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Restorative therapy using autologous bone marrow derived mononuclear cells infusion intra-arterially in patients with cerebral palsy: An open label feasibility study

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Abstract

Cerebral Palsy is a common and devastating neurological disorder, with no medical treatment apart from physiotherapy regimes to alleviate the functional disability. Regenerative medicine using stem cells has gained momentum in recent years as a possible strategy to repair the injured brain. Present study was undertaken in a open label series to evaluate the safety, feasibility and observe for any beneficial effects of intra-arterial infusion of autologous bone marrow derived mononuclear cells in patients with cerebral palsy with moderate disability. Functional improvement was assessed using the motor power and spasticity scales, dystonia and abnormal movements scale and the activities of daily living scales by modified Barthel Index and modified Rankin Scores. Serial structural imaging with MRI and functional imaging with FDG-PET scans were done. Of the 30 patients injected with an average of 10-30 million cells into each carotid artery, improvements were observed in all clinical scales, and predominantly in the disability scores. No adverse events were noted on a 12 months follow up.

INTRODUCTION

Cerebral Palsy (CP) has been defined as a "group of permanent disorders of the development of movement and posture that cause limitations in functional activities that are attributed to non-progressive disorders which occur in the fetal or infant brain".[1] It has been classified as spastic (tetraplegia, dipelgia , hemiplegia), ataxic, dyskinetic (athetoid, dystonic) and mixed type. As the disease evolves, the clinical symptoms develop into spastic paralysis, deformities, joint degeneration, osteoporosis, fatigue, pain, gait disturbances and others.[2,3] In a study conducted by Singhi *et al* 2002, on 1000 cerebral palsy cases, spastic quadriplegia was found in 61%, spastic diplegia in 22%, and dyskinesia in 7.8%. Apart from clinical and functional deficits, mental retardation was consistently associated in 75% of cases.[4]

The management paradigms of CP involve physiotherapeutic intervention to treat the residual motor deficits that help in enhancing functional independence in self care, mobility, personal hygiene and leisure activities. Neurodevelopmental techniques are followed to achieve the goals of intervention.[5] Since there is no definitive "cure" available in the medical armamentarium, CP constitutes a tremendous socio-economic burden in the society besides causing an enormous emotional and psychosocial upheaval in the parents so there is an urgent need to explore any possible curative strategy for this otherwise untreatable condition. Recently there has been a lot of emphasis and interest on the role of restorative therapies such as stem cell transplantation in incurable and disabling neurological disorders. Contrary to the earlier belief regarding the incapability of the central nervous system for regeneration after injury, studies have shown new migrating cells in rodent's brain and in the human hippocampus, capable of neural regeneration. There are reports of bone marrow-derived cells developing neuronal and vascular phenotypes and aiding in repair of

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injured brain. These findings have fuelled interest in regenerative medicine for neurological diseases, arguably the most difficult diseases to treat being static encephalopathies including cerebral palsies, spinal cord injury and other chronic debilitating illnesses.[6,7]

Mononuclear stem cells are hematopoietic cells capable of self renewal, differentiation and migration.[8,9,10] They are abundantly found in the bone marrow. They are characterized by CD 34+ cells and are hypothesized to help in neurogenesis and angiogenesis. It has been observed in preclinical and clinical studies that bone marrow mononuclear stem cells are capable of neurogenesis and angiogenesis by modulating the 'host environment'.[11,12] Although initially, the belief was that cell therapy may work by a 'cell replacement' mechanism, subsequently a large body of evidence has emerged regarding the role of stem cells in providing trophic or 'chaperone' support to the injured tissue and brain.[13]

The main objectives of this study were (i) to evaluate the feasibility and safety of trans-arterial injection of autologous bone marrow derived naïve mononuclear cells (MNCs) in the carotid artery, (ii) to explore the therapeutic potential of adult stem cells in patients with static encephalopathies with special reference to cerebral palsy, (iii) to help establish future guidelines regarding the proper selection of patients and to look for complications specific to stem cell therapy.

METHODS

We recruited 30 patients with cerebral palsy fulfilling the inclusion criteria (i.e., mental retardation: IQ – 30 % -75%, activities of daily living (ADL) scores: modified Barthel Index (mBI) -> 30% and < 75%, Modified Rankin Scale (mRS) ≥ 3, Spasticity as measured by Ashworth Scale ≥ 2, muscle power as assessed by MRC grade ≤ 3, ataxia with Abnormal Involuntary movement scale (AIMS) ≥ 25, and Dystonia Movement Scale ≥ 60). The exclusion criteria were progressive neurological illness, renal / hepatic failure, congestive heart failure, active infections, thrombophilias/ bleeding diathesis, prolonged PT/ APTT, protein C and S deficiency, anti thrombin III deficiency, progressive neurological diseases, and active epilepsy (seizures in last 6 months). This was an open label series. The project has been cleared from the local IRB as well as the Institutional Stem Cell Research Committee. Written informed consent was taken from the patients and in case of children, from the legally acceptable relative (LAR).

Procedure

Patients were recruited from the neurology clinic of our Institute. This was an open label series with sample size of 30 patients. The baseline measurements included clinical scores such as modified Barthel Index (mBI)[14], MRC, Ashworth, modified Rankin Scale (mRS), Dystonia Score, AIMS (abnormal involuntary movement scale) and Malin's Intenlligence scale for Indian Children and imaging. The structural and imaging scans were done on MRI scanner (Seimens, Avanto, Germany, 1.5T) to record any cerebral/cortical atrophy, ventricular dilatation, size and number of infarcts, and /or site of gliosis. The 18-FDG-PET scans were done at baseline to observe for any regions of hypometabolism. Imaging was repeated at 3, 6, 9 and 12 months, post infusion of stem cells.

Mononuclear cells aspiration and transplantation

Under general/local anaesthesia, 25-50 ml of bone marrow was aspirated from iliac crest. MNCs were separated using Ficoll density separation method. These cells were layered over lymphocyte separation medium and centrifuged at a speed of 1500 rpm for 30 min. Mononuclear stem cells were separated and washed thrice in heparinized normal saline to remove traces of Ficoll. The harvested mononuclear cells were evaluated for viability of cells by Trypan Blue exclusion test, flow cytometry and MNC morphology by Geimsa staining (figures 1, 2). The whole procedure took approximately 150±10 minutes. Patients were evaluated for safety i.e., laboratory tests and clinical examination. Two patients reported pain at the site of bone marrow aspiration, which was treated with analgesics. This procedure was same as reported in other clinical trials.[15,16]

Intra arterial infusion

Under aseptic precautions, and with local /general anaesthesia, digital subtraction angiography was performed in the angio suite and MNCs were injected over 5 minutes, into the carotid artery on each side through the femoral artery cannulation. Following injection, catheter was flushed with 1000 units of heparin. On an average 10-30 million cells were infused in each carotid artery.

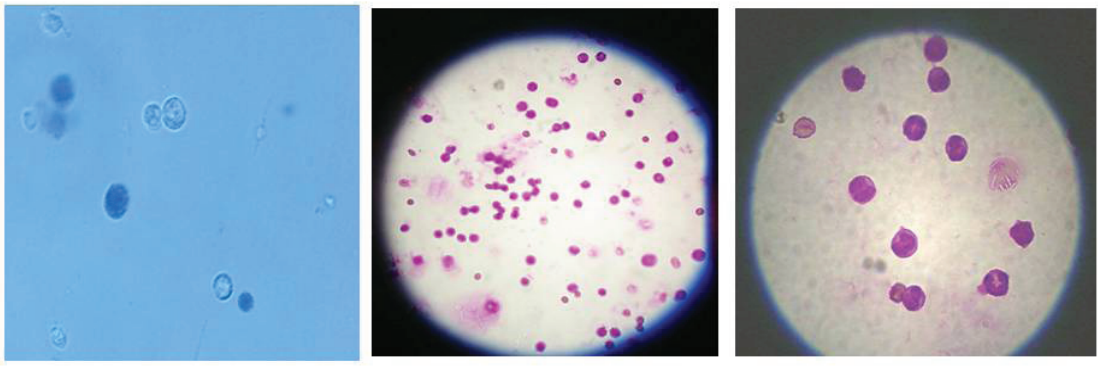

Figure 1. Trypan blue and Geimsa staining of bone marrow derived mononuclear cells

RESULTS

Safety of stem cell transplantation

Following the MNCs infusion, patients were monitored in the neuro intensive care unit. Neurological status was monitored every hour for 24 hours for any change in sensorium/headache / vomiting/seizures. There were no major immediate or delayed adverse reactions observed at the time of bone marrow aspiration, during and after MNC transplantation. The cells had an average viability of 99% before transplantation. The transplanted cells were unfractionated mononuclear stem cells but CD 34+ was quantified to evaluate the proportion of these cells in the implanted volume. The mean CD 34+ count was 0.21% with mean 15.7 x 10^6 infused intra arterially, into each carotid artery. The whole procedure took 120±10 minutes. Two patients developed fever after the stem cell infusion which was treated by standard regime. We did not observe any infection, bleeding, edema, thrombus formation, cardiovascular and neurological deterioration up to one year follow up. As the subjects were in the younger age group, post sedation drowsiness and clumsiness was observed in certain patients which resolved spontaneously over 2-6 hours.

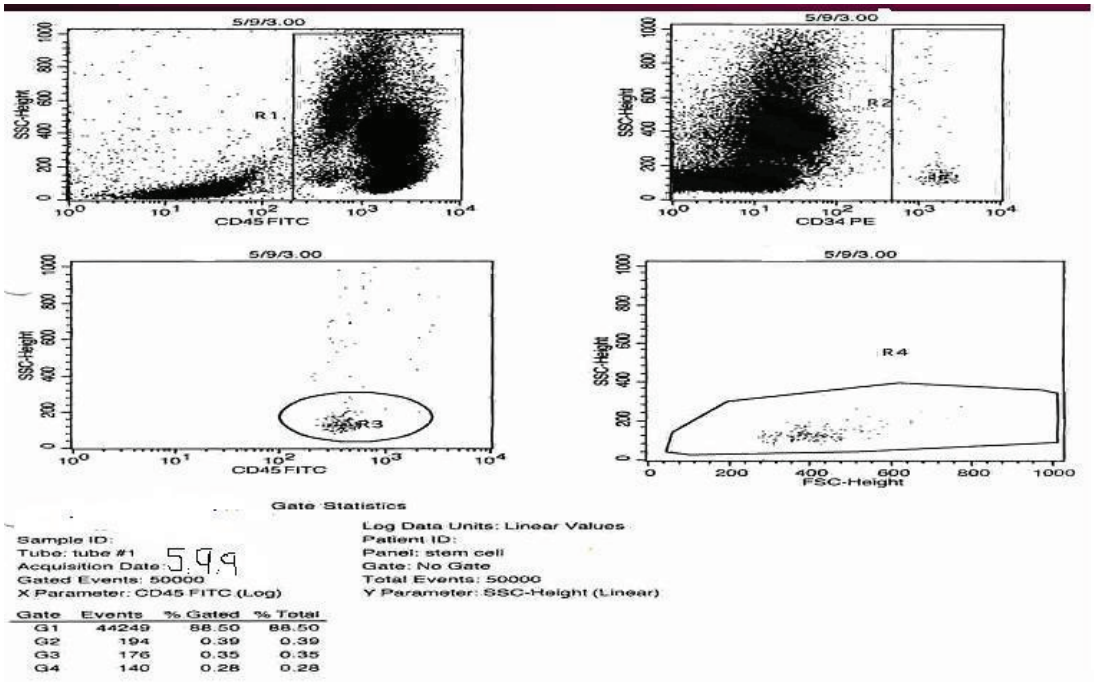

Figure 2. Phenotyping of bone marrow derived mononuclear cells

Clinical results

Out of 30 patients with CP, 19 were males and 11 females. The mean age was 10.1 years (5-25). The mean baseline mBI was 24.4, mean baseline mRS, 3.93, Ashworth scale, 2.37 and mean MRC was 1.9, which indicated moderate to severe disability in all patients (table 1). Only 7 patients presented with abnormal involuntary movements and more than 50% patients recruited had dystonia with a mean score of 20.72. The mean mBI at 3, 6, 9 and 12 months was 24.4, 33.4, 51.5 and 65.9 respectively whereas mRS were 3.93, 3.53, 3.03 and 2.86 respectively (table 2). We applied paired t-test and repeated measures ANOVA for all patients. Since this was not a controlled study design, we calculated the percentage difference between the scores. The mBI scores showed statistically significant improvement ($p< 0.05$) between baseline and 6, 9 and 12 months, with $p =0.005$ between (0-3months), $p=0.001$ between (0-9 months) and $p = 0.0001$ between (0-12 months). The MRC and Ashworth scale showed statistically significant improvement only at follow up of 12 months ($p<0.05$) and was non significant ($p>0.05$) when compared between baseline and 3, 6, 9 months respectively. We observed a gradual increase in the mBI and MRC scores and a decrease in the Ashworth and mRS scores from baseline to follow up (figures 3, 4). We did not observe a significant percent change of the scores from baseline and at 3 months, where as there was a 36.8% increase in mBI from baseline and at 6 months, 111.2% from baseline and at 9 months and about 170% at 12 months. The MRC scores showed a change of 47.3% and 73.6% from baseline and at 6 months and 12 monthly follow up respectively.

DISCUSSION

The mononuclear stem cells were easy to procure and had an average viability of 99% before transplantation which is in confirmation to previous studies.[15,16] The routine clinical examination, laboratory reports and radiological scanning were normal in all patients and we did not observe any major complications during and after the transplantation till the last follow up at 12 months. From these observations, the main objective of the study which was to evaluate the safety, feasibility and tolerance of autologous MNC transplantation in patients with CP was established.

The improvements were observed predominantly in the disability scales i.e., mBI and mRS as compared to the impairment scales (Ashworth and MRC). We observed 22.09% decrease in mRS scores between baseline and 9 months and 28.75% decrease in the scores from baseline to 12 months follow up. There was a decrease of 15.6% in Ashworth score from baseline to 9 months follow up and 28.3 till 12 months follow up. This can be hypothesized that being a non progressive static disease, cerebral palsy afflicted subjects may not show improvements in the impairments as fast as perceived. Dystonia score consisted of 5 point scale with 0= no spasm, 1= mild barely noticeable spasm, 2= moderate, no functional impairment, 3= moderate spasm, moderate functional impairment, 4 = severe or incapacitating spasm. We did not find a significant improvement in this scale till 6 months from baseline but a gradual decrease in spasm scale at 12 monthly follow up.

When introduced into the injured brain, stem cells can have a positive influence through intrinsic neuroprotective capacities due to the production of neurotrophic factors, stimulation of endogenous neurogenesis, and modulation of neuro-inflammation.[17] Stem cells are also endowed with a multipotent differentiation profile, suggesting that a positive outcome could result from the replacement of non-neuronal cell types, in particular astrocytes and oligodendrocytes. It is also postulated that stem cells operate not only through a unidirectional mechanism (e.g., generating neurons) but as cellular mediators of many of biological activities that could provide a favorable outcome for diverse neurological disorders.[18,19]

Stem cells can be delivered intra-lesionally into the brain or are infused by an intravenous or intra-arterial route. Stem cells can be mobilized by approaches in which endogenous stem and progenitor cells are mobilized by cytokines such as granulocyte colony stimulatory factor (GCSF) or chemokines such as SDF-1; trophic and growth factor support, such as delivering brain-derived neurotrophic factor (BDNF) or glial-derived neurotrophic factor (GDNF) into the brain to support injured neurons. These approaches may be used together to maximize recovery.[20,21]

In our study, we found that the beneficial effects of cell transplantation were seen after 9 months to one year of stem cells infusion. Increasing angiogenesis with adult stem cell approaches in rodent models of stroke lead to preservation of neurons and improved functional outcome.[22,23] We hypothesize the improvement observed in our patients to either increased angiogenesis or to improved "internal milieu" conducive for initiation

Table 1: Demographics and clinical data in cerebral palsy patients

Patient	Age /sex	No. of MNC	% of CD 34+	Baseline mBI	Baseline mRS	Baseline Ashworth	Baseline MRC
1	5/F	11x 10^6	0.14	12	4	3	2
2	7/F	14 x 10^6	0.12	22	3	3	2
3	6/F	8 x 10^6	0.2	16	4	2	2
4	5/F	12 x 10^6	0.08	18	4	2	1
5	10/F	15 x 10^6	0.13	23	3	3	2
6	11/F	16 x 10^6	0.15	48	3	3	3
7	17/M	9 x 10^6	0.21	34	3	2	3
8	5/M	22 x 10^6	0.11	13	5	2	1
9	14/M	20 x 10^6	0.07	38	4	3	2
10	5/M	12 x 10^6	0.11	8	5	3	1
11	16/M	8 x 10^6	0.09	8	5	1	0
12	10/M	21 x 10^6	0.15	26	3	2	2
13	10/M	25.4 x10^6	0.18	20	3	2	2
14	5/M	23.4 x10^6	0.09	22	4	2	2
15	18/M	14 x 10^6	0.12	17	3	1	2
16	7/F	16 x 10^6	0.08	34	4	2	2
17	11/F	18 x 10^6	0.20	34	4	1	2
18	9/M	11 x 10^6	0.09	45	4	1	2
19	18/F	13 x 10^6	0.18	13	3	2	1
20	7/M	14 x 10^6	0.19	19	5	3	1
21	9/F	13 x 10^6	0.21	26	4	2	2
22	11/M	12 x 10^6	0.06	31	4	2	2
23	14/F	11 x 10^6	0.05	28	4	2	2
24	10/M	21 x 10^6	0.09	15	5	3	2
25	25/M	26 x 10^6	0.19	16	5	4	2
26	7/M	22 x 10^6	0.12	17	4	4	2
27	10/M	19 x 10^6	0.13	44	3	1	3
28	5/M	17 x 10^6	0.11	12	5	4	2
29	10/M	15 x 10^6	0.08	34	4	3	3
30	12/M	14 x 10^6	0.09	39	4	3	2
Mean	**10.1 yrs**	**15.7 x 10^6**	**0.21**	**24.4**	**3.93**	**2.37**	**1.9**

MNC: mononuclear cells; mBI: modified Barthel Index; mRS: modified Rankin Scale; MRC: Medical Research Council

Table 2: Modified Barthel Index and modified Rankin Scale at 3, 6, 9 and 12 months

Patient	Modified Barthel Index				Modified Rankin Scale			
	3 month	6 month	9 month	12 month	3 month	6 month	9 month	12 month
1	12	18	27	45	4	4	3	3
2	22	30	43	58	3	3	3	3
3	16	24	44	58	4	4	3	3
4	18	28	48	62	4	4	3	3
5	23	23	44	68	3	3	3	3
6	48	52	68	78	3	3	3	2
7	34	40	58	65	3	3	3	3
8	13	20	32	45	5	4	3	4
9	38	48	68	79	4	3	2	2
10	8	15	23	38	5	5	4	3
11	8	35	52	69	5	5	4	3
12	26	30	54	72	3	3	2	2
13	20	30	58	75	3	3	3	3
14	22	30	54	75	4	3	3	3
15	17	22	54	75	3	3	3	3
16	34	44	62	82	4	3	2	2
17	34	44	64	76	4	3	2	2
18	45	58	76	89	4	3	2	2
19	13	22	38	48	3	3	4	3
20	19	22	38	51	5	5	4	3
21	26	35	57	69	4	3	3	3
22	31	42	63	81	4	3	3	3
23	28	42	68	79	4	3	3	3
24	15	24	38	48	5	4	4	3
25	16	23	39	49	5	4	4	3
26	17	24	41	58	4	3	3	3
27	44	59	70	85	3	3	3	3
28	12	20	31	43	5	5	4	3
29	34	48	65	78	4	4	3	3
30	39	50	68	80	4	4	2	3
Mean	**24.4**	**33.4**	**51.3**	**65.9**	**3.93**	**3.53**	**3.03**	**2.86**

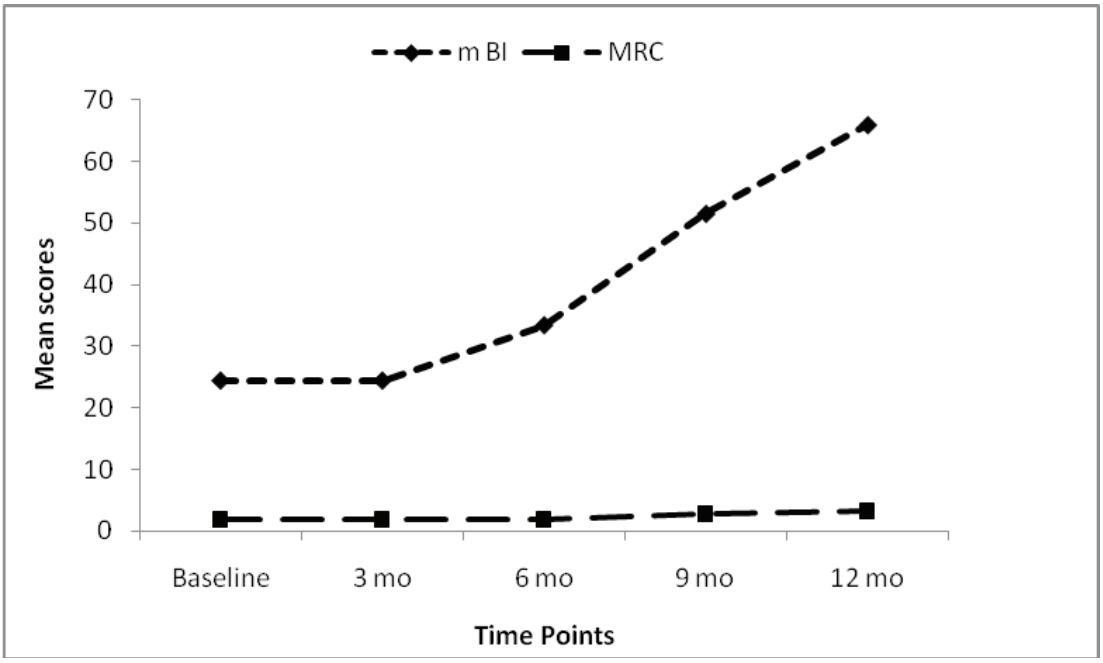

Figure 3. Mean mBI (modified Barthel index) and MRC (Medical Research Council) score at baseline, 3, 6, 9 and 12 months.

of the reparative process. We had no means to objectively prove this hypothesis since there was no method available to either "tag" the cells to observe their "homing" capability or survival or integration into the host cytoarchitecture or histologically prove the same.

Motivated by the ambitious expectation to achieve functional neuronal replacement, several studies have already evidenced a potential benefit of mononuclear stem cell grafts in animal models and clinical trials.[29,15,16] Nevertheless, growing evidence suggests that the effects

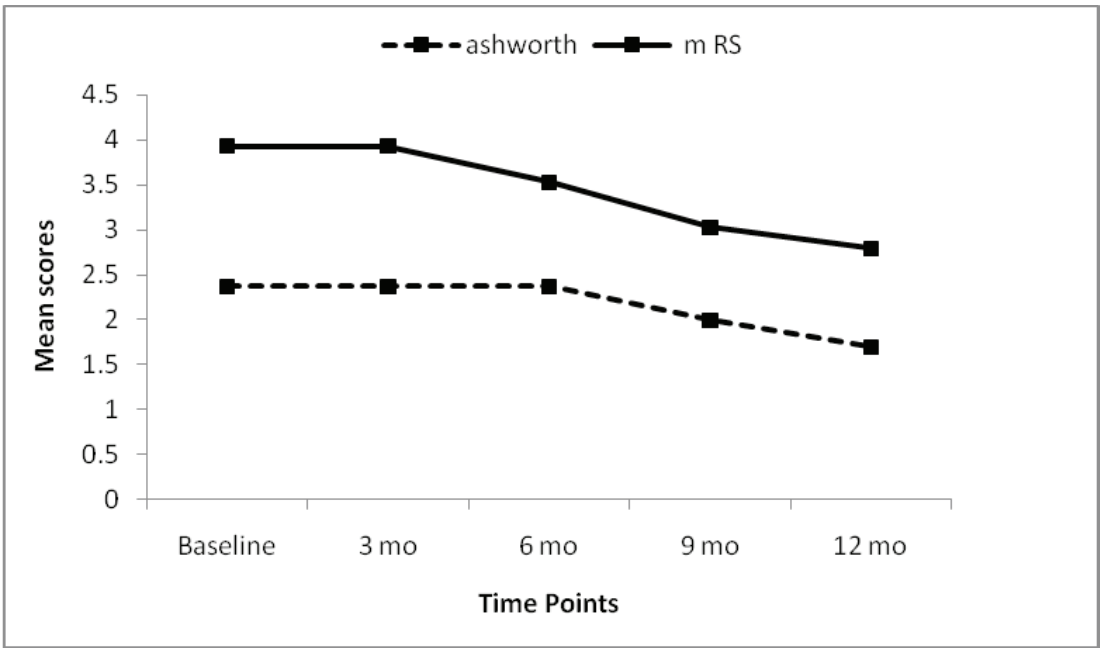

Figure 4. Mean Ashworth tone grade and mRS (modified Rankin Scale) at baseline, 3, 6, 9 and 12 months.

orchestrated by stem cells, are not necessarily associated with the generation of new neurons only but also augmentation of endogenous repair mechanisms.[30]

In our patients, MR imaging revealed variously periventricular leukomalacia, multicystic encephalomalacia, cortical and subcortical atrophy, cortical and subcortical atrophy, brain malformation, periventricular leukomalacia, multicystic encephalomalacia, porencephaly and delayed myelination, which were very similar to the earlier reports.[24] We did not find any change in the structural and anatomical changes in the MRI, PET scans at the regular time intervals.

It has been demonstrated that tripotential neural stem/progenitor cells (NSPs) participate in the regenerative response to perinatal hypoxic ischemic injury in rats by an increased proliferation of the cells at 2nd day.[25] It was also observed that the proliferating cells expressed nestin, membrane receptors Notch1, gp-130, and the epidermal growth factor receptor, as well as the downstream transcription factor Hes5, which stimulated NSP proliferation.[26]

The demyelinating diseases / hypoxic ischemic injuries of the brain are especially attractive targets for cell-based therapeutic strategies, since they are caused by the loss oligodendrocytes.[27] Oligodendrocytes are the sole source of myelin in the adult CNS, and their loss or absence lead to variety of brain dysfunctional disorders, thus it becomes necessary to develop newer therapies either replacing myelinogenic cells directly by oligodendrocyte replacement, or supporting their viability through the introduction of non-oligodendrocytes able to restore missing enzymes to an otherwise deficient environment.[28]

Owing to the reason of non progressive static disease, CP afflicted subjects may not show improvement in the impairments as fast as perceived but there have been improvements in both the impairment and disability scales. At three months follow up when the data was analysed, there was little or no change in the outcome measures. But the data at 9 and 12 months follow up suggested some improvement in the m BI and m RS scales (Figure 3 and 4). Nevertheless it was also observed that the improvements were greater in patients who were moderately affected (baseline MRC: 2-3/5, tone =1-2) as compared to those severely affected group (MRC: 0-1/5. Tone: 3-5, M-ISIC =0-4).

We assessed the patients on MRC, Ashworth, BI, and mRS which are the outcome measures to evaluate impairment, functionality and disability. We did not assess patients on Gross Motor Function Measure which is an assessment tool for Cerebral palsy in many trials[29], instead we used the above mentioned assessment tools which would also evaluate the motor function and daily living activities. Gross Motor Function Measure assesses developmental milestones, functional movements and activities of daily living and since there was no control group to evaluate efficacy, and this being primarily a safety trial, GMFM was not an assessment tool. As this is an open label, safety and feasibility study of intra arterial mononuclear stem cell transplantation, we have tried to establish the safety of intra arterial mononuclear stem cell therapy.[30]

We conclude that it is feasible and safe to transplant autologous bone marrow derived naïve MNC in the carotid artery in patients with non progressive static encephalopathies with special references to CP. This research managed to explore the therapeutic potential of adult stem cells and may also help establish future guidelines regarding the proper selection of patients, dosage, and route of delivery for stem cell therapy.

DISCLOSURE

Conflict of Interest: None

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