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Case Study

Impact of Human Embryonic Stem Cells on the Clinical Syndrome and the Genome in Genetic Disorders: Case Series

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Abstract

Gene therapy is a technique for correcting defective genes responsible for disease development. The basic principle of gene therapy is the introduction of a normal, functional copy of a gene into a cell in which the defective gene is present. The problems in gene therapy that are necessary to overcome include the failure to transfer the appropriate gene into a target. The potential of rapid growth, to mature into multiple cell types *in vitro*, human embryonic stem cell (hESCs) has captured the imagination of scientists. hESCs might prove useful as a possible treatment for genetic disorder. The present study reported the safety and efficacy of hESC therapy in patients with genetic disorders. hESCs administered to the patients in the present study were obtained from a single, spare, expendable, pre-implantation stage fertilized ovum taken during a natural *in vitro* fertilization (IVF) cycle. Genomic integrity studies showed an improvement in the affected gene after the hESC therapy. All the patients showed improvement in their clinical condition also. No adverse events were reported in the patients. hESC therapy helped in stabilizing and improving the condition of patients both at the genotypic and phenotypic level.

Keywords: *Gene Therapy; Human Embryonic Stem Cells (hESCs); In Vitro Fertilization; Genetic Disorders; Immunogenicity*

Abbreviations

hESCs: Human Embryonic Stem Cells; IVF: *In Vitro* Fertilization; AEs: Adverse Events; IEC: Institutional Ethics Committee

Introduction

Gene therapy is a novel treatment technique which involves insertion, alteration, or removal of genes within an affected individual's cells to treat the disorder as the conventional drug compounds are not useful [1]. In gene therapy, DNA encoding a therapeutic protein is enveloped within a "vector", which transports the DNA inside the cells within the body. The cell machinery treats the disease by the expression of inserted DNA with least toxicity. Gene therapy could help in the treatment of genetic disorder, block neurological disorders, eliminate cancerous cells, and even eliminate infectious pathogens. Mainly, there are two types of gene therapy; i.e. somatic cell and germline gene therapy [2-4]. The major obstacles in gene therapy which need to be overcome include, the failure to transfer the appropriate gene into a target, non-germ-cell tissue, which further helps in treatment of disease [5].

Viruses are the commonly used vectors in gene therapy. After the manipulation of the viral genome, the therapeutic genes are introduced into the target organ. Though, viruses can cause various adverse events (AEs) such as toxicity, immune and inflammatory responses as well as gene control and targeting issues. The potential of the stem cells to replace and mature into multiple cells has raised a new hope in the treatment of genetic disorder. Stem cells are primitive cells that have the potential to differentiate into multiple mature cell types as well as they possess self-renewal capacity. Because of their ability of unlimited expansion and pluripotency, they are useful in regenerative medicine. The stem cell treatment replaces all the defective cells in different tissues [6,7]. Due to the potential of rapid growth, ability to mature into multiple cell types *in vitro* and the remarkable stability of hESCs has captured the imagination of scientists [7]. These hESCs might prove as potential tool for the treatment of genetic disorder. Darabi., *et al.* reported the therapeutic efficacy of embryonic stem (ES) cells in the treatment of autosomal dominant forms of muscular dystrophy. They transplanted the Pax3-induced ES-derived myogenic progenitors into Frog1 mice [8].

The previously published studies of patients with genetic disorder such as Down's syndrome and Duchenne muscular dystrophy (DMD) have showed remarkable improvement after hESC therapy [9,10]. The present study aimed at reporting the use of hESCs in patients affected with different genetic disorders.

Case Report

The study included patients (2 to 65 years) with genetic disorders who either approached our facility or were referred by other hospitals. A written and video informed consent was obtained from the patients prior to the start of the treatment. The study was approved by independent Institutional Ethics Committee (IEC). All the patients were investigated with standard diagnostic procedures for their disorders.

Process of Microarray Analysis of DNA of Patients with Genetic Disorders Treated with hESC Therapy

Cell culturing

The hESCs administered to the patients in the present study were obtained from a single, spare, expendable, pre-implantation stage fertilized ovum obtained during natural *in vitro* fertilization (IVF) process with due consent. The cell lines were cultured and maintained as per our patented in-house technology (United States Granted Patent No US 8592, 208, 52) in the Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) and Good Tissue Practice (GTP) compliant laboratory. The composition, methods of preparation and methods of using hESCs have been presented elsewhere [11]. The safety of our hESC cell line has also been assessed [12].

DNA Isolation

DNA samples were isolated from different blood culture and different patient's blood using Qiagen DNA mini Kit Cat. No. – 51304 (QIAGEN Ltd.-UK) as per the standard protocol given.

Genomic integrity

The process of maintaining genomic integrity consisted of four steps as follows:

Digestion

Firstly, 10x Restriction Enzyme Buffer and Bovine Serum Albumin (BSA) (included in the SureTag Complete DNA Labeling Kit and the SureTag DNA Labeling Kit) were thawed between the palms of the hand. The tube was then flicked to briefly mix the contents and spun in a microcentrifuge at 2400 rpm. Following to this, the digestion master mix was prepared by mixing the components (by pipetting up and down) in ice, based on the microarray format (Table 1). Thereafter, 5.8 µL (for 4-pack microarrays) of digestion master mix was added to each reaction tube containing the gDNA to make a total volume of 26 µL (4-pack microarrays) and mixed well by pipetting up and down. Lastly, the sample tubes were transferred to a thermal cycler and ran at 37°C for 2 hours, 65°C for 20 minutes and held at 4°C.

Components	Per reaction (µL)	X 16 rxns (µL)	X 48 rxns (µL)	X 96 rxns (µL)
Nuclease-free water	2.0	34	100	200
10x restriction Enzyme Buffer	2.6	44.2	130	260
BSA	0.2	3.4	10	20
Alu 1	0.5	8.5	25	50
Rsa 1	0.5	8.5	25	50
Final volume of digestion master mix	5.8	98.6	290	580

Table 1: *Digestion Master Mix.*

Sample labeling

After the digestion, the samples were spun in a centrifuge for 1 minute at 6,000 × g to drive the contents off the walls and lid. Random Primer (5 µL) was added of to each reaction tube (for 4-pack microarrays), containing 26 µL of gDNA to make a total volume of 31 µL. The contents were mixed well by pipetting up and down gently. The sample tubes were transferred to a thermal cycler and ran the program (98°C for 3 minutes and held at 4°C). For 1-pack, 2-pack and 4-pack microarrays:

- Mixed the components in ice to prepare one cyanine-3 and one cyanine-5 (Table 2).
- Added 19 µL (or 21 µL) of Labeling Master Mix to each reaction tube containing the gDNA to make a total volume of 50 µL. Mixed well by gently pipetting up and down.
- Transferred sample tubes to a thermal cycler. Programmed the thermal cycler (37°C for 2 hours, 65°C for 10 minutes and held at 4°C) and ran the program.

Component	Per reaction (µL)	x 8 rxns (µL) (including excess)	x 24 rxns (µL) (including excess)	x 48 rxns (µL) (including excess)
Nuclease-Free Water	2.0	17	50	100
5x Reaction Buffer	10.0	85	250	500
10x dNTPs	5.0	42.5	125	250
Cyanine 3-dUTP or Cyanine 5-dUTP	3.0	25.5	75	150
Exo (-) Klenow	1.0	8.5	25	50
Final volume of Labeling Master Mix	21.0	178.5	525	1050

Table 2: *Labeling Master Mix.*

Purification of labeled gDNA

- Spun the labeled gDNA samples in a centrifuge for 1 minute at 6,000 × g to drive the contents off the walls and lid. Added 430 µL of 1 × Tris-EDTA (TE) (pH 8.0) to each reaction tube. For each gDNA sample to be purified, placed a column into a 2-mL collection tube and labeled the column appropriately. Loaded each labeled gDNA on to a column and covered the column with a cap and spun for 10 minutes at 14,000 × g in a microcentrifuge at room temperature. Discarded the flow-through and placed the column back in the 2 mL collection tube.
- Added 480 µL of 1×TE (pH 8.0) to each column and spun them for 10 minutes at 14,000 × g in a microcentrifuge at room temperature.

- Discarded the flow-through and inverted the column into a fresh 2-mL collection tube that was appropriately labeled. Spun for 1 minute at 1,000 × g in a microcentrifuge at room temperature to collect the purified sample. Approximately 20 to 32 µL of volume per sample was obtained.
- In a fresh 200 µL thin-walled tube, combined test and reference sample using the appropriate cyanine-5-labeled sample and cyanine-3-labeled sample volume 21 µL and 39 µL, respectively.
- The 1.5 mL RNase-free microfuge tube was used as container. The labeled DNA can be stored up to one month at -20°C in the dark using this method.

Microarray Processing

Microarray processing was further divided into three steps, which included: Hybridization, Microarray Wash and Microarray Scanning and Analysis.

Hybridization

Preparation of the 10x Blocking Agent

To prepare blocking agent, added 1,350 µL of DNase/RNase-free distilled water to the vial containing lyophilized 10 x aCGH Blocking Agent. This was left at room temperature for 60 minutes and mixed on a vortex mixer to reconstitute sample before use or storage.

Preparation of Labeled gDNA for Hybridization

Equilibrated water baths or heat blocks to 98°C and 37°C or use a thermal cycler. To prepare the Hybridization Master Mix the components were mixed according to the microarray format (Table 3).

Component	Volume (µL) per hybridization	x 8 rxns (µL) (including excess)	x 24 rxns (µL) (including excess)	x 48 rxns (µL) (including excess)
Cot-1 DNA (1.0 mg/mL) *	5	42.5	125	250
10x aCGH Blocking Agent	11	93.5	275	550
2x HI-RPM Hybridization Buffer	55	467.5	1375	2750
Final Volume of Hybridization Master Mix	71	603.5	1775	3550

Table 3: *Hybridization Master Mix for 4-pack microarray.*

Added the appropriate volume (71 µL) of the Hybridization Master Mix to the 1.5 mL RNase-free microfuge tube that contained the labeled gDNA to make a total volume of 110 µL.

Mixed the sample by pipetting up and down. The sample was quickly spun in a centrifuge to drive contents to the bottom of the reaction tube. Transferred the sample tubes to a thermal cycler and ran the program (98°C for 3 minutes and 37°C for 30 minutes).

Removed the sample tubes from the water bath, heat block, or thermal cycler and spin for 1 minute at 6000 × g in a centrifuge to collect the sample at the bottom of the tube. The samples were hybridized immediately.

Preparation of hybridization assembly

A clean gasket slide was loaded into the Agilent SureHyb chamber base with the gasket label facing up and aligned with the rectangular section of the chamber base. 100 µL (for 4-pack microarray) of the hybridization sample mixture was slowly dispensed onto the gasket well in a "drag and dispense" manner. A microarray slide "active side" was put down onto the gasket slide in a manner that the numeric barcode side was facing up and the "Agilent"-labeled barcode was facing down.

The SureHyb chamber cover was put onto the sandwiched slides and slides the clamp assembly onto both pieces. The assembled chamber was vertically rotated to wet the slides and assess the mobility of the bubbles. The assembly was tapped on a hard surface if necessary to move stationary bubbles.

Hybridization

Each assembled chamber was loaded into the oven rotator rack starting from the center of the rack (position 3 or 4 when counting from the left) and rotated at 20 rpm. The hybridization was done at 67°C for 24 hours (4-pack and 8-pack microarrays) and 40 hours (1-pack and 2-pack microarrays).

Microarray Wash

Microarray wash was done as described in the Agilent Manual. Briefly, Agilent Oligo aCGH/ChIP-on-Chip Wash Buffer 2 was pre-warmed overnight. Then, washed with Milli-Q ultrapure water and cleaned with Acetonitrile (Wash Procedure B Only). Stabilization and Drying Solution were also pre-warmed (Wash Procedure B Only).

Microarray Screening and Analysis

Microarray screening was done as described in the Agilent manual. Firstly, the microarray slides were scanned. Then, the microarray images were analyzed. Both the *in vivo* and *in vitro* tests have also been done.

In vivo analysis

For *in vivo* analysis, 1 ml of patient blood was collected, and DNA was isolated for microarray analysis after hESC treatment.

In vitro analysis

For *in vitro* analysis, 1 ml patient blood was cultured with hESCs in a 50 ml flask. After culturing, DNA was isolated from the mixture and microarray analysis was performed.

Dosage Schedule

All the patients were assessed for hypersensitivity reaction to hESC by a subcutaneous (*s.c.*) test injection of 0.25 mL hESC. Following the hypersensitivity evaluation, all the patients underwent one treatment session of hESC therapy, following an already established treatment protocol [11]. All the patients were transplanted with hESCs daily to help cells reach the affected area for repair and regeneration. Patients were observed regularly for the occurrence of any side effect by the in-house physicians and nurses during the therapy.

Patient 1

A female patient aged 59 years was admitted to our facility on 31 January 2006 with complaints of severe back pain for 2 years, severe stiffness in back and inability to roll over on bed due to severe stiffness in back. The patient history revealed that she was diagnosed with ankylosing spondylitis having mutation in human leukocyte antigen (HLA)-B gene on short (p) arm of chr6 at 21.3 location (Chr 6p21.3). HLA-B is part of gene family known as HLA complex. This complex helps the immune system differentiate the body's own proteins from proteins made by foreign invaders such as viruses and bacteria. A variation in HLA-B gene increases the risk of developing ankylosing spondylitis [13].

The patient underwent hESC therapy at our facility. After the hESC therapy, the patient was discharged with satisfactory condition with an improvement in back pain, muscle stiffness of back and can easily roll over on bed with no resistance. She was pain free, able to walk easily, could bend forward and was fine clinically. After *in vivo* microarray analysis, it was observed that Chr 6p21.3 had reverted to normal. No mutation was observed (Table 4).

Patient 2

A female patient aged 16 years was admitted to our facility on 16 November 2015 with complaints of delayed growth, subnormal understanding, mood swings, aggressive behavior, lack of social interaction, short attention span, delayed and slurred speech, and limping gait. The patient was diagnosed as a case of Down syndrome with hypothyroidism. The chromosomal analysis revealed Trisomy 21 in the patient. After undergoing hESC therapy, the patient is more social, has started talking to people around her. Her mood swings have improved, and she has started interacting. Her memory was better and continually improving. Both *in vivo* and *in vitro* followed by *in vivo* microarray analysis showed lesser amplification in chromosome 21 after the treatment as shown in table 4 and 6.

Patient 3

A male patient aged 16 years was admitted to our facility on 26 April 2010 with complaints of muscle weakness in lower limbs (LLs) and upper limbs (ULs), cannot independently stand and walk without wheel chair, weakness in back, and slurring of speech. The laboratory examination at our facility found that patient had DMD gene mutation on short (p) arm of chrX at p21.1 location (chrXp21.1) of about 15 kb having Creatine phosphokinase (CPK) level 512 IU/L. The patient was diagnosed with DMD. Following the therapy, the patient was able to move his fingers and hands to a larger degree than before, stiffness in bilateral LL has decreased, is able to take large steps with support, body balance has improved and speech has improved and is clear. After *in vivo* and *in vitro* microarray analysis, no mutation was observed in the previously affected gene (Table 4 and 5). The CPK level decreased to 31 IU/L which showed improvement in patient.

Patient 4

A female patient aged 65 years was admitted to our facility on 30 December 2003 with complaints of difficulty in respiration, slurred speech, fine motor skills impaired, decreased power in limbs, restricted movement in limbs, wheelchair bound and decreased level of response. The patient was diagnosed with Olivopontocerebellar atrophy (OPCA). After the laboratory examination, it was found that patient had a deletion of ATXN1 gene at short arm of chromosome 6 on location 22.3 (Chr 6p22.3). The ATXN1 gene helps in the production of a protein called ataxin-1 that is found throughout the body [14].

Following the therapy, the patient has improved respiration, motor function of limbs and no further deterioration noted. The *in vivo* micro array analysis revealed normal genetic constitution after the treatment (Table 4).

Patient No.	Disease name	Cytogenetic location (OMIM)	Genomic study of patient blood			Genomic study of patient blood after treatment			Remarks
			Bp Size (kb)	Probes	A/G/L/D	Bp Size (kb)	Probes	A/G/L/D	
1	Ankylosing Spondylitis	Chr 6p21.1	Detected abnormality			Abnormality not detected			Chromosome 6p21.1 was normal, no mutation was observed.
2	Down Syndrome Trisomy 21	q11.2 - q22.3	32, 581	1346	0.6693299	Detected abnormality			The amplification was observed very low in Chr21.
3	DMD	Chr Xp21.1	15	3	-3.803253	Abnormality not detected			The mutation in DMD gene was not present.
4	Olivopontocerebellar atrophy	Chr 6p22.3	Detected abnormality			Abnormality not detected			The Chr 6p22.3 was normal in report.
7	FSHD	Chr 4q35	Detected abnormality			Abnormality not detected			No mutation was present on FSHD gene (DUX4 was not present).

Table 4: *Blood from patient before and after treatment (in vivo).*

Patient 5

A male patient aged 2.5 years was admitted to our facility on 15 September 2015 with complaints of inability to sit, walk and stand without support, had involuntary jerky movements, decreased muscle tone, impaired speech, and multiple episodes of seizures. The laboratory examination at our facility found that patient had deletion of FOX G1 genes on its nearby location at long (q) arm of chr14 at11.2. The single-photon emission computed tomography (SPECT) scan on 15 September 2015 showed moderate hypoperfusion in bilateral (b/l) parieto occipital region. Severe hypoperfusion was reported in b/l fronto temporal region and b/l cerebellar region. After examination, it was observed that deletion of gene at chr14q11.2 was the main cause of all the associated problems in the patient.

After the treatment, the patient could balance his trunk and neck for few seconds with support and was able to sit without support for 3 - 4 seconds. The SPECT scan on 5 November 2015 after therapy showed mild to moderate hypoperfusion in b/l temporal and b/l cerebellar region. A significant improvement (> 60%) was observed in degree of hypoperfusion seen in the cerebellar region (Figure 1). The *in vitro* microarray analysis revealed that the deleted genes were not present on affected location after the treatment (Table 5).

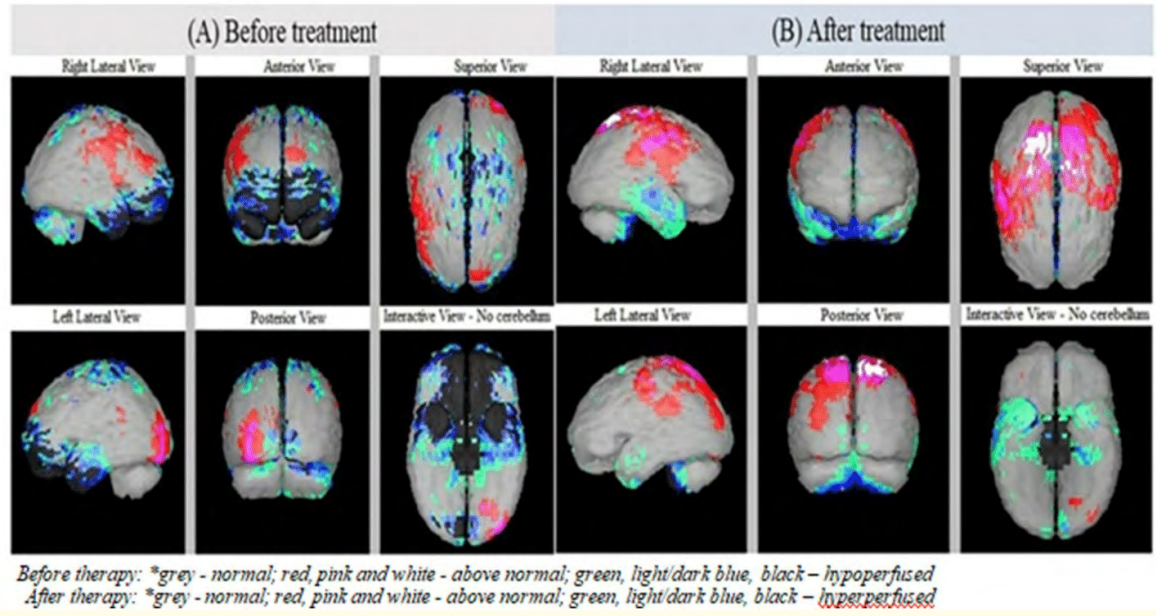

Figure 1: *SPECT Scan of Patient number (5) before and after therapy.*

Patient 6

A 2-year male patient was admitted to our facility on 28 July 2015 with developmental delay, facial dysmorphia, speech and communication issues, vision problem, and poor feeding. After the laboratory examination at our facility, it was found that patient was suffering from Williams's syndrome having deletion of several genes (CLIP2, ELN, GTF2I, GTF2IRD1, and LIMK1) at chromosome 7 on long arm (q) at location 11.23. Studies suggested that deletion of CLIP2, GTF2I, GTF2IRD1, and LIMK1 leads to characteristic difficulties with visual-spatial tasks, changes in behavioral characteristics, and other cognitive difficulties in people with Williams syndrome [15].

After undergoing hESC therapy, the patient initiated showing interest in his surroundings and increase in curiosity, had better understanding, feeding is better, growth has improved, child has started walking and has increase in activity. The *in vitro* microarray analysis showed the Chr7q11.2 to be normal after the therapy (Table 5).

Patient No.	Disease name	Cytogenetic location (OMIM)	Genomic study of patient blood			Genomic study of patient blood after treatment			Remarks
			Bp Size (kb)	Probes	A/G/L/D	Bp Size (kb)	Probes	A/G/L/D	
3	DMD	Chr Xp21.1	2268.773	134	1.063	Abnormality not detected			The mutation in DMD gene was not present.
5	FOXG1 deletion	Chr14q11.2	596	21	-0.489077	Abnormality not detected			The deletion of Fox G1 gene was not present.
6	William Syndrome	Chr7q11.23	1612	97	0.896697	Abnormality not detected			The Chromosome 7q11.23 was normal in the report.

Table 5: *Blood from untreated patient cultured with hESC (in vitro).*

Patient 7

A male patient aged 24 years was admitted to our facility on 19 December 2013 with complaints of difficulty in getting up from sitting position, unable to lift arms and forearm above shoulder levels, weakness of facial muscles and loss of muscle mass and winging of scapula. The patient was diagnosed with Facioscapulo humeral muscular dystrophy (FSHD) at Ganga Ram Hospital, Rajinder Nagar, New Delhi, having mutation of DUX4 gene in long (q) arm of chromosome 4 at location 35 (Chr 4q35). The active DUX4 gene influences the activity of other genes. The changes in the activity DUX4 gene damages or destroys the cells, leading to progressive muscle weakness and atrophy [16].

After the hESC therapy, the patient had significant increase in muscle mass, facial expressions have improved, is able to lift both arms above shoulders and overall strength has increased significantly. The *in vivo* microarray analysis did not show mutation of DUX4 gene at Chr4q35 (Table 4).

Patient 8

A 27-year-old male patient was admitted to our facility on 16 January 2016 with complaints of difficulty in getting up from sitting position, more weakness in LLs as compared to ULs, slurring of speech and gait disturbance. The patient was diagnosed with hereditary spastic paraplegia 8 years back. After laboratory examination at our facility, it was found that patient had deletion of interferon regulatory factor-4 (IRF4) at short arm (p) of Chr 6 at location 22.3 (Chr 6p22.3). The patient was diagnosed as a case of cerebellar ataxia. The SPECT scan showed moderate to severe hypoperfusion in b/l temporal regions left > right. Mild hypoperfusion was noted in cerebellar region. Electromyography (EMG) showed neurogenic changes in tested muscles.

Following the therapy, patient showed decreased spasticity in LLs, no more tremors in b/l hands, lower back pain was relieved, strength in both LLs had increased, walking with crutches has improved, speech has improved and getting up from bed has become easier. SPECT scan showed significant improvement (>60%) in degree of cerebral and cerebellar perfusion (Figure 2). The *in vitro* microarray analysis followed by *in vivo* analysis revealed that gene deletion was not present at the affected location (Table 6).

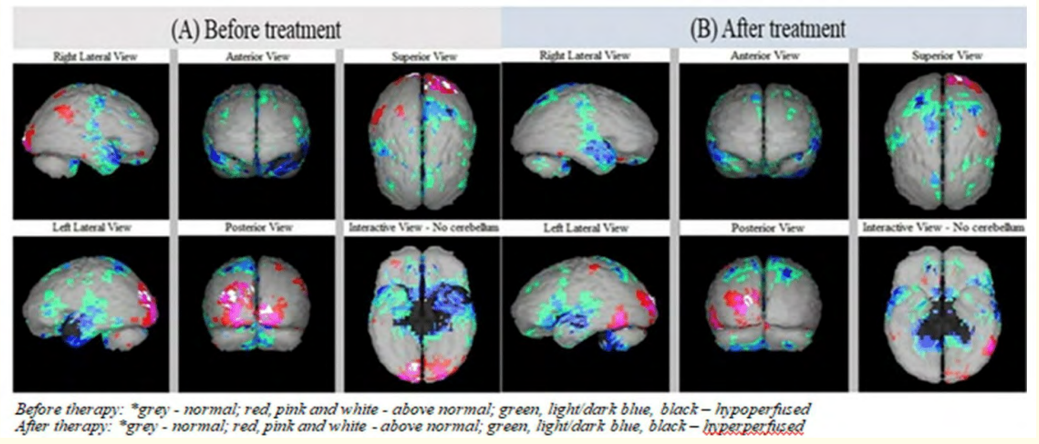

Figure 2: *SPECT Scan of Patient number (8) before and after therapy.*

Patient No.	Disease Name	Cytogenetic Location (OMIM)	Genomic study of patient blood			Genomic study of patient blood after treatment					Remarks
				Genomic study after growing blood with hESC			Genomic study of patient after treatment				
			Bp size (kb)	Probes	A/G/L/D	Bp size (kb)	Probes	A/G/L/D	Bp size (kb)	Probes	A/G/L/D	
2	Down Syndrome Trisomy 21	Chr21q11.2 - q22.3	33,434	1582	-0.592700	33,365	1427	-0.269656	No abnormality detected			No mutation has been seen on Chr21 in "*in vivo*" report. In "*in vitro*" report, the deletion in Chr21 was very less.
8	Cerebellar Ataxia	Chr 6p22.3	277	33	-0.370104	No abnormality detected			No abnormality detected			No mutation has been seen in both reports "*in vivo* as well as *invitro*".

Table 6: *In vitro followed by in vivo*

Discussion

Gene therapy is a practice for correcting defective genes that are responsible for disease development [1]. It introduces a normal, functional copy of a gene into a cell in which that gene is defective. It is associated with various issues such as gene identification, gene expression and gene delivery [17]. Currently, stem cells are being explored by scientists in regenerative medicine for treating various diseases due to certain characteristics, primarily an ability for long term self-renewal and the capacity to differentiate into multiple cell lineages [18,19]. These cells are responsible for the development and maintenance of tissues and organs and differentiate in response to a combination of biochemical signals and biomechanical stimuli provided by the stem cell niche [20]. At the injured area, stem cells produce different trophic factors and reduce the cell loss, promote host regeneration and restore the function [21]. Recently, the combination of stem cell therapy with gene therapy has emerged as a hopeful approach for the treatment of disease [22].

Bank., *et al*. reported the efficacy of hematopoietic stem cell (HSC) gene therapy in the treatment of various human hematopoietic diseases, such as sickle cell disease [23]. Fischer (2003), reported that HSC have potential to treat all genetically determined blood diseases by relevant gene transfer into HSC [24].

The successful isolation, ability to proliferate and differentiate into all tissue cell types has raised the hope that hESCs may provide a universal tissue source to treat many human diseases [25]. Berberi., *et al*. reported that hESC may be indirectly beneficial for cellular gene therapy. They observed that hESC-derived stem cells may be utilized in genetic manipulation techniques [26].

Bak., *et al*. reported when hESC-derived mesenchymal stem cells (MSCs) coupled with the herpes simplex virus thymidine kinase (HS-Vtk)/ganciclovir prodrug cancer gene therapy system showed a killing effect [27].

Studies till now focused on transfer of gene through stem cell to the targeted tissue. But, in the present study hESCs were directly transplanted to the patients. These hESCs produce various therapeutic proteins or large number of normal cells. The hESCs can be delivered to accessible and receptive site for transplantation, even if the tissue is not the normal site of production of the protein of interest. Such proteins show their effect only at appropriate site. In genetic disorder, when the genes are mutated or deleted from the particular location of the chromosome, the hESCs with their regulated genetic characteristics differentiate to the normal cell type and correct the affected location [28]. The present study reported the use of in-house cultured hESC therapy in the treatment of patients with different genetic conditions (ankylosing spondylitis, Down syndrome, DMD, OPCA, FOX G1 deletion, FSHD, cerebellar Ataxia, and William syndrome). The blood of the patients was first cultured *in vitro* with hESCs and then the DNA was taken of the mixture. The genomic integrity studies showed an improvement in the affected gene. In few cases the patients' blood was taken both before and after the therapy and then studied. It was observed that the affected genes were corrected in the *in vivo* sample. A few patients also got their *in vitro* and then *in vivo* tests done with correction seen in the genetic studies in both. Most importantly, these patients also showed an improvement in their clinical condition. No AEs were reported during and after the study. Various studies have also reported the gene expression profiling of differentiated hESCs-derived embryoid bodies. When hESCs are differentiated, a set of genes are up-regulated and 'stemness' genes are down regulated [29].

Jiang., *et al*. used patient derived stem cell to silence the extra copy of chromosome 21 in Down syndrome. They reported that the RNA gene X-inactive specific transcript (XIST) present on X chromosome is responsible for turning off one of the two X chromosomes found in women. The XIST gene produces a large piece of RNA that coats X chromosomes and contracts it into an inaccessible bundle. It can be used to shut down chromosome 21 in trisomic cells [30]. Different studies have reported that XIST accumulates around every X chromosome in human embryos of both male and female [31]. In the present study, hESCs might have activated the genes present on the affected chromosome(s), which might have corrected the chromosomal defects present in the patients.

Conclusion

The use of hESC therapy was found to be safe and effective clinically. The patients showed improvement in their condition after the treatment. hESC therapy was well tolerated among all the patients and no one experienced serious AEs during the study. hESC therapy also corrected the genomic aberrations in the patients. However, future clinical studies with large number of patients are needed to collect evidences supporting the use of hESCs in the treatment of genetic disorders.

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Conflict of Interest

The authors declare no competing interest associated with the publication of this manuscript.

Bibliography

1. Patil PM., *et al*. "Review article on gene therapy". *International Journal of Genetics* 4.1 (2012): 74-79.
2. "Gene Therapy" (2016).
3. "Third World Network [Internet]. Human gene therapy: A cure for all ills?" (2016).
4. Mountain A. "Gene therapy: the first decade". *Trends in Biotechnology* 18.3 (2000): 119-128.
5. Kay MA., *et al*. "Gene therapy". *Proceedings of the National Academy of Sciences of the United States* 94.24 (1997): 12744-12746.
6. Alenzi FQ., *et al*. "Review: Stem cells and gene therapy". *Laboratory Hematology* 16.3 (2010): 53-73.
7. Rideout WM., *et al*. "Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy". *Cell* 109.1 (2002): 17-27.
8. Darabi R., *et al*. "Engraftment of embryonic stem cell-derived myogenic progenitors in a dominant model of muscular dystrophy". *Experimental Neurology* 220.1 (2009): 212-216.
9. Shroff G. "Human embryonic stem cells in the treatment of patients with Duchenne muscular dystrophy: a case series". *Journal of Neurology Research* 5.1-2 (2015): 186-191.
10. Shroff G. "Human embryonic stem cells in the treatment of patients with down syndrome: a case report". *Journal of Medical Cases* 7.4 (2016): 123-125.
11. Shroff G. "Establishment and characterization of a neuronal cell line derived from a 2-cell stage human embryo: Clinically tested cell-based therapy for neurological disorders". *International Journal of Recent Scientific Research* 6.4 (2015): 3730-3738.
12. Shroff G and Barthakur JK. "Safety of human embryonic stem cells in patients with terminal/incurable conditions- a retrospective analysis". *Annals of Neurosciences* 22.3 (2015): 132-138.
13. "Ankylosing spondylitis" (2016).
14. "U.S. National Library of Medicine [Internet]. ATXN1 gene" (2016).

15. "U.S. National Library of Medicine. "Williams syndrome" (2016).

16. U.S. National Library of Medicine [Internet]. "Facioscapulohumeral muscular dystrophy" (2016).

17. Kumar A., *et al*. "Gene therapy: an updated review". *European Journal of Biotechnology and Bioscience* 1.3 (2013): 42-53.

18. "Gene therapy: introduction and methods".

19. Shroff G., *et al*. "Therapeutic potential of human embryonic stem cell transplantation in patients with cerebral palsy". *Journal of Translational Medicine* 12 (2014): 318.

20. Price FD., *et al*. "Stem cell-based therapies to treat muscular dystrophy". *Biochimica et Biophysica Acta* 1772.2 (2007): 272-283.

21. Baraniak PR and McDevitt TC. "Stem cell paracrine actions and tissue regeneration". *Regenerative Medicine* 5.1 (2010): 121-143.

22. Mahdi E. "Application of Stem Cells in Disease and Gene Therapy". *Der Pharmacia Lettre* 4 (2012): 734-740.

23. Bank A. "Hematopoietic stem cell gene therapy: selecting only the best". *Journal of Clinical Investigation* 112.10 (2003): 1478-1480.

24. Fischer A. "Hematopoietic stem cell gene therapy". Stem Cell Technology and Other Innovative Therapies (2003): 127-133.

25. Yabut O and Bernstein HS. "The promise of human embryonic stem cells in aging-associated diseases". *Aging (Albany NY)* 3.5 (2011): 494-508.

26. Barberi T., *et al*. "Derivation of multipotent mesenchymal precursors from human embryonic stem cells". *PLoS Medicine* 2.6 (2005): e161.

27. Bak XY., *et al*. "Human embryonic stem cell-derived mesenchymal stem cells as cellular delivery vehicles for prodrug gene therapy of glioblastoma". *Human Gene Therapy* 22.11 (2011): 1365-1377.

28. Meregalli M., *et al*. "Chapter 17- Stem cell therapy for neuromuscular diseases. In: Gholamrezanezhad A, editor". *Stem Cells in Clinic and Research* (2011).

29. Bhattacharya B., *et al*. "A review of gene expression profiling of human embryonic stem cell lines and their differentiated progeny". *Current Stem Cell Research* 4.2 (2009): 98-106.

30. Jiang J., *et al*. "Translating dosage compensation to trisomy 21". *Nature* 500.7462 (2013): 296-300.

31. Sloka JS and Stefanelli M. "The mechanism of action of methylprednisolone in the treatment of multiple sclerosis". *Multiple Sclerosis Journal* 11.4 (2005): 425-432.

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