

# A Case of Non-Progressive Congenital Myopathy: Efficacy and Clinical Outcomes of the Wharton's Jelly Derived Mesenchymal Stem Cell Transplantation

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Received: 12 Aug. 2021; Accepted: 14 Mar. 2022

**Abstract-** Non-Progressive Congenital Myopathy is a disease characterized by muscle weakness, and unfortunately, there is no conventional treatment. In the last decade, regenerative medicine practices have become a rising value, and Mesenchymal Stem Cells (MSCs) have fascinating outcomes in regenerative medicine with their high regenerative capacities, their ability to regulate with paracrine secretions, and their immunological properties. Based on our experience in our previous clinical studies, Wharton's-Jelly-derived (WJ)-MSCs are the most suitable source for muscle diseases among all MSC sources. In this study, we evaluated the outcomes of 10 doses of WJ-MSC transplantation to the patient diagnosed with Non-Progressive Congenital Myopathy. A 17-year-old female with a SPEN-1 mutation, Non-Progressive Congenital Myopathy patient received 10 times as  $1 \times 10^6$  /kg in the intra-arterial, intramuscular and intravenous administration of allogenic WJ-MSC. Before and after the treatment, the patient was followed-up with the upper extremity scale, Vignos lower extremity scale, muscle strength scale, functional independence measure, and evaluation of Serum creatine kinase (CK) levels. Improvement in both upper extremity scale and Vignos lower extremity scales, increasing in muscle strength, and decreasing in CK-level were detected. Although transplantation of WJ-MSC cannot treat any genetic-based diseases, they may benefit in alleviating clinical outcomes of disease. More importantly, WJ-MSC transplantation may offer a better quality of life by alleviating the symptoms of this rare disease with no treatment option that can be provided in conventional methods.

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*Acta Med Iran* 2022;60(4):249-253.

**Keywords:** Non-progressive congenital myopathy; Mesenchymal stem cells; Muscle fibrosis; Muscle-fiber regeneration

## Introduction

Non-progressive-congenital-myopathy is a muscular disorder characterized by hypotonia, motor development retardation, and proximal muscle weakness without any conventional treatment. Pathophysiology of congenital myopathy is related to protein defects that play a significant role in skeletal muscle contraction via their interaction with myosin, calcium homeostasis of skeletal

muscle, or sarcomere proteins in skeletal muscle (1).

Mesenchymal stem cells (MSC) are emerging biological sources used in regenerative medicine with their capacity for self-renewal, differentiation potential, anti-inflammatory, antiapoptotic, regenerative, and immunomodulatory abilities, and the preferable cellular source for therapeutic applications (2). MSC can be derived from different sources (3); Wharton-jelly-derived MSC(WJ-MSCs) are promising therapeutic sources for

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## Wharton's jelly derived mesenchymal stem cell transplantation to a non-progressive congenital myopathy

the treatment of myopathies by preventing muscle cell death via repressing apoptosis (3) with their paracrine activity such as secreting cytokines, releasing exosomes, and reaching the damaged host cells and by repopulating promote muscle regeneration and so improved muscle function and pathology (4).

The cellular therapy for non-progressive congenital myopathy using allogenic MSCs underlies the improvement of myogenic differentiation (5) reverses myopathy via increasing the satellite cell pool. This clinical study aimed to consider the efficacy of allogeneic WJ-MSC treatment for the rare disease of non-progressive-congenital-myopathy.

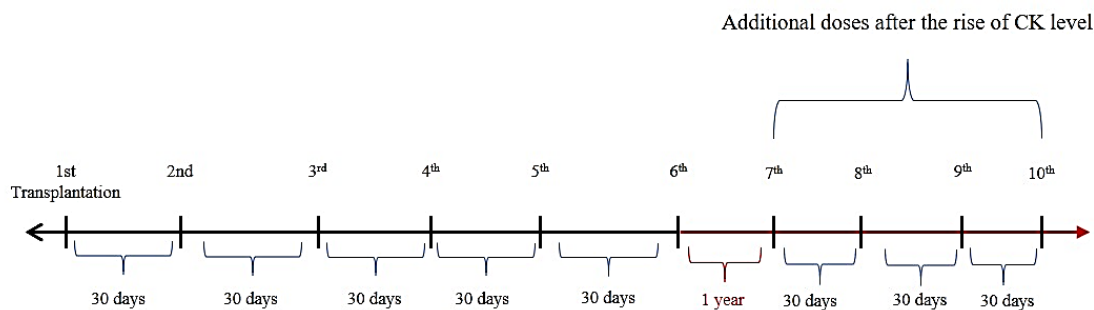
### Case Report

A 17-year-old female patient got diagnosed with non-progressive-congenital-myopathy with a muscle biopsy at the age of 10 was treated. Histological sections showed a slight diameter difference between muscle fibers, atrophy, several regenerated fibers, and rare central nuclei fibers. There was a significant increase in the prismatic adipose tissue and a focal increase in the endomysial connective tissue. Spectrin, merosin, dystrophin (1,2,3), alpha, beta.

üçç, gamma, and delta sarcoglycan were positive in immunohistochemistry staining also confirmed myopathic changes.

This patient had a homozygous deletion in the 6<sup>th</sup> exon of the *SPEN1* gene, and her healthy mother-father-brother was heterozygous for the same deletion. The study was approved by the Ethical Committee of the Ministry of Health Republic of Turkey (protocol number:56733164/203), was performed in accordance with the Helsinki Declaration, and informed consent was received from the patient's family.

The patient was treated with allogeneic WJ-MSC with a total of ten doses with four additional doses as  $1 \times 10^6/\text{kg}$  once a month. The treatment was initially planned as 6 doses. The three doses (1<sup>st</sup>, 4<sup>th</sup>, and 6<sup>th</sup>) were planned the combination of intra-arterial, intra-muscular, and intravenous; the other three doses (2<sup>nd</sup>, 3<sup>rd</sup>, and 5<sup>th</sup>) of intramuscular injections of WJ-MSC into abdominal muscles, deltoid, quadriceps, tibialis anterior and gluteal muscles by using ultrasonography (Figure 1). Nine months after 6 doses of planned therapy, an increase in Creatine Kinase (CK) level was detected. So, we decided to add additional 4 doses of WJ-MSc transplantations by following the first treatment protocol design.



**Transplantation design:** -1<sup>st</sup>, 4<sup>th</sup>, 6<sup>th</sup> were intra-arterial;

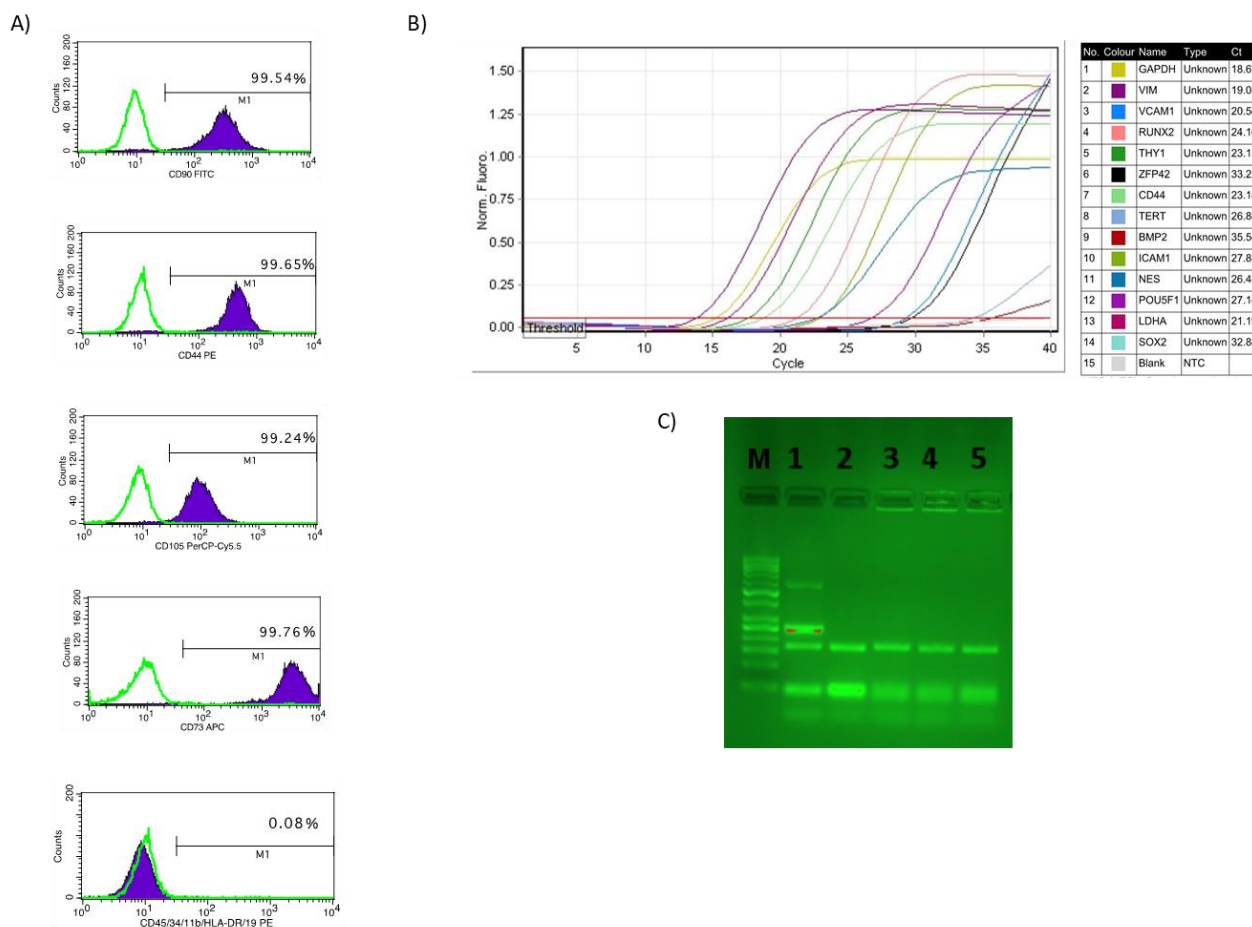
-2<sup>nd</sup>, 3<sup>rd</sup> and 5<sup>th</sup> were both intramuscular and intravenous injections.

**Doses:**  $1 \times 10^6/\text{kg}$  for each route.

**Figure 1.** Designing and timeline of stem cell transplant therapy for Non-Progressive Congenital Myopathy patient

Transplanted MSCs were isolated, expanded, and characterized based on our previous protocols (6). These

tests results were detected in the reference range required for transfer to the patient (Figure 2).



**Figure 2.** A) Flow cytometer analysis of WJ-MSC. A1; Positive expressions of CD90, CD105, CD44, CD73 markers and A2; negative expressions of CD45/34/11b/HLA DR/19 marker cocktail. B) Gene expression profile of WJ-MSC. C) Mycoplasma PCR results on the agarose gel. M: M: DNA ladder; 1: positive control; 2: negative control; 3: donor

The efficacy of allogeneic WJ-MSC treatment for our patient was evaluated by five clinical evaluation test parameters pre-/post-treatment measurements; Brooke upper extremity scale, Vignos lower extremity scale, muscle strength scale, functional independence measure, and evaluation of Serum creatine kinase (CK) levels.

Based on comparison measurements taken at the pre- and the post-term of treatment, the patient's Brooke upper extremity scale had decreased from 6 to 3. When the general condition of the patient is evaluated, it was not possible or even difficult to do daily hand skill activities before the treatment. It was seen that there was little difficulty or no difficulty in individual skills such as writing, combing hair, eating after the treatment. The Vignos lower extremity scale had dropped from 10 to 5,

so the patient showed improvement in independent walking. It was recorded that the 6-meter area walked in 95 steps and approximately 2 minutes after the treatment, while it was difficult to take 3-4 steps before the treatment. The muscle strength scale elevated from 1 to 3, so there was a significant increase in muscle strength. The functional independence measure was increased from 3 to 27 out of 40. The patient has become capable of activities that are restricted before the treatment, such as dancing, rarely sitting at the table, eating, walking around the house, writing, and writing. Although the CK level was decreased from 232U/L to 140U/L, 9 months after the treatment, it increased to 408U/L again. After the four additional doses, the CK level was re-declined to 136 U/L (Figure 3).

Additionally, no serious side effects were observed in the patient, who was observed by physicians on-site

consistently throughout the study.

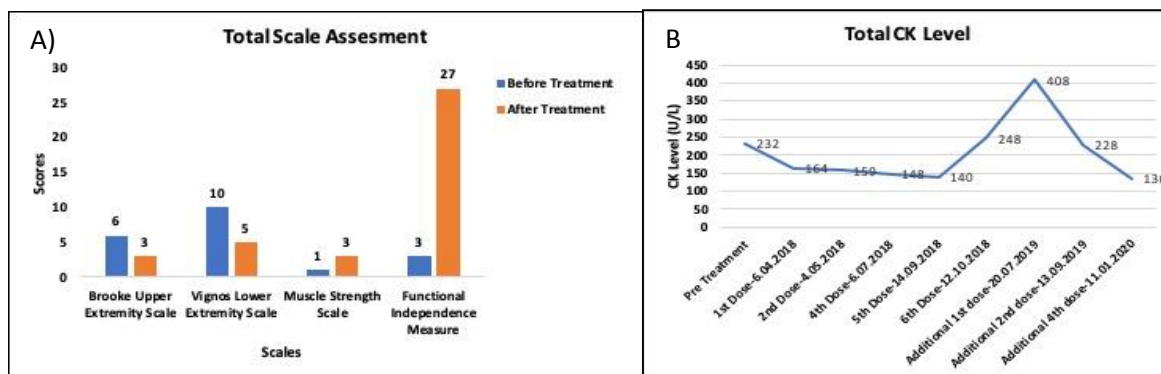


Figure 3. Overall results of the evaluations tests. A) Total scale assessment of muscle function tests. B) The results of CK level after MSC injections

## Discussion

Our patient, diagnosed with Non-progressive congenital myopathy, is a rare disease, got a homozygous deletion in the *SPEN1* gene at the 6<sup>th</sup> exon that causes embryonic lethality due to defects in many vital tissue and organ system formations, including the muscles (7). Combining both the genetic background and the clinical outcomes of the patient, *SPEN1* mutations cause atrophy of the adductor magus and biceps femoris muscles, which have a major role in the grade of walking ability. As seen, the increase in the Vignos-scale in post-treatment showed the patient started walking without support. Likewise, it has been reported that the CK levels in *SPEN1* related muscle disorders were measured 300-500IU/L (8). Considering the effect of the increase in CK level causes inflammation and necrosis of myofibers, the decrease in CK level after allogenic WJ-MSC transplantation would support the regression of the effects of the disease. As seen in Figure 3, the CK level peaked again between the 6<sup>th</sup> dose and the additional 1<sup>st</sup> doses. Although each dose was injected in monthly doses on average, the interval between these 2 doses is 9 months. This gap reversed the CK level with the increase of fibrosis. Decreasing with the additional doses can be explained by immune evasive and anti-inflammatory characteristics of MSCs via suppressing T-cell functions by secreting *PGE2*, secreting anti-inflammatory cytokines such as IL-10, increasing the secretion of TNF- $\alpha$ , IL1 $\alpha$  and IL1 $\beta$  (6). However, the increase in CK level shows us that repeated MSC injections in genetic-based muscle disease are how important and necessary.

One of the important factors determining the

effectiveness of the treatment depends on the transplantation route of MSCs. Our cellular therapy for this case was designed as three different transplantation routes intra-arterial, intramuscular and intravenous. The reason for choosing these routes were the anti-inflammatory effects of both intramuscular and intravenous injections; by the intramuscular injection, a local regeneration as the lost tissue caused by the disease triggers differentiation and triggers the formation of new myofibrils can be provided (9), and a wider systemic effect of intra-arterial (10).

The use of MSCs in the treatment of irreversible muscle diseases has a significant place in regenerative medicine. According to the National Institute of Health Clinical Trials data, the importance of using MSC in the translational treatment of muscular diseases is increasing. There are 13 MSC treatment records for myopathy or muscular disorders; four of them are WJ-MSC. This increased acceleration of preferring WJ-MSC for muscle diseases is the triggering proliferation by anti-apoptotic effect, endogenous muscle cell precursors, and its immunosuppression role for preventing fibrosis through on myofibroblast via regulating the Extra Cellular Matrix via its paracrine secretions (2,3). Besides the paracrine secretions, MSCs also transfer their genomic material. As we report, the 10-Duchenne Muscular Dystrophy patients treated with WJ-MSC showed the restoring dystrophin expression by cellular fusions explained by fusing with the recipient cells to transfer its genomic material. So, the deteriorated gene expression pattern related to the myogenic differentiation may be impaired by *SPEN1* might be regulated (6).

WJ-MSC transplantation may offer a better quality of

life by alleviating the symptoms of this rare disease with no treatment option that can be provided in conventional methods. Although transplantation of WJ-MSC cannot treat any genetic-based diseases, they may offer a more comfortable life with slowing the progression rate, regression of fibrosis, and immunoregulation (6). For all that, new technologies, such as CRISPR/Cas9, might be offering advanced these MSCs therapies with combining the gene-editing approaches in the years to come.

## Acknowledgments

The authors acknowledge Gökay Görmeli, Assoc. Prof. Dr.; Sacit Karamürsel, Prof. Dr. and Gülşen Günel, MSc. for their technical and clinical assistance.

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