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Intrathecal Infusion of Umbilical Cord Derived Allogeneic Mesenchymal Cells for the Treatment of Refractory Epilepsy in a Patient with Genetic Proved Cerebrotendinous Xanthomatosis: a Case Report

Alireza Rezayi[1], Bita Shalbafan[2]*, Fatemeh Khodaei[3], Vahideh Nasr[4] and Mandana Mohyeddin Bonab[5]

[1]*Child neurology, clinical neurophysiology and epilepsy, Skull Base Research Center, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Iran*

[2]*Consultant Neurologist, Clinical Research Development Unit, Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Iran*

[3]*Master of Midwifery, Skull Base Research Center, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Iran*

[4]*General practitioner, Labbafinejad Hospital, Clinical Research Development Unit, Shahid Beheshti University of Medical Sciences, Iran*

[5]*Immunogenetics Research Centre, Department of Immunology, Tehran University of Medical Sciences, , Iran*

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***Corresponding author:** Bita Shalbafan, Consultant Neurologist, Labbafinejad Hospital, Clinical Research Development Unit, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Cerebrotendinous Xanthomatosis (CTX) is a rare autosomal-recessive metabolic disorder caused by mutations in the CYP27A1 gene. Mutation of this gene leads to decreased synthesis of the mitochondrial enzyme sterol 27-hydroxylase, which is involved in bile acid synthesis. It causes excess production of cholestanol and consequent accumulation of cholestanol in tissues. CTX presents with neurological and non-neurological signs and symptoms. We report a 23 years old man with CTX and refractory myoclonic epilepsy and a family history of CTX. He had generalized epilepsy with a prominent myoclonic feature. He also had progressive ataxia, bulbar and pseudobulbar palsy, feeding and swallowing disorders, and cataract. Brain MRI showed dentate hyperintensity with cerebellar atrophy. Intrathecal injection of Umbilical Cord Allogenic Mesenchymal Stem Cells (UC-MSC) collected through Umbilical cord followed by enzymatic isolation and laboratory cellular culture was performed. Neurological status, cognitive function, antiepileptic effect, and brain MRI were monitored for 12 months. Immediately after the procedure, the patient had a good condition but laboratory analysis revealed leukocytosis without fever or increasing in Erythrocyte Sedimentation Rate (ESR). During the next months, some improvements in swallowing problem, manual performance, and cognitive functioning were observed. Interestingly, no sign of seizure was observed during the first-year follow-up of the patient.

Keywords: Intrathecal Infusion; Wharton's jelly; Umbilical Cord Allogenic Mesenchymal Stem Cells (UC-MSC), Refractory Epilepsy; Cerebrotendinous Xanthomatosis (CTX), CYP27A1 gen

Introduction

Cerebrotendinous Xanthomatosis (CTX) is a rare autosomal recessive inborn error of metabolism caused by mutations in the CYP27A1 gene (OMIM *606530), which lead to the accumulation of increased deposition of cholesterol and cholestanol in multiple tissues. Since the first report in 1937 of a case of CTX, more than several hundred cases have been reported [1,2,3]. This condition is characterized by tendon xanthomas, refractory diarrhea, juvenile cataracts, and progressive neurological dysfunctions, including Intellectual Disability (ID) or dementia, neuropsychiatric symptoms, pyramidal signs, cerebellar ataxia, peripheral neuropathy, extrapyramidal manifestations, learning difficulties, behavioral changes, and refractory seizures [4,5]. Clinical symptoms of CTX have been reported to improve by treatment with chenodeoxycholic acid (CDCA) [6]. Here we report a patient with CTX presenting with refractory myoclonic epilepsy as an initial manifestation in which seizures did not respond to

anti-epileptic drugs (AEDs) and CDCA therapy. The goal of our project was to evaluate effects of MSC therapy on the neurological outcome, including its safety and efficacy in patients with CTX. We expected to improve the cognitive, gate function, and other neurological manifestations of the patient by UC-MSC therapy, but seizure control was the main outcome.

Clinical presentation

A 23-year-old Iranian man born from a consanguineous marriage was referred to our epilepsy center with a diagnosis of drug-resistant epilepsy and progressive ataxia. He had good mental ability and he could achieve high school academic performance. At the age of 10, he had a car crash. Myoclonic seizures have been started at the age of 17 and were intractable to multiple AEDs treatment. Ataxia and gait abnormalities followed by Carbamazepine therapy but after ceasing the Carbamazepine, ataxia had not been improved and he had experienced progressive ataxia. Bilateral Cataracts were removed by surgery at the age of 17. First Neurological evaluation in our clinic at the age of 21, the patient had bulbar palsy (dysphagia, nasal speech, dysphonia), pseudobulbar palsy (uncontrollable laughing and facial movements), dysarthria and dysmetria, spastic paraparesis, hyperactive deep tendon reflexes with clonus and bilateral Babinski sign. Figure1 demonstrates the pathological forced laughter in the patient's face. Brain MRI showed Abnormal signals as hyperintensity in T2 sequences and hypointensity of dentate nuclei in FLAIR and T1 sequences (Figure 2). There were also abnormal signals of bilateral white matter. Plasma cholestanol concentration was increased (2,43 mg/dl; n.v.< 1 mg/dl). The patient had a suspicious index of 275 due to diagnostic indicators described by Mignarri et al, (consanguineous parents, juvenile cataract, spastic paraparesis, dentate nuclei signal alterations, intellectual disability, and epilepsy) [7]. Sequencing the 8 exons of the CYP27A1 gene revealed the presence of unreported homozygous variation change, c.1263+4A>T in intron 7 (Figure 3), corresponding to a novel splice site mutation that presumably results in the skipping of exon 7, p.(R395fs). There were also the same results of molecular genetics study in other affected families (Figure 4). With the diagnosis of CTX disease, the patient was subjected to stem cell therapy. After obtaining the informed consent, Allogeneic UC-MSC was discussed with the transplant team. This procedure was approved by the Pajouhan Research information system, Shahid Beheshti University of Medical sciences. The patient was introduced to the Sina Cell stem cell group after performing the virological tests, HIV, HCV, HBV, HTLV by real-time PCR, and tumor examination included abdominal and pelvic and with the answer of routine biochemistry, hematology, and serology tests.

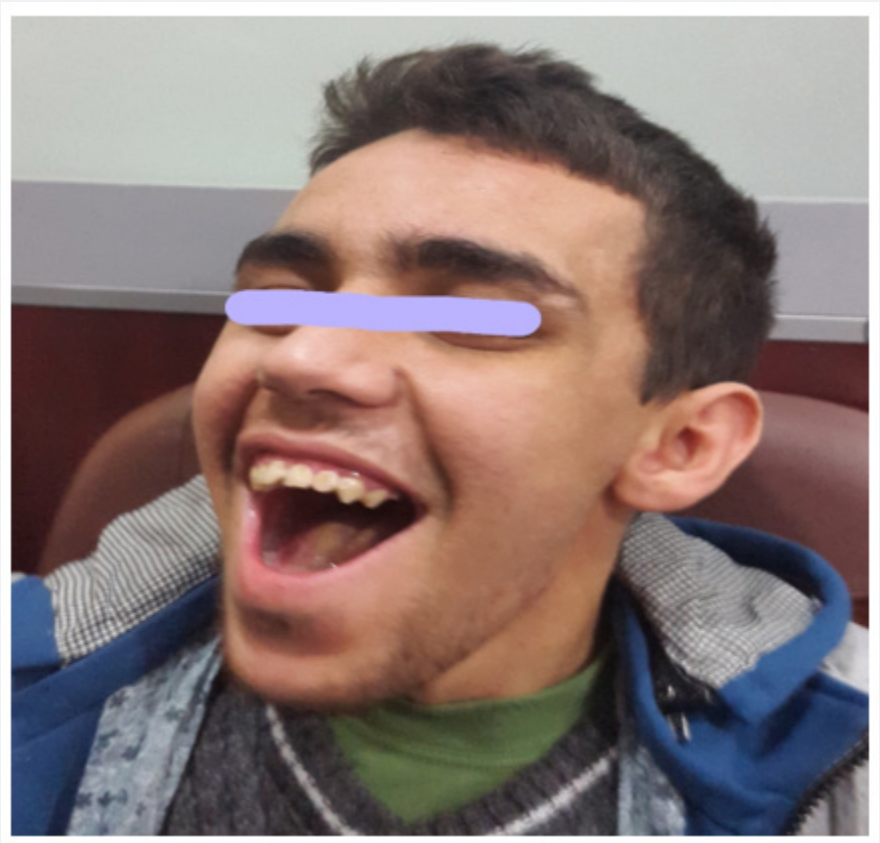

Figure1: Forced laughter of patient's face represents pseudobulbar palsy.

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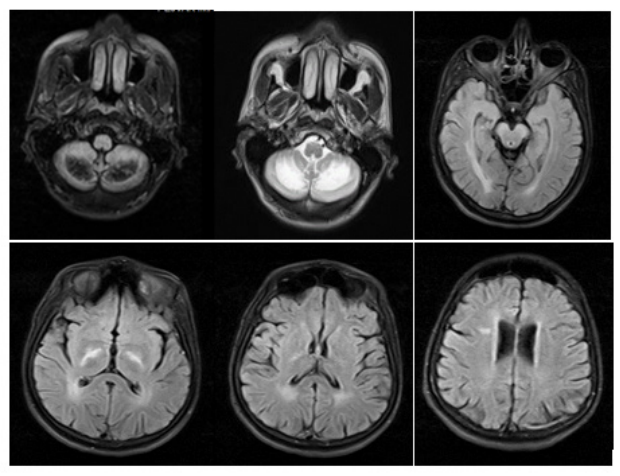

Figure 2: Axial Brain MRI of the patient (A) FLAIR sequence showed hypointensity of dentate nuclei (B) T2 Sequence showed hyperintensity of dentate nuclei. (C, E, F) Axial FLAIR sequence showed abnormal hyperintensity of white matter and (D) abnormal hyperintensity of bilateral basal ganglia.

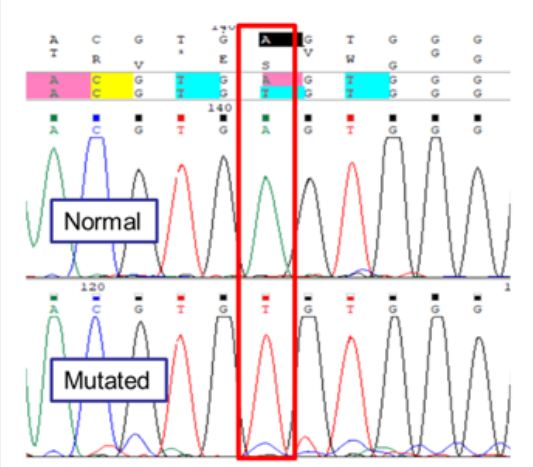

Figure 3: Electropherogram revealed homozygous c.1263+4A>T mutation (red box).

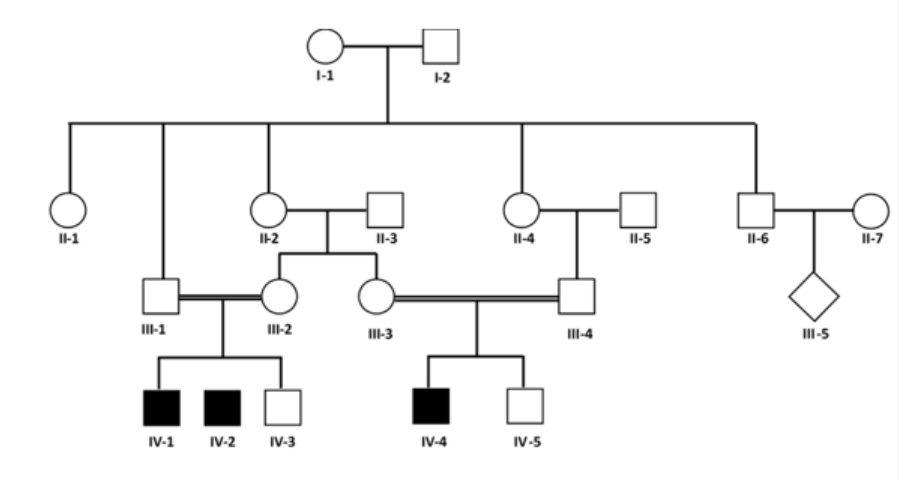

Figure 4: Pedigree diagram of the family carrying the c.1263+4A>T mutation.

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Sample collection and MSC expansion

Umbilical cord was obtained from the cesarean delivery with the signed informed consent of a healthy mother. MSCs isolation and expansion was performed according to our previously described protocol (R) briefly; the umbilical cord was washed with Dulbecco's Modified Eagle Medium (DMEM) for removing the blood and cut to 1-2 cm pieces. Then we removed the blood vessels and isolated the Wharton's jelly. Isolated Wharton's jelly pieces were put in a 50ml sterile tube in DMEM medium containing collagenase (2mg/ml) and Hyaluronidase (0/5mg/ml) and incubated for 16-18 hours in a CO_2 incubator. After that, the digested Wharton's jelly was transferred into the culture flasks and fed with a complete medium (DMEM medium, 10% FBS, and 1% L-glutamine) incubated at 37° C in a humid chamber containing 5% CO_2 incubator. The medium of Culture flasks was replaced every 4 days until the fibroblast-like cells reach confluence at the base of flasks. When the cells were confluent, they separated with trypsin, and re-cultivation was done. In the final passage when the cells were confluent, the medium was replaced with DMEM without FBS and incubated for one more day in the CO_2 incubator. After that, the cells were detached using trypsin and washed with normal saline containing 2.5 % HAS by centrifugation two times. The final sediment was prepared with the normal saline containing 2.5 % HSA, and 1U/ml heparin as a cell suspension with a concentration of 2-4 × 10^6 cells/ml.

Safety assessment and Identity validation

To make sure of expanded MSCs are safe we did the mycoplasma, endotoxin, sterility, and karyotype test. The percentage of viability of the cells was evaluated by trypan blue dye exclusion method. To evaluate the cell surface markers and the purity of the cultured cells, flow cytometry analysis was done for CD105, CD90, CD73, CD34, CD45, HLA DR, markers. We also evaluated the differentiation potential of our cell to adipocyte and osteocyte.

Injection of MSCs

Due to the leukodystrophy and cerebellar involvement, Injection was performed intrathecally. Intrathecal stem cell injections were performed through lumbar puncture. Lumbar needle was placed at the level of the L4 or L5 vertebrae, so that the introducing needle entered below the level of spinal cord end. First, 5 ml of cerebrospinal fluid was collected for protein, sugar and lactate level and Red Blood Cell (RBC) and White Blood Cell (WBC) count. Next, through the same cannula, cell suspension (4 ml) was injected over 2 minutes followed by saline injection (1 ml over 30 sec). Total cell dose was 1×10^8 which was injected two times (each 0.5×10^8 cells) with 28 days interval. The procedure lasted about 10 minutes and monitored vital signs for 6 hours after administration. The patient received an injection of 8mgr dexamethasone, 30 minutes before the cell therapy. The cells' viability in the first injection was 80 percent and in the second injection was 90 percent. Immediately after the procedure, the patient had a good condition, but laboratory analysis revealed leukocytosis without fever or increasing in Erythrocyte Sedimentation Rate (ESR).

Results

All parameters which were assessed for MSC safety like mycoplasma, endotoxin, and sterility tests were negative and the last passage of MSCs had a normal karyotype. Cell surface markers were positive more than 90% for CD105, CD90, and CD73 and negative (≤2%) for CD34, CD45, HLA DR. Adipocyte and osteocyte differentiation ability of MSCs were confirmed by Oil Red O and alizarin red staining. The patient had no significant complications in the post-transplant period. No immediate or delayed side effects following UC-MSC infusion were observed. He developed neither malignancy nor unwanted cells or any infectious complications 12 months after the transplantation, we performed a Cerebral MRI showed stable cerebral lesions but his gate and balance did not improve. Anti-HLA Antibody measurement confirmed that the patient's immune system was not stimulated by injected cells. Concerning his neurological symptoms, Scale for the assessment and rating of ataxia (SARA), The Multiple Sclerosis Functional Composite Measure (MSFC), Quality of Life Assessment (QOL), and Cognitive Functioning Status (ACE-R), the patient did not have further deterioration of his previous clinical status in the follow-up period of 12 months with some improvement of cognitive and swallowing function. Without changing in AEDs regiment myoclonic seizures constantly improved and after a month he did not have any seizures. Unfortunately, he died after 12 months of mesenchymal cell therapy due to unexpected myocardial infarction and aspiration pneumonia.

Discussion

The most common seizure semiology was reported is convulsive Tonic-Clonic seizure. This patient had refractory myoclonic epilepsy which is a rare manifestation in CTX patients. Recently an Indian family with a progressive myoclonic epilepsy-like presentation of CTX was reported [8]. Early-onset refractory epilepsy also is a rare condition in CTX and to date, was reported in a case without cataract and diarrhea. Although they have been reported a case of drug-resistant epilepsy who had a significant reduction in seizure frequency after chenodeoxycholic acid supplementation [6], our patient did not respond to the CDCA therapy and ataxia was aggravated. In recent years, cellular therapies offered a great assurance for the treatment of Neurodegenerative Disorders (NDs). Stem cell replacement in widespread neuronal loss can stabilize neuronal networks in the nervous system. Furthermore, cell replacement may also help the remaining cell and also prevent the accumulation of toxic factors that play a significant role in neurodegeneration [9].

There was not any MSC therapy for CTX to the best of our knowledge and we have a lack of knowledge in this area. This patient's myoclonic seizures were improved and stopped in a short period after MSC therapy which was not reported previously

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to the best of our knowledge, however, a trial of MSC therapy on refractory epilepsy has been reported. The study revealed that MSC therapy can improve seizure control outcome and cognitive function in patients with autoimmune refractory epilepsy [10].

Conclusion

Because of the MSC capacity for de novo neurogenesis, brain regeneration, and immunomodulatory effects, this study suggests that MSC therapies could be relatively effective and safe in patients with CTX or other neurodegenerative diseases who have refractory epilepsy. But limited information is available regarding functional outcomes of MSC therapy. Thus, further human studies are necessary to confirm the emerging findings to the clinical application of MSC for CTX and neurodegenerative disease with refractory epilepsy.

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