Safety evaluation of umbilical cord tissue-derived mesenchymal stem cells (UC-MSCs) in the treatment of patients with multiple sclerosis

Mohamad Mahdi Esmaeili Araghi[1], Mehrdad Moosazadeh Moghaddam[2], Hadi Esmaeili Gouvarchin Ghaleh[3], Mehdi Raei[4], Bahman Jalali Kondori[5, 6], Akbar Ghorbani Alvanegh[7], Javad Hosseini Nejad[1*]

1. Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Tissue Engineering and Regenerative Medicine Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
3. Applied Virology Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
4. Health Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
5. Department of Anatomical Sciences, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran.
6. Baqiyatallah Research Center for Gastroenterology and Liver Diseases (BRCGL), Baqiyatallah University of Medical Sciences, Tehran, Iran.
7. Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

[*]**Corresponding author:** Javad Hosseini Nejad, Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. Email: javadhoseini1360@gmail.com

Abstract

Multiple sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system, leading to symptoms like fatigue, mobility issues, and cognitive decline. Available treatments include disease-modifying therapies (DMTs) and symptom management options. However, many patients experience limited effectiveness, side effects, or inadequate response, highlighting the need for innovative therapeutic approaches. Given the potential of mesenchymal stem cells (MSCs) to modulate immune responses and promote neuroprotection, this study aims to assess safety of UC-MSCs, paving the way for future therapeutic applications in MS management. In this study, five patients with MS were selected based on McDonald criteria and specific inclusion criteria, including age, EDSS score, and absence of certain medical conditions. Pre-injection assessments included ECG, MRI, and comprehensive blood and urine tests. Patients received UC-MSCs in normal saline over 20 minutes, followed by hydrocortisone. Post-injection, patients were monitored in the hospital for 24-48 hours, with vital signs checked every 1 to 3 hours. Blood and urine tests were repeated 24 hours after injection and again one month later to evaluate safety and monitor for adverse effects. Our result showed that the differences in means for all inflammation and infection, liver function, kidney function and blood tests over the four time points were not statistically significant. Given the absence of significant side effects associated with the utilization of UC-MSCs, it can be confidently concluded that these cells represent a promising therapeutic option for the effective management of MS. Their safety profile enhances their potential as a viable treatment alternative for patients.

Keywords: Umbilical Cord Tissue, Mesenchymal Stem Cells, Multiple Sclerosis, Safety, Cell Therapy

1. Introduction

Multiple sclerosis (MS) is a chronic, relapsing condition characterized by the formation of plaques in the brain and spinal cord. In MS, the immune system of the body targets the myelin sheath surrounding nerve cells in the central nervous system. This attack results in dysfunction of the nervous system, which presents with a range of clinical symptoms [1]. In many patients, the clinical presentation of multiple sclerosis is characterized by reversible neurological symptoms, known as relapsing-remitting MS. Following this phase, individuals may transition into a secondary progressive stage, where they experience lasting neurological deficits and a gradual increase in disability, referred to as secondary progressive MS [2]. The mechanism underlying this disease is autoimmune in nature, with T cells playing a crucial role. Additionally, macrophages and microglia are found in the demyelinated plaques, contributing to the inflammatory processes associated with the condition [3]. Most current treatments focus on managing acute attacks and alleviating symptoms by modulating the immune system. Standard therapies can lower relapse rates, slow down or prevent the progression of disability, and, in some cases, even reduce existing disability [4]. MSCs, which originate from the mesoderm, have the remarkable ability to self-renew. These cells can differentiate into various lineages, allowing them to develop into mesodermal, ectodermal, and endodermal tissues. This versatility makes them a valuable resource in regenerative medicine and tissue engineering [5]. The therapeutic effects of MSCs are closely linked to their ability to differentiate and their paracrine effects. These cells secrete a variety of substances, including cytokines and microRNAs, which play significant roles in mediating their beneficial effects in tissue repair and regeneration [6]. MSCs are immune system modulators and, in addition to their anti-inflammatory properties, they also play a role in stopping apoptosis [7, 8]. Due to the unique properties of MSCs, numerous studies have been conducted across various

countries and years. Some of these studies utilized MSCs sourced from the patient's bone marrow or adipose tissue, while others employed fetal UC-MSCs for treatment. The research has explored different doses and frequencies of injections to assess their efficacy and safety in therapeutic applications [9-12]. In various studies, injections of MSCs were administered via different routes, including intrathecal (IT) and intravascular (IV) methods. Some studies even combined both IV and IT injections. Notably, no serious adverse events or deaths were reported in any of the reviewed studies prior to the project's initiation. However, several adverse events were recorded, such as headaches, infections, cardiovascular complications, and injection site issues [13-19]. Based on the information, the aim of the present study is to evaluate the safety of the purchased UC-MSCs in this research for conducting the subsequent phases of the clinical trial. This assessment is conducted to ensure the safety and usability of these cells in the treatment of patients with multiple sclerosis.

2. Materials and Methods

2.1.Patient Selection

Five patients with MS diagnosed according to the McDonald criteria were included in the study based on the inclusion criteria. The inclusion criteria included: age between 18 and 55 years, EDSS score between 2 and 7, patients with secondary progressive MS, absence of hepatitis B and C and HIV diseases and any active infection (WBC above 11,000), no history of tuberculosis, no kidney problems (creatinine above 2.5) and cardiovascular and mental problems, no poorly controlled diabetes (HbA1 above 8.5), no history of organ transplantation and no pregnancy in women. Patients were given explanations regarding ethical issues and were told that participation in this study was voluntary and that they could withdraw from the study at any time and that they would not be deprived of their usual treatments.

2.2.Pre-injection checks

A complete history was taken from all patients and the following tests were performed on them. Before the injection, all patients underwent an electrocardiogram and MRI imaging. Blood and urine biochemical tests including CBC, creatinine, BUN, Blood sugar, bilirubin, liver enzyme levels including AST and ALT, urine U/A test, and ESR and CRP inflammatory tests were performed.

2.3.UC. MSCs injections

Umbilical cord-derived mesenchymal stem cells (UC-MSCs) were purchased from Cell Tech Pharmed Company in Iran and transported to the neurology department of Baqiyatallah Hospital in 5 ml vials containing 60 million cells at a temperature below 8 degrees. The contents of each vial were injected into the patients in 100 ml of normal saline over a period of 20 minutes. After each injection, 100 mg of hydrocortisone was slowly injected into each patient.

2.4.Post injection Follow up

All patients were hospitalized for 24 to 48 hours after the injection, and vital signs were checked every 1 to 3 hours. Twenty-four hours after the injection, blood and urine biochemical tests, including CBC, creatinine, BUN, blood sugar, bilirubin, liver enzyme levels (including AST and ALT), urine U/A test, and ESR and CRP inflammatory tests, were repeated before the patients were discharged. All tests were repeated one month after the injection.

2.5.Statistical analysis

Data are expressed as mean ± standard deviation. Data before and after mesenchymal stem cell injection over time were compared using a repeated measures ANOVA. A P value of < 0.05 was considered to be statistically significant. All data were analyzed with SPSS 16.0 software.

3. Results

The mean and standard deviation of the Hematological and Biochemical Parameters measured at four time points and the trends of their changes are illustrated in Table 1 and Figure 1. The analysis showed that the differences in means for all inflammation and infection, liver function, kidney function and blood tests over the four time points were not statistically significant ($P>0.05$).

Table 1: Mean and Standard Deviation of Hematological and Biochemical parameter before and Post-Infusion

	Before intervention	1 Day after intervention	A Few days after intervention	1 month after intervention	
Parameters	Mean ± standard deviation				p-value
White Blood Cell Count	7.42±1.76	7.92±2.43	7.06±1.37	8.62±3.77	0.51
Erythrocyte Sedimentation Rate	11.4±7.70	6.20±3.11	18.80±21.14	12.40±18.20	0.50
C-Reactive Protein	4.16±5.51	8.50±15	25.20±31.57	8.02±14.53	0.52
Aspartate Aminotransferase	20.80±3.56	17.40±3.64	29.5±15.86	24.20±8.01	0.36
Alanine Aminotransferase	19.4±6.84	17±3.53	30±19.51	21.6±3.84	0.37
Blood Urea Nitrogen	13.36±3.52	13.66±2.29	12.5±1.87	15±5.83	0.39
Creatinine	0.86±0.11	1±0.12	0.89±0.07	0.97±0.02	0.12
Hemoglobin	14.66±1.93	13.74±1.42	14.7±1.95	15.44±1.65	0.25
Platelet Count	302±103.68	278±61.81	246.6±20.37	290.6±71.55	0.23

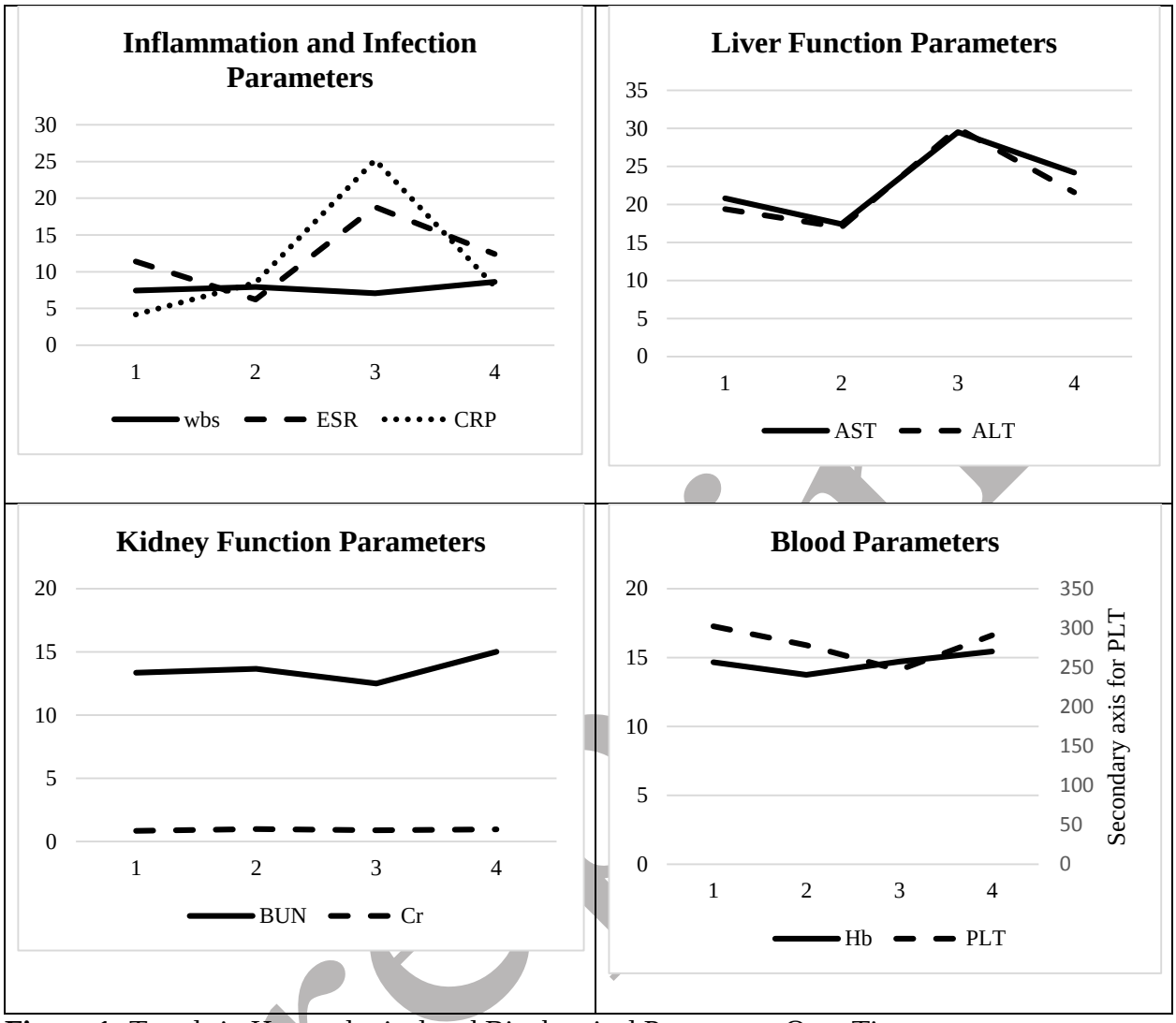

Figure 1: Trends in Hematological and Biochemical Parameters Over Time

4. Discussion

Numerous studies have been conducted in various regions to assess the safety of using MSCs in patients with MS. These studies have shown considerable variability in several factors, including the source of the MSCs, the number of cells injected, the frequency of injections, and the method of administration (whether intravenous, intrathecal, or both). Additionally, the medications taken

by patients, the safety tests performed, and the duration of follow-up after injection have all differed across studies. As a result, it's not surprising that there are discrepancies in the reported complications related to MSCs injections in these patients. Riordan et al., (2018) involving 20 patients, each participant received seven doses of UC-MSCs, with each dose consisting of 20×10^6 cells, totaling 140×10^6 cells. Throughout the one-year follow-up of patients who participated in the study, no serious adverse events were reported. The side effects observed were mild to moderate, including headaches, fatigue, and cardiovascular and gastrointestinal issues, with a total of 6 moderate and 66 mild cases documented. Importantly, none of these side effects persisted beyond one year, and none led to the discontinuation of the study [17]. Lu et al., (2020) the safety of infusions of UC-MSCs in patients with progressive relapsing MS was examined. Five patients received low-dose MSCs, with four intravenous infusions and three intrathecal injections. The treatment protocol included an initial dose of 40×10^6 cells on the first day, followed by 20×10^6 cells intravenously combined with 20×10^6 cells intrathecally every seven days. Over a 10-year follow-up period, no serious complications, such as organ dysfunction or tumor formation, were reported. Consequently, the researchers concluded that intravenous and intrathecal infusions of UC-MSCs appear to be safe and feasible [18]. Jamali et al. (2024) conducted a study involving 35 patients with MS to evaluate the safety and efficacy of UC-MSCs injections. The patients were divided into two groups: the first group received two doses of UC-MSCs, while the second group received one dose. Additionally, both groups were administered supernatant fluid derived from MSCs three months after the initial injection. The results indicated that all patients tolerated the MSCs injections well, with no severe side effects reported during the one-year follow-up. However, mild side effects, such as headaches and mild fever, were noted, but these resolved within the first month [19]. In this study involving 5 patients, the one-month follow-up revealed

that 3 patients reported no complaints at all. One patient was hospitalized twice: once due to fever and chills, and another time for a lung infection, but he received treatment and recovered. Additionally, another patient was monitored in the hospital for several days due to abnormal test results following the injection. However, this patient was discharged once the results improved and did not report any new issues unrelated to their existing condition during the one-month follow-up. Among the 5 patients in the study, 2 experienced changes in their laboratory results, specifically in inflammatory tests such as ESR, CRP, and WBC count. Of these two patients, one showed no findings on examination or culture results indicating infection and was discharged in good general condition after necessary evaluations. Within a month, their laboratory results returned to nearly normal levels. The second patient was hospitalized initially with fever, chills, and laboratory changes following the injection, but they recovered within 24 hours. However, this patient was treated again about two weeks later for pneumonia. It remained unclear whether this infection was related to the injection or was merely coincidental. Similar to the previous study, fever was noted as a complication in one patient. Additionally, changes in inflammatory tests were observed in two patients, which could potentially be linked to the injection or a mild non-severe infection. Overall, no severe complications were reported among the 5 patients during the one-month follow-up, suggesting that injecting UC-MSCs may be feasible and safe for patients with multiple sclerosis in the short term. However, to draw more definitive conclusions, a more comprehensive study involving a larger population and extended follow-up period is necessary.

The limitations of this study include a small sample size, which may affect the generalizability of the results, and an insufficient follow-up duration to assess long-term safety and efficacy. Additionally, the lack of an appropriate control group may lead to bias in evaluating treatment

effects. Variability in treatment protocols and safety assessment methods could also result in inconsistent outcomes, and patient selection may limit the applicability of the findings.

In summary, we investigated the feasibility and safety of administering 60 million UC-MSCs to patients with MS who were also undergoing treatment with monoclonal antibodies. Our results indicate that this method could be a promising strategy for improving treatment outcomes in MS patients. Nonetheless, additional research with larger sample sizes and extended follow-up is essential to comprehensively evaluate the long-term safety and effectiveness of UC-MSCs in this setting.

Acknowledgment

The authors wish to thank all the staff of Central Research Laboratory and neurology department of Baqiyatallah University of Medical Sciences Tehran, Iran., for their cooperation in implementing experimental procedures, analysis of the data and Cell Tech Pharmed Company, Tehran, Iran., for providing the UC-MSCs.

Authors' Contributions

Conceptualization: J.H.N
Data curation: M.M.E & H.E.G.G
Formal analysis: M.R & A.G.A
Methodology: M.R, A.G.A & B.J.K
Software: M.R, A.G.A & B.J.K
Validation: M.R and M.M.M
Investigation: M.M.E & H.E.G.G
Writing - original draft: M.M.E & H.E.G.G
Writing - review & editing: M.M.E, M.M.M, H.E.G.G, M.R, B.J.K, A.G.A and J.H.N

Funding

This study was fully sponsored by the Baqiyatullah Hospital, Tehran, Iran.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The study was approved by the Baqiyatullah university of medical science, Tehran, Iran (ethical code: IR.BMSU.BAQ.REC.1402.031, IRCT20230806059052N1)

Consent for publication

N/A.

Competing interests

The authors declare that they have no conflicts of interest.

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