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Human Embryonic Stem Cell Therapy in Cerebral Palsy Children with Cortical Visual Impairment: A Case Series of 40 Patients

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Abstract

Background: Use of human embryonic stem cells (hESCs) has been explored for treatment of cortical visual impairment (CVI) in children with cerebral palsy (CP).

Objectives: The study evaluated efficacy and safety of hESC therapy in 40 CP children with CVI.

Methods: The study had four treatment phases (T1, T2, T3, and T4) separated by gap phases. Patients were evaluated for visual impairment using Nutech Functional Scores (NFS).

Results: Of 40 patients included in study, 8 had NFS level "1" (were blind/ had no perception of light); 16 had level"2" (perception of light); 10 had level "3" (could identify blurred images); and 6 had level "4" (could see objects up to a distance of 25 cm from the eye). After therapy, 27 patients gained normal vision; 10 patients could see objects 25 cm from the eye; 2 patients could see blurred images; and 1 had perception of light. Overall, 39 patients showed improvement in NFS by at least one level. In SPECT scan, 2 patients had normal perfusion, 18 had significant improvement and 3 had moderate improvement.

Conclusion: Use of hESC therapy in patients with CVI showed beneficial results for treatment of CVI in children with CP.

Keywords: Cerebral palsy; Human embryonic stem cells; Transplantation; Hypoperfusion

Abbreviations:

hESCs: Human Embryonic Stem Cell; CVI: Cortical Visual Impairment; CP: Cerebral Palsy; NFS: Nutech Functional Score; SPECT: Single Photon Emission Computed Tomograph; IEC: Independent Ethics Committee; IC-SCRT: Institutional Committee of Stem Cell Research and Therapy; GCP: Good Clinical Practice; HMPAO: Hexa Methyl Propylene Aminoxime; CSE: Correlated Signal Enhancement; GMFCS: Gross Motor Function Classification System

Introduction

Cerebral Palsy (CP), a neurological disorder which affects the brain is caused by hypoxia resulting in many symptoms among which there could be cortical visual impairment (CVI) [1]. Garcia et al. showed that 13% of the patients with optic nerve hypoplasia have CP [2]. Most children with CP show decreased accommodative function which may or may not result in impaired learning [3].

Treatment of visual impairment in children with CP is challenging, recent research in this area has shown considerable improvement with the use of stem cells in combination with neurotrophic agents [4]. The use of human embryonic stem cells (hESCs) has been explored recently for the treatment of CVI in children with CP. Although very few studies evaluating the role of hESCs are available due to controversial source and isolation technique of hESCs, a study on visually impaired rates showed 100% improvement with the use of hESCs [5]. The present study assessed the efficacy and safety of hESC therapy in 40 CP children with CVI. Single Photon Emission Computed Tomography (SPECT) scan and an in-house developed scoring system; Nutech Functional Scores (NFS) was used to evaluate the extent of improvement in perfusion before and after treatment with hESCs.

Materials and Methods

Study characteristics

This was a retrospective subgroup analysis of a single cohort study of patients having CP with CVI (diagnosed elsewhere) and treated by us with hESCs conducted from 01 October 2007 to 31 July 2013 in New Delhi, India. The data was collected from patients having CP with CVI retrospectively.

The study protocol was approved by the Independent Ethics Committee (IEC). The Institutional Committee of Stem Cell Research and Therapy (IC-SCRT) of Nutech Mediworld, a GCP certified center for stem cell therapy reported all the work with respect to embryonic stem cells to National Apex Body. A written informed consent which

was videographed was obtained from the study patients prior to the treatment.

Study population

Inclusion Criteria: Patients less than 16 yr, and with a documented diagnosis of CP with cortico-visual impairment and willing to provide a written informed consent were included in the study.

Exclusion Criteria: Patients above the age of 16 yr were excluded from the study. Patients who have received other form of stem cell therapy elsewhere were not given treatment at Nutech Mediworld within 12 months of such treatment.

Cell culture and differentiation

The cell culture consisted of two directed cell lines "neuronal" and "non-neuronal". The non-neuronal cell lines included progenitor cells for hematopoietic stem cells, insulin producing stem cells, mesenchymal stem cell, epithelial stem cells, hepatocyte stem cell, cardiac stem cells.

The hESCs used in our study have been obtained from a single, spare expendable, pre- implantation stage fertilized ovum taken during a natural IVF process after consent from the donor. The fertilized ovum was suspended in a small amount of minimal essential media and broken mechanically. The product was incubated in carbon-dioxide (CO_2), water jacketed incubator with essential media Dulbecco's Modified Eagle's Medium (DMEM; Himedia Labs, Mumbai, India) and Roswell Park Memorial Institute medium (RPMI; Himedia Labs, Mumbai, India) with addition of progesterone (16-64 µl of 250 mg/ml, Sun Pharma, Mumbai, India) and β-HCG (16-64 µl of 500 IU/ml, Serum Institute of India, Pune, India), in aerobic conditions. After a time span of 24 hr, the product was divided into two different flasks and DMEM and RPMI were added in a ratio of 1:3.5 to 1:35 volume by volume. The cells obtained were re-incubated in a water jacketed incubator at 34-38°C with an atmosphere of 3.5-6% CO_2 for 24 hr in anaerobic conditions. The cells were divided in to three aliquots- one aliquot was reincubated in anaerobic condition with either RPMI or DMEM; second aliquot was stored at freezing temperature and the third aliquot was made ready to injection (RTI) (Patent-WO2007/141657A PCT/1 B 2007; published Dec 2007). Patent-WO 2007/141657A PCT/1B 2007.

Various marker studies were done to characterize the cells were which include whether the cell were SSEA3+ve; OCT4 +ve; NANOG +ve; β actin +ve; SOX +ve; β –HCG +ve; alkaline phosphatase +ve; Nestin +ve, CD 34 +ve; NeuN+ve; GAF +ve; GATA +ve and TRA –ve. The detailed cell culture and differentiation techniques have been elaborated elsewhere (unpublished data, submitted for publication).

Study design

The study consisted of four treatment phases separated by gap phases. After diagnosis of CP was established and SPECT scan was done to document perfusion in the brain; patients were examined by an ophthalmologist, and a pediatrician. The patients were then tested for hypersensitivity reactions with hESC (0.05 ml hESC injected subcutaneously).

Following safety evaluations, patients entered the first treatment phase (T1, 8 weeks) in which patients received 0.25 ml hESC daily intramuscularly to "prime" the body and allow for the recipient immune system not to reject the stem cells and 1 ml hESC twice within 7 days intravenously (IV) to "home in" to the affected area. In addition, patients received hESCs through retrobulbar route (every 10-21 days), eye drops, and/or nasal spray of hESCs as required. The patients received eye drops twice a week. The eye drops were given three times in each session with a gap of 45 min between each dose. After the eye drops were administered, the patients were requested to keep their eyes closed for a few minutes [6,7]. The patients received predominantly non neuronal cell lines through i.m route and predominantly neuronal through i.v, nasal spray, eye drops and caudal routes.

After a gap period of 3-6 months, patients entered subsequent treatment phases (T2 and T3) which lasted for 4 weeks separated by a gap phase of 3-6 months. Patients received same dosage regime as T1. Patients then entered fourth treatment phase (T4) after a gap period of 6-12 months with similar dosage regimen as that of T2 however; the IV dose of hESC was increased by 1 ml. The treatment phase was repeated annually if required. All the patients received physiotherapy, occupational therapy and rehabilitation program in addition to hESC therapy. The detailed study design has been elaborated elsewhere (unpublished data, submitted for publication).

Variables for analysis

Nutech Functional Scores (NFS)

All patients were monitored for functional vision throughout the study. The level of visual impairment was assessed using specially designed scoring system namely Nutech Functional Scores (NFS):

Level 1- no perception of light;

Level 2- perception of light present;

Level 3- can identify objects but the images are blurred and have finger counting ability depending on age

Level 4- can see objects up to a distance of 25 cm from eye; and

Level 5- normal vision

This score has been developed based on the clinical observations and improvements. A team of ophthalmologists, occupational therapists, and specialists performed the clinical evaluation process before and after the therapy.

Data validation

The data for all the patients was validated by Quality Austria Central Asia Pvt Ltd (DOC NO Q ACA/OCT/2013/26) Accreditation Company. They were allowed to examine the medical and statistical data present at the institute and were also able to meet the patients.

SPECT scan

The SPECT scan (Millennium MG, GE) was carried out before or within 7 to 10 days of hESC therapy initiation and thereafter at the end of each treatment phase. The patients received an IV injection of hexamethyl propylene aminoxime (HMPAO) into antecubital vein before the SPECT scan. After 15 min to 2 hr of receiving the injection, the patients were placed in a supine position with the orbitomeatal line positioned vertically centered in the field of view. Dual Digital Correlated Signal Enhancement (CSE) detectors, a gamma camera with a crystal thickness of 8.5 mm and energy window between 55 keV and 540 keV was used to obtain the SPECT scan. Patients showing

10%-30% changes were considered to have mild improvement, 30%-60% changes to have moderate improvement and 60%-90% changes as significant improvement.

Results

Study patients

Of the 40 visually impaired CP patients included in the present subgroup analysis, most were male (n-24) between the age group of 0 to 16 yr (0-2 yr: 6 patients; 2-4 yr: 7 patients; 4-6 yr: 10 patients; 6-12 yr: 15 patients; and 12-16 yr: 2 patients). Eight patients had NFS "1" (were blind/ had no perception of light); 16 had NFS "2" (perception of light); 10 had NFS "3" (could identify blurred images); 6 had NFS "4" (could see objects up to a distance of 25 cm from the eye); and no patients had NFS "5". Of the 24 patients with an NFS of level 1 and level 2; 7 had squint; 3 had night blindness; 4 had nystagmus; and 4 had restricted eye movements (Table 1).

Improvement in vision at the end of treatment

At the end of all treatment phases (331 treatment days), 39 patients showed an improvement in NFS by at least one level. A total of 27 patients regained their normal vision; 10 patients could see objects up to a distance of 25 cm from the eye; 2 patients could see blurred images; and 1 patient had perception of light.

NFS Levels	At Admission (n)	At Discharge					
		No Change	NFS 1	NFS 2	NFS 3	NFS 4	NFS 5
NFS 1	8	-	-	1	1	3	3
NFS 2	16	-	-	-	1	6	9
NFS 3	10	-	-	-	-	-	10
NFS 4	6	1	-	-	-	-	5
NFS 5	0						
Overall	40	1	0	1	2	9	27

NFS 1- no perception of light; NFS 2- perception of light present; NFS 3- can identify objects but the images are blurred and has finger counting ability depending on age; NFS 4- can see objects up to a distance of 25 cm from eye; and NFS 5- normal vision

Table 1: Improvement in Vision among Patients at Final Discharge

Of the 8 patients who had NFS 1, 3 patients transitioned to NFS 5, 3 patients transitioned to NFS 4; and one each to NFS 3 and NFS 1 at the end of all treatment phases. Of the 16 patients who had NFS 2 at the beginning of the study, 9 patients transitioned to NFS 5, 6 patients transitioned to NFS 4, and one patient to NFS 3. All 10 patients who had NFS 3 at the beginning of the study transitioned to NFS 5 at the end of all treatment phases. Five of 6 patients who had NFS 4 transitioned to NFS 5 however; one patient showed no change at the end of treatment phases (Table 1). Example of a patient included a 13 yr old male child diagnosed with CP having poor cognition, coordination, articulation, unable to see, read, or watch television was admitted to the hospital in Apr 2011. After receiving hESC therapy, this child showed an improvement in cognition was able to pass school examination, was able to read from more than 15 cm, could see colors, and watch television. The SPECT scan of this patient has been illustrated in Figure 1 and 2.

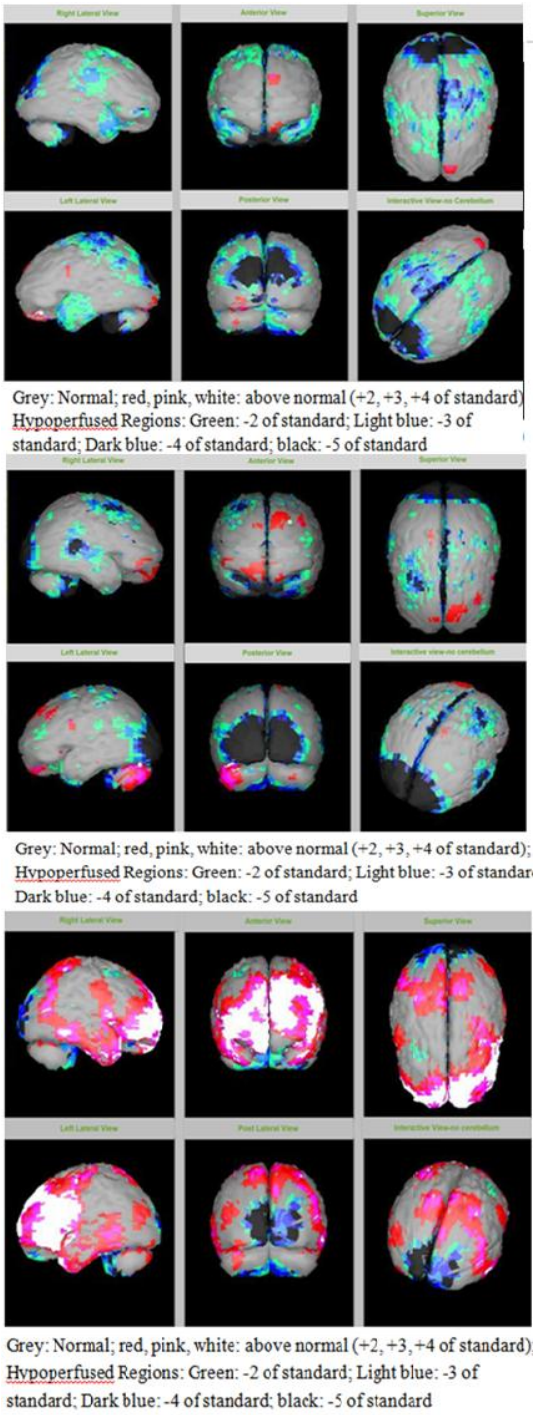


Figure 1: Improvement in perfusion of the brain before receiving hESC therapy.

SPECT scan

At the beginning and the end of the study, 23 patients underwent SPECT scan. Hypoperfusion was either observed in the occipital lobes or frontal lobes or both lobes. Of the 23 patients who underwent SPECT scan, 5 patients showed hypoperfusion in the occipital lobes, 7 patients showed hypoperfusion in the frontal lobes, and 11 patients showed hypoperfusion in both occipital and frontal lobes.

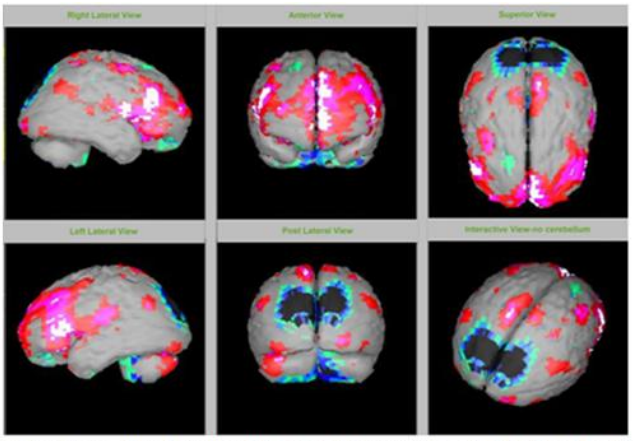
Overall, 2 patients had normal perfusion by the end of the therapy, 18 patients (12 male and 6 female patients) showed a significant improvement in perfusion (>60%) and 3 patients showed moderate improvement in perfusion (30-60%). Most of the patients who had severe hypoperfusion in their frontal and occipital lobes prior to receiving hESC therapy showed an improvement after receiving hESC therapy. The GMFCS scores of 23 patients for whom SPECT scan reports were available showed an improvement after the hESC therapy (Table 2).

Safety evaluation

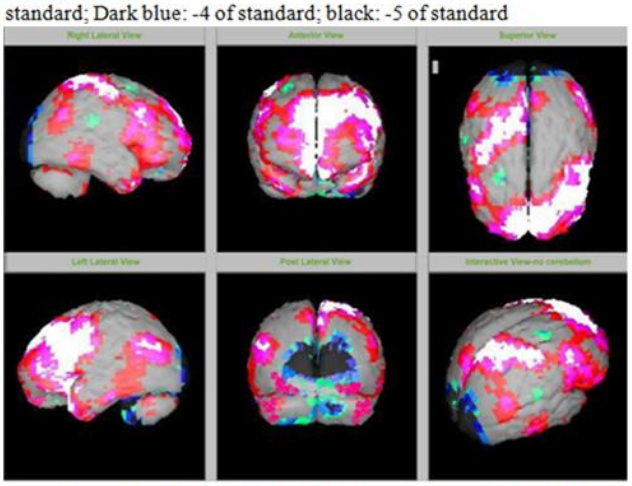
No adverse events were reported during the study period. None of the patient had teratomas as a result of the therapy.

S.No	Visual (SCORE)								Improvement from Baseline	GMFCS before	GMFCS after
	Occipital Lobe				Frontal Lobe						
	Left		Right		Left		Right				
	Before	After	Before	After	Before	After	Before	After			
1	-1	2	-1	2					Significant	4	1
2					-2	-1	-2	-1	Moderate	5	2
3					-3	2	-3	2	Significant	3	1
4	-5	-4	-5	-3					Significant	2	1
5							-2	2	Significant	1	1
6					-2	2	-2	2	Significant	2	1
7	-2	0	-2	0	-2	2	-1	4	Significant	2	1
8			-3	2					Significant	5	1
9					-2	-1	-3	-2	Significant	3	3
10	-2	1	-5	-2					Significant	3	1
11					-2	2	-2	2	Normal	4	2
12	-1	1	-5	2	-2	2	-5	-5	Moderate	3	2
13	-2	1	-3	1					Significant	5	2
14	-3	3	-4	3			-2	4	Significant	5	2
15			-2	-2	-5	-3	-5	-3	Moderate	5	2
16	-5	-2	-5	-3	-5	3	-5	1	Significant	5	3
17							-2	2	Significant	5	3
18	2	2	2	3	2	3	2	2	Significant	5	2
19	3	4	3	4	2	4	1	4	Significant	4	2
20	0	2	3	4	2	4	2	4	Significant	2	1
21	3	4	3	4	0	4	2	4	Significant	2	2
22	2	2	3	4	3	4	2	2	Normal	2	1
23	0	3	2	4	0	1	1	2	Significant	5	3

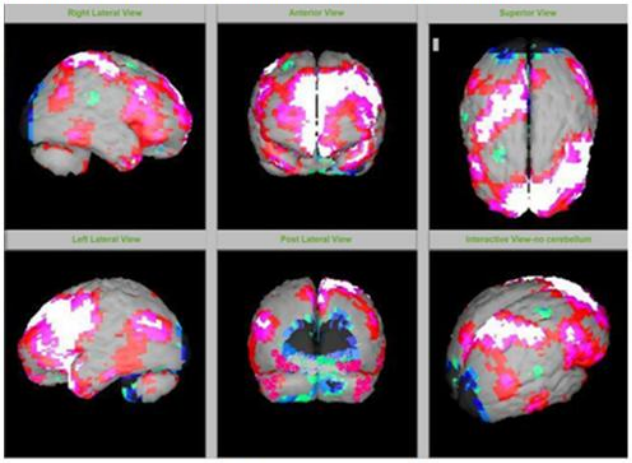
Table 2: SPECT scan and GMFCS scores in patients before and after hESC therapy.



Grey: Normal; red, pink, white: above normal (+2, +3, +4 of standard); Hypoperfused Regions: Green: -2 of standard; Light blue: -3 of standard; Dark blue: -4 of standard; black: -5 of standard



Grey: Normal; red, pink, white: above normal (+2, +3, +4 of standard); Hypoperfused Regions: Green: -2 of standard; Light blue: -3 of standard: Dark blue: -4 of standard: black: -5 of standard



Grey: Normal; red, pink, white: above normal (+2, +3, +4 of standard); Hypoperfused Regions: Green: -2 of standard; Light blue: -3 of standard: Dark blue: -4 of standard: black: -5 of standard

Figure 2: Improvement in perfusion of the brain after receiving hESC therapy.

No immunosuppressant was given to the patients. An immune response to hESCs was not observed in any patient included in the study. All patients were carefully examined frequently for any adverse events by in-house physicians and nurses. During the treatment, trained medical personnel including doctors and nurses monitored the patient. They examined the patient and also recorded the patient's statements. Specialists were consulted if the patient had any symptoms. Injection sites were regularly checked, vitals monitored and blood tests (CBC) were done to check for leukocyte count. Any untoward general/symptoms were also recorded and treated. There were relevant radiological screenings done (such as MRI SPECT) which recorded the efficacy and helped analyze the safety. The total period of hESC transplantation has been over 12 years and as such no untoward effects have been recorded.

Discussion

CVI is the visual loss which occurs due to disturbance in the posterior visual pathway or the visual cortex. Lack of oxygen to the brain is the most common reason for the occurrence of CVI. Apart from this, CVI may result due to intracranial pressure/hydrocephaly, brain malformations/head injury, central nervous system infections, poisoning/drug exposure, prematurity/birth trauma, cerebral palsy, or seizures/epilepsy [8]. Huoet al. reported that 25.9% of the children with CVI have CP [9]. Other studies have also reported most children with CVI have concomitant CP [10].

Damage to the anterior vision pathway, lateral geniculate bodies, or the occipital cortices in the brain among patients with CP may cause visual impairment [3]. Presently, there are no medications or surgical therapies available for the treatment of CVI except rehabilitation therapy [11]. In the present study, we attempted to evaluate the effectiveness of hESCs in the treatment of CVI in children with CP. Almost all the children (39 of 40) showed improvement in vision by atleast one grade. Of the 8 blind patients included in the present study, all showed improvement in vision (greater than 2) with only one patient having perception of light at the end of the study. More than half of the patients regained normal vision at the end of the study after receiving hESC therapy.

After being transplanted in the body, the hESCs start growing in the affected area to replace the degenerated cell type. In the present study, the newly differentiated cells replaced the damaged cells and improved perfusion was reflected in the SPECT scan [12]. The patients received hESC therapy with gap period between two treatment phases because hESCs require time to grow and regenerate the affected part to a maximum extent. In addition, the gap phase gives the physician an understanding of the patient's condition and requirement for further treatment. Stem cells have the potential to communicate with the other cells of the body from the area of damage and "home" at the site of injury. Chemokines, cytokines, and growth factors released from the site of injury communicate with the stem cells administered in the body. This process is initiated by the upregulation of selectins and integrins on the surface of the stem cells which enable their migration to the damaged site leading to regeneration [13]. Previous studies have shown that the route through which mesenchymal stem cells were administered influenced their potential to migrate and home at the site of injury [14]. In addition, studies investigating the potential of stem cells for degenerative disorders have shown that a gap period between two doses of stem cells is essential because the stem cells require time to start multiplying into the desired cell type [15]. Penhaet al. showed that a constant improvement was observed over a period of 3 months

after transplantation of MSCs in dogs with SCI and a remarkable improvement was seen 18 months after the transplantation MSCs [16]. Jensen et al. showed improved eye sight of a 5 yr old CP patient with cord blood stem cell therapy at 2 months [17]. Neural stem cells, hematopoietic stem cells and embryonic stem cells have shown potential to restore normal vision by repopulating the damaged areas and by preventing further degeneration of the cells [18-20].

Lack of oxygen supply to the brain is one of the leading causes of CVI in children with CP. SPECT scan is an effective tool in the detection of cerebral impairment, cerebral blood flow, and hypoperfused areas of the brain. SPECT scan detects the hypoperfused areas of the brain due to reduced tracer uptake in these regions. The extent of tracer uptake shows the extent of perfusion in those areas. The regions of the brain with decreased tracer uptake represent hypoperfused areas, those with absence of tracer uptake represent areas with no perfusion, and an increased tracer uptake represents hyperperfusion [21]. We used SPECT scan as a prognostic tool for detecting the extent of improvement in perfusion among the patients in this study. Brain SPECT scan is an effective monitoring tool for response to hESC therapy in patients with CP. It detects changes occurring at molecular level [22].

Sasmalet al. showed that 20.7% of the patients with CP have concomitant CVI and early ophthalmologic examination is necessary for optimal management [23]. Other prevalence studies have shown high proportion of patients (61.9%) with CP to be completely blind. Of the different types of visual impairments observed in patients with CP, 47.7% of them experience CVI [24]. Impairment of vision also results in the overall disability of the children with CP [25].

Treatment with hESC derived retinal pigment epithelium has shown improvement in vision on the electroretinogram in rats with retinal disease [5]. The use of hESCs has not been clinically viable in the past due to difficulty in harvesting these cells in a xeno free environment. However; we used a patented (United States Granted Patent No-WO 2007/141657A PCT/1B 2007 Published 13 Dec 2007)in-house methodology for the isolation of hESCs to explore their potential in the treatment of visually impaired patients with CP. Treatment of an 11-year old patient who had CP and visual impairment with bone marrow mesenchymal stem cells showed improvement in electrophysiologically examined vision after treatment for a period of 6 months [26]. The use of hESC therapy in the treatment of visual impairment in patients with CP has not been experimented extensively however; the use of hESCs in the treatment of ocular diseases of varying etiologies has shown improvement in vision [27-29]. Neural stem cells (NSCs) derived from hESCs preserve photoreceptors and visual function without formation of tumor cells [28]. Preclinical animal studies have demonstrated effective transplantation of NSCs in hypoxic-ischemic brain with cortical damage [30].

Neural stem cells are known to restore normal vision by repopulating the damaged areas and by preventing further degeneration of the cells [18,19]. The hESCs initiate regeneration of damaged cells by multiplying into similar cell type. Otaniet al. showed that hematopoietic stem cells restored retinal function and vascularization in mice with retinal degeneration disease [20]. Ameeleet al. showed that transplantation of embryonic stem cells in the brain results in corticogenesis and improvement in vision within a period of 6-8 days in a rodent model [12]. When the newly differentiated cells replaced the damaged cells, improved perfusion was observed in the present study which reflected in the SPECT scan. Jensen et al. showed improved eye sight of a 5 yr old CP patient with cord blood stem cell therapy at 2 months [17].

Vision plays a crucial role in development of gross and fine motor skills, visual-motor coordination, and walking capabilities in children. The development of prehension is lacking in visually impaired children due to lack of eye and hand or foot interlock [31]. As compared with cerebral palsy children having normal vision, the cerebral palsy children with vision impairment need a lot of assistance in their daily activities [32]. We analyzed the GMFCS E & R scores of our 23 patients in whom SPECT scan was available and observed that there was a remarkable improvement in their scores before and after improvement in vision. Though, improvement in GMFCS E & R scores might be multi-factorial but this aspect cannot be completely ruled out.

In conclusion, the use of hESC therapy in patients with CVI has shown beneficial results. Considering fewer therapeutic options for the treatment of CVI in children with CP, hESC therapy has provided hope to several patients with this condition. Although the results of the present study have shown the effectiveness of hESC therapy; further studies with larger sample size are a necessity for making this therapy available clinically.

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