transplantation with various therapies offers an augmented beneficial response. Future studies may be planned with a control group for these individual therapies.

5. Conclusion
Despite accumulating evidence of the safety of cellular therapy, there is a dearth of published human clinical studies.
Autism has been in discussion since a long period and the increasing incidence has established a need to find a definitive treatment modality. Therefore, the transition of cellular therapy from benchside to bedside is warranted. Though autologous BMMNCs may not be a cure for autism, they definitely possess the potential to manage overall disease severity and improve the quality of life. This study is a preliminary demonstration of the safety and efficacy of autologous BMMNCs in autism. The minimal invasiveness, simplicity of the procedure, and autologous nature of the cells render it as a promising therapeutic potential. This is the first clinical study on the application of cellular therapy in patients with autism which may provide future directions for larger randomized controlled trials.

Conflict of Interests

The authors declare that they have no conflict of interests.

References


Intrathecal Autologous Bone Marrow Mononuclear Cell Transplantation in a Case of Adult Autism

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Abstract

Autism is a complex neurodevelopmental disorder with a worldwide prevalence of 1 in 88. With greater understanding of mechanism of action of cellular therapy it is now possible to address the pathogenesis of autism. Recent findings of cellular therapy offering immunomodulatory, angiogogenetic and paracrine effects make it a lucrative option for treatment of autism. We administered a 33 year old adult patient of autism intrathecally, with autologous bone marrow mononuclear cells (BMMNCs), twice with an interval of six months. On follow up at 3, 6 and 9 months post first intervention, he was re-evaluated clinically and no major or minor side effects were observed. At 6 and 9 months objective outcome measures of Indian Scale for Assessment of Autism (ISAA) and Clinical Global Impression (CGI) were used and they showed significant improvement. At the end of 9 months, on ISAA, the score improved from 94 to 64. 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 affected areas. The changes observed on the PET CT scan correlated with clinical improvements. MRI remained same at 6 months thereby, indicating that PET CT scan may serve as a better monitoring tool for effects of cellular therapy. In this case study, we hypothesize that cellular therapy has repaired the neural connections and achieved balance in the excitatory and inhibitory neuronal cells by various mechanisms of neuroprotection, neuromodulation and neurorestoration. Cell therapy holds great potential and randomized controlled trials may be conducted to study their long term effects in treating autism.

Keywords: Autism; Cellular therapy; Autologous; Bone marrow mononuclear cells; PET CT

Introduction

Autism is a neurodevelopmental condition with cognitive and neurobehavioral issues leading to impairments in socialization, verbal and non-verbal communication and behavior. The symptoms are noticed at around twelve–eighteen months. However, at times autism is diagnosed at adulthood [1]. The prevalence of autism is estimated to be about 1 in 88 [2]. Adults with autism are deprived of social relationships, employment and a better quality of life [3]. Development of effective interventions is thus required to help the amalgamation of these adult patients into the wider society.

All the present available treatments for autism are targeted towards specific symptoms. Cell therapy as a treatment for autism is currently being explored. Adult stem cells have shown positive benefits in various neurological disorders such as cerebral palsy, stroke, etc [4,5]. Various mechanisms have known to be responsible for the positive effect of cellular therapy which include a combination of trophic factor delivery, neuroplasticity, neuromodulation, neuroprotection, angiogenesis amongst others [6]. With an aim to further study the effect of cell therapy in adult autism, we administered an adult patient of autism with autologous bone marrow mononuclear cells (BMMNCs), twice at an interval of six months. To our knowledge, there is no published case study of cell therapy in adult autism. Thus, this case report provides an insight of application of cell therapy in adult autism.

Case Report

Case presentation

A thirty three years old male was diagnosed with autism at the age of eighteen years. He achieved normal motor milestones except speech. His speech developed only after the age of three years. At six to eight years of age, hyperactivity developed along with behavioral issues like laughing without reason, hitting others, poor eye contact and running without reason. By the age of fourteen he became more aggressive and started showing temper tantrums and poor social interaction skills. Responses were delayed due to which he required repetitive commands. At the age of eighteen years he was diagnosed as autism. He lacked empathy, had difficulty understanding other person’s feelings. There was a failure to establish relationships including sexual relationships. Absence of understanding of making a family. Since the age of eighteen he underwent rehabilitation including behavior therapy, occupational therapy and speech therapy. He was trained to become independent in activities of daily living. Vocational rehabilitation was provided, so he could stitch, make boxes and work on a computer. Presently, his eye contact and social interaction was affected. He stayed aloof and did not maintain peer relationships. Emotional responses were also poor. He used stereotyped and repetitive language. Behavior was aggressive and he threw temper tantrums.

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Pre-intervention assessment

MRI brain showed mild cerebral and cerebellar atrophy. The EEG showed no epileptogenic focus. On Indian Scale for Assessment of Autism (ISAAS) he scored 94 (mild autism with 60% disability). On Clinical Global Impression (CGI) scale, severity of illness (CGI-I) was scored 3 which was mildly mentally ill. PET CT scan was performed using the Siemens Biograph CT 64 slice high speed scanner - 3D PET True V wide detector (Siemens-CTI, Knoxville, Tenn., USA), with a resolution of 0.6-mm full width at half maximum (FWHM) and the images of 45-50 contiguous transverse planes with a field of view of 21.6 cm axial PET FOV with True V (Figure 1). The mean standardized uptake values (SUV) are given in the Table 1.

Isolation and administration of autologous bone marrow mononuclear cells

The protocol is based on the inclusion criterion as per the World Medical Associations Helsinki declaration [7]. It has been reviewed and approved by the Institutional committee for Stem cell Research and Therapy (IC-SCRT). The patient’s parents were informed about the procedure and a duly filled informed consent form was obtained. 300 mcg of G-CSF injections were administrated 48 hours and 24 hours before bone marrow derived mononuclear cell transplantation, to stimulate CD34+ cells and increase their survival and multiplication. Bone marrow (100 ml) was aspirated from the anterior superior iliac crest bone using the bone marrow aspiration needle. Mononuclear cells (MNCs) were obtained from the bone marrow by density gradient separation method. Viable count of the isolated MNCs was taken and cells (MNCs) were obtained from the bone marrow by density gradient separation method. Viable count of the isolated MNCs was taken and approximately 56×10⁶ MNCs were immediately injected post separation, intrathecally at L4-L5 level using an 18G Touhy needle and epidural catheter. Simultaneously, the patient was administered with one dose of 30 mg/kg methylprednisolone intravenously.

Neurorehabilitation

The patient was also given multidisciplinary therapies which included occupational therapy, psychological therapy, applied behavior analysis, sensory integration and speech therapy. It included Vestibular activities like tandem walking, peg transfers on vestibular board/swiss ball etc, kneel walking, ball throwing in standing etc, therapeutic activities like grouping, sequencing, improving eye hand coordination and visual perceptual skills were included. Social interaction and skills were emphasized during the therapy sessions. During his psychological sessions, cognitive rehabilitation, family counseling and psychological education was emphasized.

Outcome measures used

On follow up at three, six and nine months post first intervention, he was re-evaluated clinically and no major or minor side effects were observed. At six and nine months objective outcome measures of ISAAS and CGI were used. PET CT scan was repeated at six months after the intervention.

Results

After the procedure, the patient had no major or minor side effects. There was improvement in eye contact within a week. After three months of follow up, reduction in hyperactivity and reduced repetitive speech was observed. On six months follow up, improvements further continued. Attention span increased along with decrease in temper tantrums. His tongue movements improved and he could eat without spilling due to improved eye-hand coordination. Reduction in the behavioral issues such as self- talking, laughing loudly was also noted. He was reassessed on ISAAS scale wherein the score reduced from 94 (Mild autism with 60% disability) to 79 (Mild autism with 50% disability). On ISAAS, he improved in language and communication, behavior pattern and sensory aspects (Table 2). On repeating his PET CT scan after six months, it was observed that various areas of brain showed reduction in FDG uptake (Table 1). MRI of the brain

<table>
<thead>
<tr>
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<th>Mean SUV (POST)</th>
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<td>Cerebellum Right</td>
<td>5.6</td>
<td>4.56</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Comparison of pre and post intervention SUV values in 18 FDG PET CT scan and correlation with clinical symptom improvement in the case study.

Figure 1: (A) PET CT scan of the patient carried out before cell therapy, (B) PET CT scan of the patient carried out after cell therapy. Comparison of PET CT scans demonstrating improvements after cell therapy i.e., the yellow areas decreased in the post intervention scan as indicated by SUV values in Table 1.
neurons. The dysfunction of cortical interneurons is also believed autism [10]. Cortical interneurons make up to 20% of the cells in cortex. Suppressed GABAergic inhibition thus could be one of the etiologies of glutamatergic transmission or suppressed GABAergic inhibition. autism [9]. Elevated levels of excitation could be due to increased glutamatergic transmission or suppressed GABAergic inhibition. Suppressed GABAergic inhibition thus could be one of the etiologies of autism [10]. Cortical interneurons make up to 20% of the cells in cortex. This cells modulate the firing activities of the excitatory projection neurons. The dysfunction of cortical interneurons is also believed to be the underlying pathophysiology of autism [11]. Neuroimmune abnormalities may be reflected as neuroglial activation in the brain [12]. Suzuki et al. [13] in their recent study, have indicated excessive microglial activation in multiple brain regions in young adult subjects with ASD.

Neuroanatomical studies suggest that frontal lobes, mesial temporal lobe (especially amygdala) and cerebellum are also involved in the pathology of autism [14]. In a multicentre study conducted on adult men with autism, it was found that there is a significant increase in gray matter volume in the anterior temporal and dorsolateral prefrontal regions along with significant reduction in the occipital and medial parietal regions as compared to the control group [15]. Brain volume in the frontal lobes is larger, whereas the occipital lobes are smaller in size in individuals with autism [16]. On studying the brain function of language processing in children and adults with autism it was found that left middle temporal, left pars triangularis, left pars opercularis, left medial frontal, and right middle temporal were the most affected. The autism group differed from the control group in the degree of network coordination and the dynamic recruitment of regions in response to stimulus [17]. All the above findings are observed in some patients with autism. In autism, the trajectory of brain development is more disturbed.

Many treatment options have been studied for adults and children with autism such as behavioral and developmental interventions, vocational therapy, pharmacotherapy, etc [18,19]. Vocational therapy and other rehabilitation interventions alone have not shown desirable benefits in adolescents and young adults with autism [20]. Medications are used to treat the comorbid conditions such as anxiety, seizures, depression and not the core symptoms of autism [21]. Since 2006, few drugs have been approved by the FDA. But, adverse events including weight gain, sedation and increased risk of mortality among adults have been recorded with use of these drugs [22].

In neurological disorders, it has been observed that stem cells improve the damaged neuronal function which is also one of the underlying pathogenetic mechanisms of autism [23]. With an aim to study the benefits of stem cells in autism, we transplanted autologous BMMNCs in an adult with autism. Bone marrow cells are safe and are widely available with no ethical or carcinogenic issues involved as compared to embryonic/lethal cells. BMMNCs are a mixed population of differentially matured B-cells, T-cells and monocytes, along with progenitor cells such as hematopoietic stem cells (HSC), mesenchymal stromal cells (MSC), endothelial progenitor cells (EPC) and very small embryonic-like cells (VSEL) [24]. It has been observed that the whole BMMNCs are more effective compared to the fractionated parts [25]. Studies suggest that these cells improve oxygenation, as they reduce the brain hypoperfusion by stimulating angiogenesis. BMMNCs can modulate the immune system by inhibiting the proliferation of CD3+, CD4+, CD8+ T cells and NK cells [26]. The transplanted cells migrate and home onto the damaged areas of the brain and produce factors such as VEGF, BDNF, FGF2, activating the endogenous repair mechanism and contributing to recovery of lost neuronal function [27,28].

The intervention is safe as being autologous; the cells evade the possibility of any reaction. Intrathecal administration of cells is relatively less invasive procedure. These cells migrate via the CSF to the affected areas in the brain. The transplanted cells home into the damaged sites by crossing the blood brain barrier. It has been seen that various mechanisms including inflammation alters the permeability of the blood brain barrier in autism [29,30]. In this case study, after autologous BMMNC transplantation we observed significant

<table>
<thead>
<tr>
<th>Sub-components of ISAA</th>
<th>Score Pre-treatment</th>
<th>Score at 6 month follow up</th>
<th>Score at 9 month follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social Relationship and Reciprocity</td>
<td>30</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>1 Poor Eye contact</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2 Lacks social smile</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>3 Remains aloof</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>4 Does not reach out to others</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5 Unable to take turns in social interaction</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>6 Does not maintain peer relationships</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Speech Language and Communication</td>
<td>14</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>1 Engages in stereotyped and repetitive use of language</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2 Unable to initiate or sustain conversation with others</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3 Unable to grasp pragmatics of communication (real meaning)</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Behaviour Patterns</td>
<td>18</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>1 Shows hyperactivity and restlessness</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2 Exhibits aggressive behavior</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3 Throws temper tantrums</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sensory Aspects</td>
<td>12</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>1 Stares into space for long periods of time</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2 Has unusual vision</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2: Table giving details of the improvements in different sub components on ISAA at follow up of 3 months and 18 months.
improvements in social relationship, behavior along with language and communication. On correlating these improvements, it can be assumed that there was improvement in temporal, amygdala and Wernicke’s area of the brain.

PET CT scan was used as a monitoring tool. It is a non-invasive and a relatively safe functional neuroimaging technique which examines the association between the metabolic activity in the brain and the mental processes. The 18-FDG dye used for the PET CT scan is an analogue of glucose which provides functional information of the cells based on the glucose uptake [31]. Reduced glucose uptake reflects reduced metabolic activity of those brain cells. The standardized uptake value (SUV) is used as a relative measure of FDG uptake [32]. These values are compared to a normal controlled SUV and a standard deviation (SD) value is calculated which indicates the areas of the brain functioning beyond the normal limits. Hence, the hyperfunctioning areas of the brain will show hypermetabolism and hypofunctioning areas will show hypometabolism. A reduction in hyperfunctioning areas or increase in hypofunctioning areas may reflect as a clinical functional improvement. In our case, on correlating the clinical improvements to the specific areas of the brain we observed a reduction in metabolism of these particular areas after the intervention. Therefore, the reduction in metabolism is a positive indicator of clinical functional improvement (Table 1). Since, the decrease in SUV values corresponds to the clinical improvements, we may hypothesize that the reduction in metabolism has restored the function of neurons. After six months, the PET CT scan recorded changes corresponding with the clinical improvements while the MRI showed no change. Hence, PET CT scan was more sensitive to study the effect of intervention as compared to the MRI.

One of the limitations of this case report is that there is no control to compare the effect of intervention, but we can consider the case as self control due to the age of the patient and his static condition despite of regular rehabilitation pre intervention. Also, for the PET CT scan, the SUV values of the normal adults (control) were not available as it was a computerized program and the values were automatically generated and compared by the software and reported as SD values. The control group values are for normal adult population and there is a need to study and gather baseline values for autism population.

We hypothesize that cellular therapy has stimulated repair processes of the neural connections and achieved balance in the excitatory and inhibitory neuronal cells by various mechanisms of neuroprotection, neuroregulation and neurorestoration. Cell therapy holds a great potential and randomized controlled trial may be conducted to study their long term effects in treating adults with autism.

References
in the therapeutic properties of mesenchymal stem cells. Cytokine and Growth Factor Rev 20: 419-427.


An Improved Case of Autism as Revealed by PET CT Scan in Patient Transplanted with Autologous Bone Marrow Derived Mononuclear Cells

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Abstract

Autism, the most severe form of autism spectrum disorder (ASD), is a complex neurodevelopmental disorder, characterized by language developmental delay, social skills impairment, communication problems, and restricted, repetitive, and stereotyped patterns of behavior. There is no cure for Autism; hence therapies and behavioral interventions are designed to remedy specific symptoms. We used autologous bone marrow derived mononuclear cells intrathecally in a 14 yr old boy with severe autism to improve the quality of life. At six months, follow up after therapy the general impression on clinical assessment showed mild autism. It is exciting to see symptomatic improvement with shift on Childhood Autism Rating Scale (CARS) from 42.5 (Severely Autistic) to 23.5 (Non Autistic), which was also visualized as enhanced PET scan brain function. All these improvements have led to improved quality of life of the patient as well as the family. Several incurable neurological disorders have shown benefits with cellular therapy thus, autism should be explored as an indication and nuclear imaging can be used to study its effects.

Keywords: Autism; Autologous; Bone marrow; Mononuclear cells; PET

Introduction

Autism is a neurodevelopmental, disorder with a multidimensional presentation. It is noticeable by parents at the age of 3 years due to delayed or abnormal language development, deficits in social interaction, lack of eye contact, hyperactivity and repetitive behaviors and interests [1]. Autism is the most severe form of autism spectrum disorder (ASD), while other conditions along the spectrum include mild forms known as Asperger’s syndrome, childhood disintegrative disorder and pervasive developmental disorder. In every 166 children is estimated to have autism [2]. Although outcomes are specific, behavioral characteristics change over time. Most autistic children remain within the spectrum as adults and continue to experience difficulty with independent living, employment, social relationships, and mental health. Since autism is incurable, chronic long term management is required and can become progressively difficult to handle for parents. The primary goal of the treatment is to improve the quality of life of the patient by minimizing the core features and associated deficits and maximizing functional independence. Facilitating development and learning, promoting socialization, reducing maladaptive behaviors, and educating and supporting families can help accomplish these goals [3-5].

Material and Methods

Case presentation

Herein, we present a 14 year old boy with autism, who had birth history of Full Term – C – section delivery followed by normal motor milestones but delayed speech with lack of social interactions and emotional development. He was hyperactive with behavioral issues like engaging in self-injurious behavior, self-scratching and hyper aggression. He had normal vision and hearing with slurred speech. He was disoriented in time, but identified places and people. There was no history of seizures. Neurologically, he had normal muscle tone and power in trunk and limb muscles. Functionally, he was independent for most of the daily activities but needed assistance in fine motor activities. In spite of regular rehabilitation since the age of 6 years, he showed no improvements with respect to behavior and social interaction.

His intelligent quotient (IQ) was found to be 64 and Childhood Autism Rating Scale (CARS) score was 42.5 which is categorized as severely autistic.

The molecular diagnostic test of fragile X done using southern blot was negative. MRI Brain showed no significant intracranial abnormality and EEG showed bilateral episodic sharp and slow wave abnormalities. The brain PET scan showed moderately reduced metabolic activity in right broca’s, right insula, right lateral temporal pole, right calcarine, both basal ganglia and left medial prefrontal. It also showed mildly reduced metabolic activity in right parietal, right sensory motor and right Wernicke’s.

He underwent autologous bone marrow derived mononuclear cell transplantation. Our protocol is based on the inclusion criterion as per the World Medical Associations Helsinki declaration [6]. It has been reviewed and approved by the Institutional committee for Stem cell Research and Therapy (IC-SCRT). The patient’s parents were informed about the procedure and a duly filled informed consent form was obtained from them. 300 mcg of G-CSF injections were administrated 48 hours and 24 hours before bone marrow derived mononuclear cell transplantation, to stimulate CD34+ cells and increase their survival and multiplication. Bone marrow (100ml) was aspirated from the iliac bone. Mononuclear cells (MNCs) were obtained using density gradient separation method. Viable count of the isolated MNCs was taken and was found to be about 98%. The MNCs were checked for CD34+ by flow cytometry and multiplication. Bone marrow (100ml) was aspirated from the iliac bone. Mononuclear cells (MNCs) were obtained using density gradient separation method. Viable count of the isolated MNCs was taken and was found to be about 98%. The MNCs were checked for CD34+ by...
FACS analysis and was determined to be 1.92%. Approximately 56 x 106 MNCs were immediately injected post separation, intrathecally in L4-L5 using a lumbar puncture needle and catheter. The patient was also given rehabilitation therapy which included occupational therapy and psychological therapy. Rehabilitation interventions seek to promote recovery & independence through neurofacilitation. During rehabilitation sessions, effective motor learning strategies with task oriented training, for real life environment were utilized & successful attainment of functional outcomes were achieved. He was evaluated at regular intervals of three and six months. He was reassessed by repeating CARS and PET scan of brain after 6 months.

**Results**

After the procedure, the patient had no side effects. The patient showed some immediate improvements within a week and significant improvements over a period of six months to one year.

Within a week, there was improvement in his eye contact and attention. His hand-writing and fine motor activities like buttoning had improved significantly. On follow up after six months, further improvements were observed in his behavior with respect to social interaction and emotions. Aggression in activities and hyperactivity had reduced by 45 to 50%. Improvements in impulse control, reading skills, tracing, recognition of all shapes and following commands were 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 were improvements recorded in comparison with previous report. Comparative study of previous and post stem cell therapy PET CT scan showed markedly increased uptake in bilateral temporal lobes and bilateral calcarine cortices with mild increased uptake in left medial pre-frontal cortex. All the views; sagittal, transverse and coronal views (Figure 1) showed the improvements.

At one year follow up, he was found to interact more with his peers. Peer activity had increased significantly. New task learning abilities had improved which was noticed due to increased participation in household work. Comprehension and ability to follow commands had improved significantly. He had developed self insight and appropriate emotional response.

**Discussion**

The exact etiology of autism is not known, but it is likely to result from a complex combination of genetic, environmental, and immunological factors [7]. Current available treatments for autism can be divided into behavioral, nutritional, and pharmacological options, in addition to individual and family psychotherapy and other nonpharmacologic interventions [8]. However, there is no defined standard approach [9]. Currently, numerous clinical trials are being conducted with interventions ranging from hyperbaric oxygen, to administration of zinc, to drugs exhibiting anti-inflammatory properties.

Stem cell therapy offers great promise for the treatment of autism. Although several neurophysiological alterations have been correlated...
with autism, immune dysfunction and neural hypoperfusion appear to be broadly consistent. The association of altered inflammatory responses and hypoperfusion with symptomology is reported, which suggests its causative role [10]. The BMMNCs are comprised of a variety of cells which includes mesenchymal stem cells (MSCs), hematopoietic stem cells, tissue specific progenitor cells and stromal cells. Mesenchymal stem cell have an ability to modulate the immune system and restore the altered brain organization with unique property of homing, wherein the cells migrate to the site of injury and carry out the repair process [11]. The various stem cells in MNC enhance angiogenesis by producing signaling molecules such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF2). They also promote tissue remodeling, prevent apoptosis, decrease inflammation and activate the satellite cells [12]. We have previously published data wherein autologous mononuclear cell transplantation was carried out in children with various incurable neurological disorders including autism, cerebral palsy and mental retardation. Improvements were reported in quality of life of these patients [12-14]. On repeating and comparing the PET scan of brain after six months post therapy, significant changes were recorded and correlated to symptomatic improvements. In numerous studies the areas affected by hypoperfusion seem to correlate with regions of the brain that are responsible for dysfunctions in autism. For example, specific temporal lobe areas associated with face recognition, social interaction, and language comprehension, have been demonstrated to be hypoperfused in autism [10]. The functions of insulae include emotional processing, empathy, perception, motor control, self-awareness, cognitive functioning, and interpersonal experience. In our case these areas showed improvements on PET scan of brain. So the clinical and radiological improvements are well correlated.

Hence, we hypothesize that the cumulative effect of various cells in MNCs showed significant clinical improvements in this case and has altered the course of disease. We conclude, though single case, that intrathecal transplantation of autologous bone marrow derived mononuclear cells is safe, feasible; and may be beneficial in autism. The large clinical studies are an immediate need to fully explore its potential in autism and nuclear imaging can be used to study its effects.

References
Autologous Bone Marrow Mononuclear Cells may be Explored as a Novel Potential Therapeutic Option for Autism

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Abstract

Cellular therapy has been viewed as a novel therapeutic modality for many neurological disorders. Autologous Bone Marrow Mononuclear Cells (BMMNCs) used in many studies, have a safe and ethical profile. These cells have been studied in great depth and have shown angiogenetic and immunomodulatory properties in addition to other neuroprotective effects. These peculiar mechanisms may serve to be beneficial in autism. Recently, hypoperfusion and immune alteration are identified as major underlying pathogenetic mechanisms in autism. We present a case of autism with comorbid mental retardation; treated with intrathecal administration of autologous BMMNCs. Results were documented objectively on Indian Scale for Assessment of Autism (ISAA) and Positron Emission Tomography Computed Tomography (PET CT) scan. On regular follow up assessment of the patient over 18 months, there was significant clinical improvement in social relationship, communication and behavior. On outcome measure, ISAA score improved from 111 (Moderate autism) to 73 (Mild Autism). PET CT scans corroboration of pre and post therapy showed balancing effect on brain metabolism. This case provides a great insight into the clinical effects of autologous BMMNCs in autism. Though a case study, the improvements guide us to plan future studies to explore different options of cellular therapy in autism.

Keywords: Autologous; Bone marrow; Mononuclear cells; Autism; ISAA; PET CT scan

Introduction

Autism spectrum disorders are a group of neurodevelopmental conditions characterized by difficulty in verbal and nonverbal communication, impairment in social interaction and restricted repetitive obsessive behaviour or interests. The caregivers usually notice the symptoms around 12-18 months and definitive diagnosis is usually made around 24-36 months [1]. However, in some cases the diagnosis may be done in adulthood. Autism is believed to occur at a rate of about 8 cases per 10,000 children (0.08 percent) [2]. Current treatment options available for autism are behavioural therapy, occupational therapy, speech therapy, nutritional guidance and pharmacological treatment. However, these treatment options are directed towards symptomatic changes in the child. Here we describe a case of autism that was treated with conventional medical treatment and therapies yet he continued to have major behavior and communication deficits. Cellular therapy has recently been viewed as a promising therapeutic modality which aims at restoring neural connections in the brain [3]. In this case study, we have demonstrated the use of autologous BMMNCs as a beneficial therapeutic approach for autism as they are easily obtainable, safe i.e. no risk of Graft-Versus-Host-Disease (GVHD) and non-teratogenic. They do not have any ethical or moral issues. The main aim of this therapy is to reduce the severity of autism and thereby improve the overall functioning of the child.

Case Presentation

Case report

We present a case of a 14 year old male with Autism. He was a 20 days preterm baby born through normal vaginal delivery. Normal milestones were achieved but there was a regression in speech at 3½ years and was diagnosed with autism with high levels of serotonin. Parents noticed hyperactivity and poor social interaction. Stereotypical motor mannerisms, minimal social reciprocity and poor eye contact were present along with self-stimulatory behaviour like jumping, hand flapping and self-touching at private parts. Speech and language skills were deficient. He could answer questions either by pointing or by making sounds and follow simple commands. He was oriented to person and place but disoriented in time, date and day. Psychomotor activity level was decreased. Reaction time and tempo were slow. The form, content and possession of thought were also absent. His attention span and concentration were average. Neurologically, muscle tone and power in trunk and limb muscles were normal. Functionally, he was totally independent in most of his Activities of Daily Living (ADLs) but required supervision. In spite of regular rehabilitation, his attention span and concentration were average. Neurologically, muscle tone and power in trunk and limb muscles were normal. Functionally, he was totally independent in most of his Activities of Daily Living (ADLs) but required supervision.

Keywords: Autologous; Bone marrow; Mononuclear cells; Autism; ISAA; PET CT scan

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Method

Intrathecal administration of autologous bone marrow derived mononuclear cell (BM-MNC) was used as a treatment method. Our protocol was based on the inclusion criterion as per the World Medical Associations Helsinki declaration [4]. It has been reviewed and approved by the Institutional committee for Stem cell Research and Therapy (IC-SCRT). The patient's parents were informed about the procedure and a duly filled informed consent form was obtained. Pre-procedure routine blood tests, urinalysis and chest x-ray were done to rule out active infection and assess anaesthesia fitness. 300 mcg of G-CSF injections were administrated 48 hours and 24 hours before bone marrow derived mononuclear cell transplantation, to stimulate CD34+ cells and increase their survival and multiplication. Bone marrow (100 ml) was aspirated from the iliac bone. Mononuclear Cells (MNCs) were obtained using density gradient separation method. The MNCs were checked for CD34+ by FACS analysis and viable count was calculated and found to be about 98%. Approximately 56×10^6 MNCs were immediately injected post separation, intrathecally in L4-L5 using a lumbar puncture needle and catheter. The patient was also given rehabilitation therapy which included occupational therapy, psychological therapy and speech therapy. Neurofacilitation may be one of the underlying mechanisms of rehabilitation to promote recovery and functional independence. During rehabilitation sessions, effective motor learning strategies with task oriented training, for real life environment were utilized and successful attainment of functional outcomes were achieved. He was evaluated at regular intervals over the period of 18 months. After the procedure, no adverse events were recorded.

Results

The patient showed some immediate improvements within a week and significant improvements over a period of 18 months. On follow up after three months, the hyperactivity had reduced by 20% and self–stimulatory behaviour of hand flapping and touching himself in private parts diminished. His eye contact had improved. The score on Indian Scale for Assessment of Autism (ISAA) reduced from 111 (Moderate autism) to 96 (Mild Autism). On six month follow up, hyperactivity had further reduced by 70% and he could maintain meaningful eye contact. Self stimulatory behaviour had subsided completely. He could remember routes as his memory improved. He developed autonomous living. New task learning abilities, concept forming and problem solving had improved. This led to increased participation in household work and performing chores independently, able to perform tasks such as unlock and lock the door without assistance. The awareness of surroundings had increased. On 18 months follow up, hyperactivity had resolved and motor repetitive movements reduced drastically. All the other improvements were sustained. On repeating the PET scan, the areas which previously showed more FDG uptake, now showed less uptake. These areas were 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 (Figure 1). Intelligent Quotient (IQ) on The Malin’s Intelligence Scale for Indian Children increased from 44 to 49.3 (Moderate Mental Retardation). The score on ISAA reduced further from 96 to 73 (Mild Autism) with improvements in social relationship and reciprocity, emotional responsiveness, speech-language and communication, behavioral patterns, sensory aspects and cognitive component (Table 1).

Discussion

Autism spectrum disorders have many associated comorbidities such as Mental Retardation (MR); motor impairments, epilepsy, sleep dysfunction, etc. Autism and MR are closely related. About 70% of autism individuals are associated with mental retardation of varying degree [5]. Autism being one of the most complicated neurodevelopmental disorders has no definite therapeutic approach [6]. Cell therapy has been widely studied and has shown to have potential as a novel treatment for various neurological disorders [7].

This is a case study of autism treated with Autologous Bone Marrow Derived Mononuclear Cell (BM-MNC) transplantation, intrathecally. These cells are a combination of hematopoietic cells such as CD 34+ and non-hematopoietic cells such as Mesenchymal Stem Cells (MSCs), tissue specific progenitor cells and stromal cells [8]. They have a unique property of migrating to the damaged areas and carry out repair process [9]. They can alter the immune system and restore the altered brain organization. These cells also exhibit paracrine effects and induce angiogenesis by producing growth factors and other chemokines such as Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor (FGF2) [10].

Amongst all the physiological changes associated with autism, hypoperfusion and immune dysfunction are broadly studied [11]. Hypoperfusion results in hypoxia. Reversal of hypoxia may lead to self repair and neural proliferation, which is observed in many animal models of cerebral ischemia. In cerebral ischemia animal models, bone marrow stem cells have shown to repair the ischemia-damaged neural networks and restore the lost neuronal connections [12]. Hence, stem cells may be used to stimulate angiogenesis and lead to reperfusion. Also, in autism, there are severe immune alterations and excessive production of pro-inflammatory cytokines. Stem cells secrete several biomolecules with anti-inflammatory properties through
ISAA at follow up of 3 months and 18 months. Table 1: (as described in results and Table 1). ISAA of this case study has correlated well to the clinical improvements cognitive component. We observed that the change in the scores on language and communication; behavior patterns; sensory aspects and social relationship and reciprocity; emotional responsiveness; speech, This scale is based on CARS and has 40 items divided under six domains–

to diagnose or measure the severity of autism, ISAA was developed by

improvements were recorded. These were supported by change in the

incurable neurological disorders including autism. These patients

regular cell therapy for autism [11]. Previous studies have shown benefits of

prove useful for the treatment of T cell defect associated with autism

Thus, stem cells are capable of suppressing the pathological immune

paracrine effect. This tries to maintain equilibrium in the immune

system alterations and activate endogenous repair mechanisms [13]. Thus, stem cells are capable of suppressing the pathological immune responses as well as stimulating vascularisation. Cell therapy may prove useful for the treatment of T cell defect associated with autism [14]. Based on these theories, Ichim et al. proposed exploration of cell therapy for autism [11]. Previous studies have shown benefits of autologous mononuclear cell transplantation in children with various incurable neurological disorders including autism. These patients reported improvement in daily functions further improving their quality of life [15]. In the present case study, on follow up, many functional improvements were recorded. These were supported by change in the ISAA scale. As there was no scale designed specific to Indian population to diagnose or measure the severity of autism, ISAA was developed by the National Institute for Mentally Handicapped (NIMH) in 2009 [16]. This scale is based on CARS and has 40 items divided under six domains—social relationship and reciprocity; emotional responsiveness; speech, language and communication; behavior patterns; sensory aspects and cognitive component. We observed that the change in the scores on ISAA of this case study has correlated well to the clinical improvements (as described in results and Table 1).

The small increase in IQ score from 44 to 49.3 may be due to improved cognition. IQ is a higher function, so a significant change on The Malin’s Intelligence Scale for Indian Children can be expected in long term and with multiple doses of stem cell transplantation. This can be explored in the future studies.

PET is a relatively non-invasive imaging test which is able to detect abnormalities in the brain based on derangements in the metabolic functions at the cellular level [17]. PET has previously shown changes with autologous BMMNCs in cerebral palsy [18,19]. In this case of autism, comparative PET study done at nine months after the cell therapy recorded improvements. It was observed that multiple areas of hypermetabolism (more FDG uptake) seen in the pre-treatment scan had regressed (less FDG uptake) in the scan performed after the treatment. This may suggest that the hyperactive areas of the brain were suppressed which were exhibited as clinical decrease in hyper functioning symptoms of autism. Thus, striking a balance of the overall metabolic activity of the brain. These findings are supported by the Intense World Theory, which proposes hyper-functioning of local neural microcircuits leading to hyper-perception, hyper-attention, hyper-memory and hyper-emotionality in autism [20,21].

In autism, neuronal dysfunction is evident in many regions such as Dorsolateral Prefrontal Cortex (DLPFC), the Anterior Cingulate Cortex (ACC), temporal lobe, amygdala, hippocampus, brainstem, and cerebellum [22]. Abnormalities in the Broca and Wernicke areas have been associated with impairment in social communication and language. Frontotemporal regions and amygdala have been associated with abnormalities in socioemotional processing and orbitofrontal cortex and caudate nucleus (i.e, frontostriatal system) are linked to repetitive and stereotypical behaviors [23]. Decrease in number of Purkinje cells in the cerebellar cortex has been observed in autistic brain which leads to clumsiness. The cerebellum has close anatomic connections with the frontal lobe, involving not only motor functions but also cognitive functions [24]. As seen in this case study, the PET CT scan shows improved functions of the areas discussed above. This enhanced function is seen as the clinical improvements in areas of language, social communication, behavior and cognition.

This case study of autologous bone marrow derived mononuclear cell transplantation demonstrates that this method is safe, feasible and has great potential in conjunction with current rehabilitation in treatment of autism. To our knowledge, this is one of the first case studies to have documented objective improvements in ISAA and radiological improvements in the PET study. With the pool of other studies, it will form a foundation to plan future larger trials to treat disorders such as autism.

### References

<table>
<thead>
<tr>
<th>Sub-components of ISAA</th>
<th>Score Pre treatment</th>
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<td>36</td>
<td>29</td>
</tr>
<tr>
<td>1 Eye contact</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2 Remains aloof</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>3 Does not reach out to others</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>4 Unable to relate to people</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>5 Unable to respond to social/ environmental cues</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>6 Engages in solitary and repetitive play activities</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>7 Unable to take turns in social interaction</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>8 Does not maintain peer relationships</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>B. Emotional Responsiveness</td>
<td>13</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>1 Shows exaggerated emotions</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2 Engaging in self stimulating emotions</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3 Excited or agitated for no apparent reason</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C. Speech Language and Communication</td>
<td>18</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>1 Engages in echolalic speech</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2 Produces infantile squeles/unusual noises</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3 Uses jargon or meaningless words</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>D. Behaviour Patterns</td>
<td>22</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>1 Shows hyperactivity and restlessness</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2 Exhibits aggressive behavior</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3 Throws temper tantrums</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4 Insists on sameness</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>E. Cognitive Component</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>1 Inconsistent attention and concentration</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2 Shows delay in responding</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1: Table giving details of the improvements in different sub components on ISAA at follow up of 3 months and 18 months.
supportive care in acrometastasis to the hand: case series. Indian J Palliat Care 17: 241-244.


