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A Case Study on Autism Spectrum Disorder Treatment Using Allogenic Mesenchymal Stem Cells Derived from the Human Umbilical Cord

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Abstract

The goal of this study was to evaluate the efficacy and safety of allogenic mesenchymal stem cell transplantation which is derived from Wharton's jelly of umbilical cords. A clinical treatment was performed from November 2019 to January 2020. 2 children, who had >60 of Childhood Autism Rating Scale (CARS) score, were selected. Mesenchymal stem cell was isolated from umbilical cords. The cell number for injection was 1x106/kg. The procedure was repeated every week for 4~6 times. There was no side effect related with transplantation. The severity of autism spectrum disorder (ASD) was significantly reduced with the CARS score that decreased from 43.25 to 31.25. Social communication, language and emotional activity was improved remarkably for stem cell treatment. Visual, listening, and intellectual response was changed. Allogenic Umbilical cord stem cell transplantation was safe and improved in children with ASD.

Keywords: Autism Spectrum Disorder (ASD); Allogenic Mesenchymal Stem Cell Transplantation; Wharton's Jelly of Umbilical Cords Autism Rating Scale; Uncultured Stem Cell

Introduction

Autism spectrum disorder (ASD) is a complex spectrum of disorders characterized by two typical abnormalities: deficits of social communication and interaction and the presence of restricted interests as well as repetitive and stereotypic verbal and nonverbal behaviors [1,2]. ASD characterized by social interaction disorder, verbal and nonverbal communication disorder, as well as stereotyped behavior and interests in childhood. Parents are often worried about delayed language development around 18 months of age and developmental differences among peers are noticed. Some children with good intelligence and self-help function may not be diagnosed until they reach school age.

ASD is a disease caused by brain development disorders due to neurobiological causes. It expresses a comprehensive disability in sociality, language, cognition, emotional control, and sensory integration due to brain development problems. The symptoms of ASD include the use of eye contact, facial expressions, and improper or lack of. Behaviors and interests are limited and repetitive. They are highly focused on limited interests with a stereotyped aspect and are obsessed with nonfunctional work or use of gestures as a social interaction disorder. They often have trouble initiating or maintaining conversation because of the delay in spoken language development due to communication disorderscustoms. They also show stereotyped and reactionary aspects of movement. There are no ultimate treatment methods for ASD; therefore, it was impossible to improve the quality of life for the patients.

Therefore, to treat the fundamental cause of the disease, stem cell therapy has gained attention. It regenerates damaged tissues as well as increasing and inhibiting various cytokines. Researcher have studied the treatment of neuropsychiatric disorders using stem cells. Mesenchymal stem cells (MSCs) exist in various tissues of the human body, especially in connective tissues. It was first discovered in the human bone marrow and has been found in blood, fat, muscle, bone, placenta, Wharton's jelly of the umbilical cord, and cord blood [3,4]. As mesenchymal stem cells can differentiate into particular cells, control cytokines, and have immune control ability, clinical research on its application to multiple diseases is progressing. Particularly, numerous attempts have been made to apply neurogenic properties and immune modulatory effects of mesenchymal stem cells in the treatment of neurological disorders [4-6]. Recently, clinical research and disease model studies on the treatment of neurological disorders, such as Parkinson's and Alzheimer's disease, using mesenchymal stem cells derived from autologous marrow stem cells [7,8].

Our goal is to observe the improvements of the developmental level after injecting mesenchymal stem cells derived from the umbilical cord (UC-MSCs) to patients with ASD and eventually treat the autistic disorder.

Subject, Materials and Methods

Patient

Inclusion Criteria

Patients who were aged between 3 and 7 years with a confirmed diagnosis of ASD were enrolled. ASD was diagnosed according to the diagnostic criteria for ASD in the Diagnostic and Statistical Manual of Mental Disorders. All children at Hospital who had severe ASD (Childhood Autism Rating Scale [CARS] scores >60) were recruited for this study.

Exclusion criteria

Patients excluded with evidence or history of significant hematological, endocrine, cerebrovascular, cardiovascular, coronary, pulmonary, renal, gastrointestinal, immune compromising, or neurological disease, including seizure disorder.

Isolation of UC-MSCs

The umbilical cord was donated by obstetrics and gynecology of Lynn woman hospitals and was determined to be aseptic by testing for bacteria, fungi, and cytomegalovirus. We had an agreement from patient and South Korea law have been not regulated to inject with uncultured stem cell.

We confirmed the safety of the donated umbilical cord by some blood tests such as Hbs Ag, Hbs Ab, Hcv Ag, Hcv Ab, HIV, Syphilis (RPR) and HTLV-I/II Ab tests. First, the umbilical cord was sterilized and cut to isolate Wharton's jelly from the umbilical cord blood vessels and other internal elements. After removing the blood vessels, Wharton's jelly was cut into smaller pieces (0.5 cm × 0.5 cm) to extract the cells. The tissue was cut by surgical scissors and was homogenized using a disposable tissue grinder (Fisher Scientific, Pittsburgh, PA, USA). To isolate mesenchymal stem cells, the cells were treated with 0.01% collagenase, stored in a cell incubator at 37 °C, and shaken once every 10 min for 1h with minimal manipulated isolation.

The cells were counted after washing with PBS, and an aseptic test was performed afterward. The extracted cells were refrigerated at - 197°C without cell cultivation.

Characterization of UC-MSCs

UC-MSCs were resuspended in PBS. Approximately 1 × 10^6 cells were stained with 1 mg of antibody for 1 h at 25 °C and then analyzed on a fluorescence-activated cell sorting (FACS) flow cytometer (Cube 8 Sysmax Int. IL USA). The human antibodies used included CD73, CD90, CD105 as positive marker, and CD45 and CD34 as negative marker (BD Biosciences NJ USA).

UC-MSC Transplantation

Each patient performed with uncultured UC-MSC transplantations with an interval of 1 weeks. The average mononuclear cell and CD70+, CD90+, CD105+ cell counts per kg body weight were 1 × 106/kg for the transplantation respectively. The average cell viabilities before transplantations were average >95%, respectively. Each dose of cells was mixed with physiological saline to a volume of 20 mL for administration.

Results

Case I

The patient was a 5-year-old boy that weighed 15 kg and was 98 cm tall.

At the age of 18 months, the call response, eye contact, and concentration significantly fell behind those of children of the same age. He was diagnosed with ASD at the University Hospital, Department of Pediatric Psychiatry when 18 months old. Before joining our program, he was treated at a psychiatric ward, oriental medical clinic and a special education center. Uncultured UC-MSCs derived from the umbilical cord were identified using markers and tested for bacteria to ensure that they were not contaminated. We confirmed UC-MSC characterization with CD73, CD 90, CD105, CD34 and CD 45 MSC marker. The Hbs Ag, Hbs Ab, Hcv Ag, Hcv Ab, HIV, Syphilis (RPR) and HTLV-I/II Ab tests of the donated umbilical cord were negative and bacteria, fungi, and cytomegalovirus test of UC-MSC were also negative.

A total of six intravenous injections of UC-MSCs were performed from November 22, 2019 to January 8, 2020. Before UC-MSC transplantation, Case 1 patient couldn't have social contact and made eye contact that was no eye contact and not showing affection toward parents. After the treatment, sociality increased by associating with peers, and physical ability and emotional response improved. There was an improvement in verbal and nonverbal communication ability, and the patient was able to behave actively similar to the children of their age. Patient increased expressive language (Table 1). Abnormal behaviors were observed that did not improve repetitive behavior and imitation and adaptation to change was not occurred (Table 2). Sensory abnormalities improved in patient after UC-MSCs transplantation (Table 3).

Table 1: Social interaction, emotional behavior, and expressive language before and after transplantation

Domain	Before transplantation	1 week after 1st Session	1 week after 2nd Session	1 week after 3rd Session	1 week after 4th Session	1week after 5th Session	2 month after 6th Session	2 month after 7th Session
Social interaction								
Relationship with People	4	3.5	3.5	3.5	3	3	3	2
Emotional behavior								
Fear or Nervousness	4	4	3.5	3	3	3	2.5	2
Reponse	3	3	3	3	3	3	3	2
General Impression	4	4	4	4	4	4	4	4
Expressive language								
Nonverbal Communication	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2
Verbal Communication	3.5	3	3	3	3	3	3	2.5

Table 2: Abnormal behaviors before and after transplantation

Domain	Before transplantation	1 week after 1st Session	1 week after 2nd Session	1 week after 3rd Session	1 week after 4th Session	1week after 5th Session	2 month after 6th Session	2 month after 7th Session
Behaviors								
Have behavior activity	2	2.5	2.5	2.5	2,5	2.5	2.5	2.5
Interaction behaviors								
Imitation	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Adaptation to Change	2.5	2	2	2	2	2	2	2

Table 3: Sensory abnormalities before and after transplantation

Domain	Before transplantation	1 week after 1st Session	1 week after 2nd Session	1 week after 3rd Session	1 week after 4th Session	1week after 5th Session	2 month after 6th Session	2 month after 7th Session
Response								
Visual	2.5	2	2	2	2	2	2	1.5
Listening	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Taste, Smell, and Touch	2.5	2	2	2	2	2	2	1.5
Level of consistency of intellect	3	2.5	2.5	2	1.5	1.5	1.5	1.5
Use								
Body	3	3	3	3	3	3	3	2.5
Object	2	3	2.5	2.5	2.5	2.5	2.5	2

According to the results of the Autism Spectrum Quotient test, the score gradually decreased as the symptoms of ASD improved along with the treatment (Figure 1). The results indicate the positive effect of UC-MSCs in treating ASD, as there was progress in the level of development. In this case, the UC-MSCs transplanted to the patient did not present any adverse effects, immunological rejection, or any suspicious side effects while observing the symptoms after the treatment.

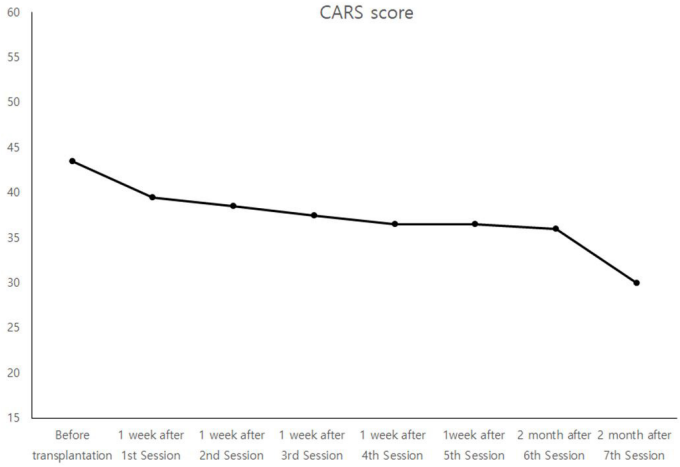

Figure 1: A graph of the Case I patient's autism spectrum quotation test scores

CASE II

The patient was a 6-year-old boy that weighed 17 kg and was 120 cm tall.

At age 2, the patient was unable to acquire a language and had poor cognitive ability in comparison to his peers. He often screamed and was out of control. The parents started questioning his condition when he was 3. He was unable to defecate or urinate properly until the age of 5 and was diagnosed with ASD at the university hospital department of psychiatry when he was 6. The patient is under speech-language therapy and behavior modification therapy at the Special Education Center; however, he has a developmental disability and is much slower than the children of the same age in development.

The purity of uncultured UC-MSCs was confirmed using markers higher than 95% and was confirmed to be free from contamination using bacterial testing. A total of four intravenous injections of UC-MSCs at 1x106/kg were performed from Sep. 19, 2019 to Nov. 28, 2019. After treatment, the relationship with people, emotional response, and physical response improved significantly, and the patient was able to imitate other people, such as singing-along. The patient had improvement of eye contact and was able to communicate smoothly with others and improved in verbal and nonverbal communication ability to increase expressive language (Table 4). Abnormal behaviors were observed that improve imitation and adaptation to change (Table 5). Sensory abnormalities improved in patient after UC-MSC transplantation (Table 6). The patient is now attending a general elementary school due to the interpersonal relationships with his friends improved.

Table 4: Social interaction, Emotional behavior, and expressive language before and after transplantation

Domain	Before transplantation	1 week after 1st Session	1 week after 2nd Session	1 week after 3rd Session	9month after 4th Session
Social interaction					
Relationship with People	3	2.5	2	1.5	1.5
Emotional behavior					
Fear or Nervousness	3	2.5	2.5	2.5	2.5
Reponse	3.5	2	2.5	2.5	2.5
General Impression	**3.5**	**3.5**	**3.5**	**3**	**3**
Expressive language					
Nonverbal Communication	3	2.5	2.5	2	2
Verbal Communication	3.5	3.5	3.5	3	2.5

Table 5: Abnormal behaviors before and after transplantation

Domain	Before transplantation	1 week after 1st Session	1 week after 2nd Session	1 week after 3rd Session	9month after 4th Session
Tereotypic/repetitive behaviors					
Have repetitive behavior	2.5	2.5	2.5	2.5	2.5
Interation behaviors					
Imitation	3.5	3	2.5	2.5	2.5
Adaptation to Change	3	3	2.5	2	2

Table 6: Sensory abnormalities before and after transplantation

Domain	Before transplantation	1 week after 1st Session	1 week after 2nd Session	1 week after 3rd Session	9month after 4th Session
Response					
Visual	3.5	3.5	3	2.5	2.5
Listening	**2**	**1.5**	**1.5**	**1.5**	**1.5**
Taste, Smell, and Touch	2	2	1.5	1.5	1.5
Level of consistency of intellect	2	2	1.5	1.5	1.5
Use					
Body	2.5	2.5	2.5	2.5	2.5
Object	2.5	2.5	2	2	2

According to the results of the Autism Spectrum Quotient test, the score decreased rapidly and remained at a certain level as the symptoms of ASD improved significantly (Figure 2). The results revealed that the UC-MSCs caused a massive improvement in the symptoms of ASD, and the sociality and language ability development sharply improved. In this case, the UC-MSCs transplanted to the patient did not present any adverse effects, immunological rejection, or cause any suspicious side effects while observing the symptoms after the treatment.

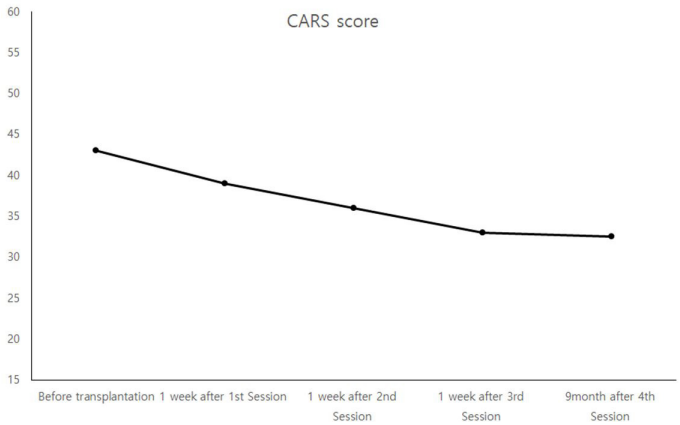

Figure 2: A graph of the Case II patient's autism spectrum quotation test scores

Discussion

The primary goal of regenerative medicine is to enable the treatment of incurable diseases. These efforts have shown therapeutic effects in various diseases, such as Parkinson's disease, spinal cord injury, polyneuropathy, myocardial infarction, and stroke. Recently, cell-based regenerative medicine therapy has spread through numerous disciplines. ASD, the most complex neurodevelopmental disorder, is occurring at a very high frequency. The causes and pathological mechanisms of this disease are still unknown. The biochemical abnormalities of ASD include oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, decreased methylation, underproduction of glutathione, intestinal dysbiosis, and toxic metal burden [9]. Additionally, organophosphate compounds and heavy metals have been reported as external factors that cause ASD [10]. It is also assumed that the functional and molecular defects of synaptogenesis due to the changes in phenotypes caused by genetic mutations cause the symptoms of ASD [11].

Recently, the degradation of cerebral hypoperfusion and immune dysfunction are known to cause ASD from a pathological perspective. The degradation of cerebral hypoperfusion damages the neuronal tissue which leads to abnormal metabolites and the accumulation of neurotransmitters. A decrease in cerebral blood flow is the major cause of the decline in intelligence quotient (IQ), the main symptom of ASD [12]. Immunodysfuction is caused by a loss of balance between pro-inflammatory and anti-inflammatory factors. The increase in macrophages produces the factors of inflammation, including neopterin, TNF-alpha, MCP-1, and IFN-gamma. Therefore, the deficiency of anti-inflammatory cytokine factors, such as IL-10 and TGF-beta, causes the impeding loss of neuronal signal transduction. The deregulation of immunoactivities during the period of nerve development causes neurological dysfunction and leads to ASD [13,14].

Cell therapy rectifies the function, structure, and molecular state of damaged neuronal tissue and exerts therapeutic effects. Stem cells have self-renewal, transdifferentiating, and paracrine effects. The paracrine effect of stem cells enables the cells to proliferate, recruit cells, and mature endogenous stem cells or progenitor cells [15].

Another key effect of stem cell is the ability to inhibit T lymphocyte pro-inflammatory cytokine production (IL-1β, TNF-α, and INF-γ) and immunomodulation through upregulation of anti-inflammatory IL-10 and TGF-β [16].

In this study, the two patients had been working on rehabilitation after they were diagnosed with ASD. Before the UC-MSCs treatment, the two patients scored 43.25 points on the Autism Spectrum Quotient test, showing a similar scale of ASD. However, when measured after the treatment, the average score of the autism spectrum dropped to 31.25, which shows the therapeutic effect of UC-MSCs. Their body movements, including restlessness, rocking, hand flapping, and jumping, decreased significantly after the treatment of UC-MSCs. Our goal was to improve the social participation and verbal communication ability of the patients. Not only were the responses to the environment and behavioral movements gradually improved, but also building relationships and verbal communication to form relationships with other people significantly improved in a short period. Particularly, the patients were able to build a sentence with 4-5 words in comparison to the beginning of the treatment, where both of them hardly used 1-2 words.

Additionally, the patients who were indifferent to songs before the treatment expressed greater interest and sang along after the procedure. Therefore, we anticipate the patients to improve their language ability and social relationship formation over time even more. However, the auditory, olfactory, and tactile responses did not improve, and the development of cognitive level was insufficient. It will take much longer to regenerate neuronal cells and properly form networks between neuronal cells. This study showed the potential for the treatment of ASD using uncultured UC-MSCs derived from the umbilical cord. Stem cell therapy for ASD will have a synergistic effect when combined with special ASD education. Currently, there is a debate over the therapeutic effectiveness of stem cells in treating diseases; however, a considerable beneficial response is expected when stem cells are used along with other therapeutic methods. Future studies should proceed with large-scale cohort clinical trials and compared to controls. Although many existing studies have proclaimed the safety and effectiveness of MSCs in humans, there is still a shortage of clinical research papers. This study is the first case of treating ASD using allogenic UC-MSCs and is expected to serve as a cornerstone for large-scale clinical trials in the future.

Conclusion

The treatment of ASD using uncultured UC-MSCs derived from the umbilical cord has improved the symptoms of ASD. Particularly, ASD patients increased social participation, building relationships, and verbal communication ability to form relationships with other people. This study shows uncultured UC-MSC have the potential for the treatment of ASD.

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