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*Research Article*

# Mesenchymal Stem Cells Derived from Different Sources and Their Secretome in Multiple Sclerosis Therapy: A Single Center Experience

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## Abstract

Multiple Sclerosis (MS) is a chronic autoimmune, inflammatory and demyelinating disease of the Central Nervous System (CNS), primarily affecting young adults. While current treatments can help manage symptoms, there remains a need for novel therapeutic approaches. Mesenchymal Stem Cell (MSC) therapy has emerged as a promising strategy for various neurodegenerative disorders due to its immunomodulatory properties and the therapeutic potential of its secretome. The MSC secretome comprises growth factors, cytokines and Extracellular Vesicles (EVs) that contribute to tissue repair and immune regulation.

This study was conducted on 10 patients with MS over a one-year period starting in November 2023. Patients were divided into two groups: Group A received bone marrow-derived MSCs and their secretome (BM-MSCs), while Group B received Umbilical Cord-Derived MSCs (UC-MSCs).

Patients in Group B demonstrated greater improvement in the Expanded Disability Status Scale (EDSS), as well as enhanced performance in speed and sustained attention indices, compared to Group A. These findings suggest that UC-MSCs may offer superior therapeutic benefits in the treatment of MS compared to BM-MSCs.

**Keywords:** Multiple Sclerosis; Mesenchymal Stem Cells; Stem Cell Therapy; Cord Tissue; Bone Marrow; Secretome

## Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease of the Central Nervous System (CNS) brain, spinal cord and optic nerves, characterized by demyelination and neurodegeneration [1]. It is one of the most common autoimmune diseases affecting the CNS, leading to a wide range of neurological symptoms and disabilities. MS affects approximately 2.8 million people globally, with higher prevalence in temperate regions and among individuals of Northern European descent. The disease typically manifests in young adults aged 20-40 years, with a higher incidence in women than men [2]. Myelin acts as an insulating layer, facilitating the conduction of nerve impulses. In MS, immune cells, particularly T lymphocytes, invade the CNS and initiate an inflammatory cascade that targets myelin. This inflammatory process leads to demyelination, disrupting nerve impulse transmission and causing neurological dysfunction. Over time, chronic inflammation can also lead to axonal loss and irreversible neurological damage [2].

Symptoms of MS vary widely depending on the location and extent of nerve damage but commonly include sensory disturbances (such as numbness or tingling), motor impairments (weakness or spasticity), visual disturbances (optic neuritis), balance and coordination problems, fatigue and cognitive deficits.

Diagnosis of MS involves a combination of clinical evaluation, neurological examination and diagnostic tests such as Magnetic Resonance Imaging (MRI) to detect characteristic lesions in the CNS. Additional tests, such as cerebrospinal fluid analysis for oligoclonal bands and evoked potentials, may also support the diagnosis [1,2]. Research into MS continues to explore its underlying mechanisms, potential triggers and more effective treatments, with the ultimate goal of finding a cure and improving outcomes for those affected by this complex neurological condition.

Mesenchymal Stem Cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types of the mesodermal lineage, such as osteoblasts (bone cells), chondrocytes (cartilage cells) and adipocytes (fat cells). They are also known for their immunomodulatory and regenerative properties, MSCs were first identified in the bone marrow as adherent, fibroblast-like cells capable of forming colonies in culture [3]. Since their discovery, MSCs have been isolated from various tissues, including adipose tissue, umbilical cord wharton's jelly and Bone Marrow [4]. Despite their diverse tissue origins, MSCs share common characteristics such as surface marker expression (e.g., CD34, CD73, CD90 and CD105) and differentiation potential under appropriate conditions.

Umbilical Cord tissue and Bone Marrow MSCs have, over time, gained significant interest for their capacity to differentiate into specific cell types and release growth factors that promote angiogenesis and tissue regeneration, what undermines their utility in tissue engineering and repair and their potential therapeutic applications due to their ability to promote tissue repair and modulate immune responses [5]. They have been investigated in treating conditions such as bone defects, cartilage injuries and myocardial infarction, through their immunomodulatory effects and the secretion of soluble factors, MSCs are a powerful candidates for treating autoimmune diseases (e.g., multiple sclerosis, rheumatoid arthritis) and inflammatory disorders (e.g., graft-versus-host disease) [4].

Numerous clinical trials have explored the safety and efficacy of MSC-based therapies across various medical conditions and specially the efficacy of their secretion Exosomes [6]. The secretome refers to the complex mixture of proteins, lipids and nucleic acids actively secreted by cells into their extracellular environment. This includes soluble factors such as cytokines, growth factors and Extracellular Vesicles (EVs), particularly exosomes. They play crucial roles in intercellular communication, immune modulation and tissue homeostasis [6]

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Understanding their origin and biological roles provides insights into their therapeutic potential and relevance in various fields of biomedical research. Exosomes carry a cargo of bio-molecular that reflects the physiological state and cellular origin. This cargo includes proteins (such as tetraspanins, heat shock proteins and cytoskeletal proteins), lipids (phospholipids, cholesterol) and nucleic acids (mRNA, miRNA, other non-coding RNAs and DNA fragments) [7]. The composition of exosomes can vary depending on the cell type, cellular stress and environmental cues. Importantly, the cargo of exosomes can influence recipient cell behavior through mechanisms such as receptor-mediated signaling, direct fusion with the plasma membrane or internalization via endocytosis. Exosomes derived from specific cell types, such as stem cells or immune cells (e.g., mesenchymal stem cell-derived exosomes) have shown regenerative properties and immunomodulatory effects in preclinical and clinical studies. In addition to their physiological roles, exosomes have emerged as potential biomarkers for disease diagnosis and prognosis due to their stability and presence in various bio-fluids [8].

One of the key mechanisms underlying MSCs' therapeutic potential in MS is that MSCs exert immunosuppressive effects by inhibiting the proliferation and function of T cells, B cells and dendritic cells and by promoting the generation of regulatory T cells (Tregs) [9]. This immunomodulatory activity helps to attenuate the autoimmune attack on myelin and reduce neuro-inflammation in MS Beyond immunomodulation, MSCs possess regenerative properties that support tissue repair and neuro-protection in MS. Preclinical studies have demonstrated that MSCs can promote re-myelination, enhance axonal survival and stimulate endogenous repair mechanisms in animal models of MS. These effects are mediated through paracrine secretion of growth factors, cytokines and extracellular vesicles (including exosomes) that promote neuronal survival, angiogenesis and modulation of the microenvironment.

**Materials and Methods**

*a. Study design and Patient Selection*

This is a retrospective, experimental study. Ten patients with MS were divided into two groups (each n = 5): Group A (BM-MSC + Secretome) and Group B (UC-MSC+ Secretome). The administration of treatment took place on an outpatient basis without the need for hospital admission. Two sessions were performed per patient at day 0 and the same injection were repeated after six months, the number of cells were $1x10^8$ diluted in 100 ml of secretome and cells were at passage three. All participants were aged between 47 and 60 years old (median age ± 53.5 years old) and were refractory to the usual conventional treatments.

All procedures conducted during the study were carried out in compliance with institutional ethical standards. Reviva Regenerative Medicine Center and the Middle East Institute of Health-University Hospital Ethics Review Boards approved the retrieval of all MSC collections (Approval Reference Number CTU-009-10) and all patients were asked to read, approve and sign an informed consent form prior to any participation.

Patient recruitment is a critical and often challenging phase in clinical research, crucial for ensuring the validity study findings. Effective recruitment strategies are essential to achieve adequate sample sizes and maintain rigorous scientific standards. Inclusion criteria typically define characteristics that participants must possess, such as age range, disease state or previous treatment history, to ensure that the sample is appropriate for the study's objectives. Conversely, exclusion criteria identify conditions or factors that disqualify potential participants, such as comorbidities, concurrent medications or other factors that could interfere with the study outcomes. Strategies such as targeted outreach through healthcare providers, community engagement and digital platforms can enhance recruitment efforts. When designing or evaluating studies on Mesenchymal Stem Cell (MSC) infusion for Multiple Sclerosis (MS), we used specific inclusion and exclusion criteria to ensure that participants are suitable for the interventions and that the results are meaningful.

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| 1. **Diagnosis of Multiple Sclerosis:**<br>○ Participants must have a confirmed diagnosis of MS, often substantiated by clinical criteria and MRI findings. This can include relapsing-remitting MS (RRMS), secondary-progressive MS (SPMS) or primary-progressive MS (PPMS) [10]<br>2. **Age Range:**<br>○ Generally, participants are required to be adults, often between 18 and 65 years old, to ensure they are within a feasible range for assessing treatment efficacy and safety [10]<br>3. **Stable Disease:**<br>○ Patients are typically required to have stable disease activity, meaning no significant relapses or worsening of symptoms for a specified period before enrollment [11]<br>4. **Previous Treatments:**<br>○ Participants may need to have experienced inadequate responses to conventional MS therapies or they may need to be on a stable regimen of disease-modifying drugs [12]<br>5. **Informed Consent:**<br>○ Individuals must be capable of providing informed consent, indicating they understand the potential risks and benefits | 1. **Severe Comorbidities:**<br>○ Individuals with severe or unstable comorbid conditions, such as severe cardiovascular disease or uncontrolled diabetes, are typically excluded to avoid confounding factors that could affect treatment outcomes or safety [14]<br>2. **Active Infection or Malignancy:**<br>○ Excluded to ensure that any observed effects are attributable to the MSC treatment rather than underlying diseases [15]<br>3. **Pregnancy or Breastfeeding:**<br>○ Women who are pregnant or breastfeeding are usually excluded due to potential risks to the fetus or infant and lack of safety data in these populations [16]<br>4. **Recent Use of Certain Medications:**<br>○ Individuals currently using high-dose immunosuppressive drugs or other medications that could interfere with MSC function or safety might be excluded to avoid interactions that could skew results [17]<br>5. **Inadequate Organ Function:**<br>○ Exclusion criteria often include significant |

| | |
|---|---|
| of the MSC treatment [13] | impairment in organ function, such as severe renal or hepatic dysfunction, which could impact the safety and efficacy of MSC treatment [18] |

**Table 1:** Study details with inclusion criteria and exclusion criteria.

*b. Collection of MSCs*

Ten collections from bone marrow and umbilical cord were made. BM aspirates were obtained by puncturing the iliac crest of participants ranging in age from 30 to 60 years at the hematology department of the Middle East Institute of Health University Hospital. UC units were collected from the unborn placenta of full-term deliveries in a multiple bag system containing 17 ml of citrate phosphate dextrose buffer (Cord Blood Collection System; Eltest, Bonn, Germany) and processed within 24 h of collection.

c. *Isolation and culture of MSC from Bone Marrow*

BM aspirates were obtained by puncturing the iliac crest of participants ranging in age from 30 to 60 years. The aspirates were diluted 1:5 with 2 mm Ethylenediaminetetraacetic Acid (EDTA)-Phosphate-Buffered Saline (PBS) (Sigma-Aldrich). The Mononuclear (MNC) fraction was isolated by density gradient centrifugation at 435 g for 30 min at room temperature using Ficoll Hypaque-Plus solution (GE Healthcare BioSciences Corp) and seeded at a density of $1 \times 10^6$ cells per $cm^2$ into T75 or T175 cell culture flasks (Sigma, Aldrich). Within 3 days after isolation, the first change of medium was accomplished. The resulting fibroblastoid adherent cells were termed BM-derived fibroblastoid adherent cells (BM-MSCs) and were cultivated at 37°C at a humidified atmosphere containing 5% $CO_2$. The expansion medium consisted of Dulbecco's modified Eagle's medium-alpha modification (Alpha-MEM) + 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 5% penicillin streptomycin-amphotericin B solution (PSA: Hyclone; GE healthcare, Logan, UT, USA). BM-MSCs were maintained in Alpha-MEM + 10% FBS and 5% PSA until they reached 70 to 90% confluency. Cells were harvested at subconfluence using Trypsin (Sigma-Aldrich). Cells at the second passage and thereafter were replated at a mean density of $1.3 \pm 0.7 \times 10^3/cm^2$.

d. *Isolation and Culture of MSC from Human Umbilical cord Wharton Jelly*

Umbilical cord was collected in PBS supplemented with 10% PSA and transferred to the laboratory in a maximum of 12 h. After washing, cord samples were cut into 1-2 cm sections, the umbilical vessels were removed (artery and veins) and Wharton's jelly was collected and minced into pieces, then digested by collagenase overnight and then cultured in flasks. Nonadherent cells were removed 12 h after initial plating. Cells were cultured in the same conditions as BM-MSCs. Adherent fibroblastoid cells only (UCMSC) appeared as CFU-F and were harvested at subconfluence using Trypsin (Sigma-Aldrich). Cells at the second passage and thereafter were replated at a mean density of $3.5 \pm 4.8 \times 10^3/cm^2$.

*e. Conditioned Media (CM) Preparation*

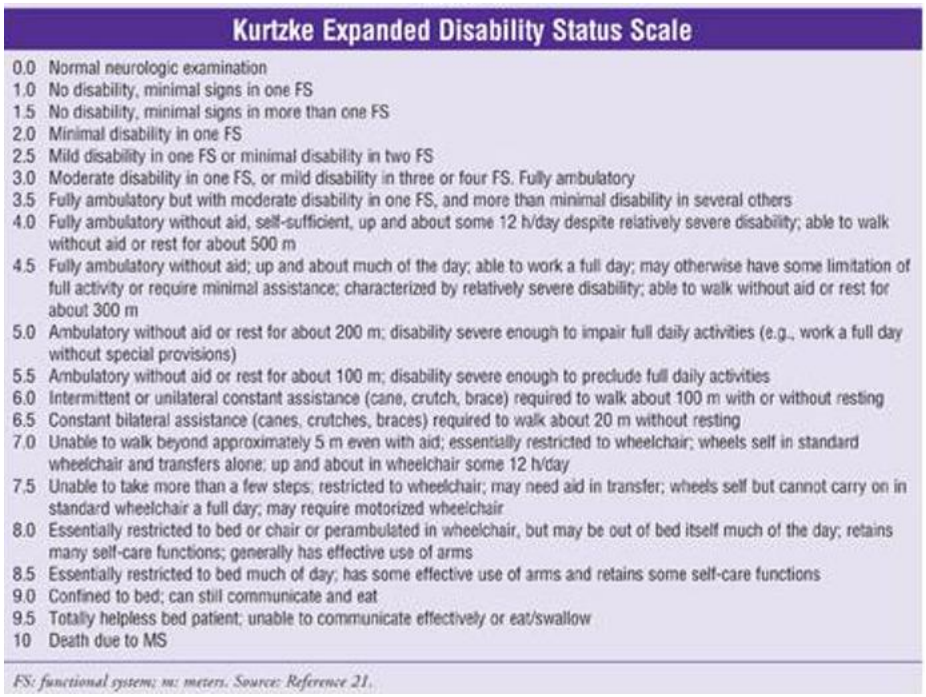
All MSCs were cultured with DMEM/F12 supplemented with 5% PSA at subconfluency. After 48h of incubation at 37 °C at a humidified atmosphere containing 5% $CO_2$, the supernatant, containing all released cytokines and chemokines was collected.

*f. Patients Screening*

*Expanded Disability Status Scale*

The primary clinical outcome measure for evaluating multiple sclerosis in clinical trials has been Kurtzke's Expanded Disability Status Scale (EDSS) (Fig. 1). It is a widely used clinical tool to assess the severity of disability in individuals with Multiple Sclerosis (MS). Developed by John F. Kurtzke in 1983, the EDSS provides a quantitative measure of disability based on neurological examination findings.

EDSS evaluation criteria and severity of MS going from 0 to 10. 0 being normal and 10 death.



**Kurtzke Expanded Disability Status Scale**

| | |
|---|---|
| 0.0 | Normal neurologic examination |
| 1.0 | No disability, minimal signs in one FS |
| 1.5 | No disability, minimal signs in more than one FS |
| 2.0 | Minimal disability in one FS |
| 2.5 | Mild disability in one FS or minimal disability in two FS |
| 3.0 | Moderate disability in one FS, or mild disability in three or four FS. Fully ambulatory |
| 3.5 | Fully ambulatory but with moderate disability in one FS, and more than minimal disability in several others |
| 4.0 | Fully ambulatory without aid, self-sufficient, up and about some 12 h/day despite relatively severe disability; able to walk without aid or rest for about 500 m |
| 4.5 | Fully ambulatory without aid; up and about much of the day; able to work a full day; may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability; able to walk without aid or rest for about 300 m |
| 5.0 | Ambulatory without aid or rest for about 200 m; disability severe enough to impair full daily activities (e.g., work a full day without special provisions) |
| 5.5 | Ambulatory without aid or rest for about 100 m; disability severe enough to preclude full daily activities |
| 6.0 | Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 m with or without resting |
| 6.5 | Constant bilateral assistance (canes, crutches, braces) required to walk about 20 m without resting |
| 7.0 | Unable to walk beyond approximately 5 m even with aid; essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 h/day |
| 7.5 | Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair |
| 8.0 | Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms |
| 8.5 | Essentially restricted to bed much of day; has some effective use of arms and retains some self-care functions |
| 9.0 | Confined to bed; can still communicate and eat |
| 9.5 | Totally helpless bed patient; unable to communicate effectively or eat/swallow |
| 10 | Death due to MS |

*FS: functional system; m: meters. Source: Reference 21.*

**Figure 1:** Kurtzke expanded disability status scale.

*Symbol Digit Modalities Test*

The Symbol Digit Modalities Test (SDMT) (Fig. 2) examines processing speed and sustained attention by primarily assessing complex visual scanning and tracking.138 Although performance is susceptible to difficulties with visual acuity or scanning, given that the test is rapidly and easily administered and scored and generally well-tolerated, the oral version of the test has been recommended to detect patients at-risk for MS-related cognitive decline.

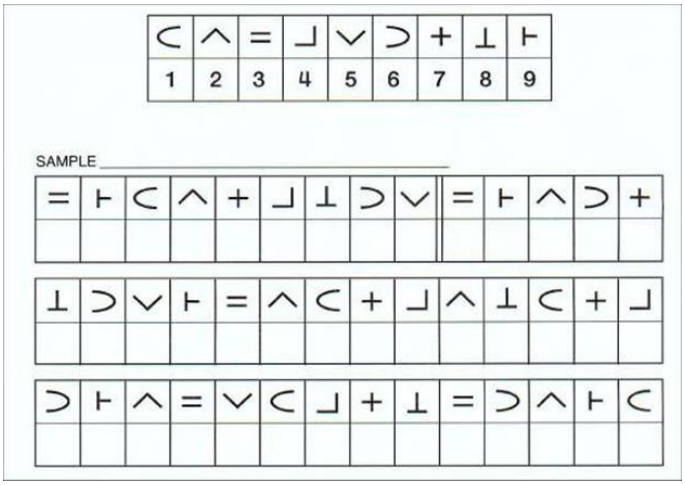



**Figure 2:** Symbol digit modalities test sample.

**Results**

Ninety-eight subjects were screened between November 2021 and November 2023, with fourteen (14.3%) meeting all eligibility criteria. Three subjects declined participation following detailed discussions and one participant withdrew consent shortly after recruitment for personal reasons. Final Number analyzed were 10 participants.

*a. Participant Characteristics*

All participants in the treatment arm had secondary progressive MS with mean disease duration of 14.4 years (SD 7.9). Mean age was 48.8 years (SD 4.1), sex ratio was 3:7 (F:M) (table 2). Nine participants had a history of clinical optic neuritis, affecting 13 eyes (65%); the remainder had electrophysiological evidence only for optic nerve involvement. One participant had been previously

treated with beta-interferon for one year with treatment discontinued due to disease progression two years before recruitment to this trial. Eight healthy controls were also recruited, matched for age (mean 43 years [SD 3.5], Student's t-test, p = 0.1124) and sex (2:6 [F:M], Fisher's exact test, p = 0.618).

*b. MSC Culture Results*

MSCs were successfully isolated and cultured to the target dose from all bone marrow aspirates (mean total cultured dose = 2.0 $\times 10^6$ cells / kg; range 1.1 to 3.7 $\times 10^6$ cells / kg). Mean culture duration was 24 days (range 20 to 30 days) with a mean cell-doubling time of 1.5 days (range 1.3 to 2.0 days). All MSC cultures were characterized to ISCT definition criteria, had normal karyotype by array-CGH and no evidence of pathogenic contamination.

*c. Baseline Assessment Results*

- Patient Based measures

Mean EDSS was 6.1 (range 5.5 to 6.5). All patients had higher MS Functional Composite (MSFC) disability scores than the National Multiple Sclerosis Society (NMSS) Task Force reference population mean (Mean MSFC z-score -1.5; range -0.4 to -5.4). Eight patients had Beck's Depression Inventory-II scores in the range for minimal depression (0 - 13), one patient scored in the range for mild depression (14 - 19) and one patient scored in the range for moderate depression (20 - 28).

- Comparative performance between patients and controls

Normalized brain volume was reduced by 11.1% in patients compared to controls (Absolute values: 1486 $cm^3$ [SD 75 $cm^3$] vs. 1671 $cm^3$ [SD 53 $cm^3$]; p < 0.00005). There were no significant differences in brain imaging MTR measures between controls and patients although there was a trend to higher MTR values in controls. Whole brain (mean) MTR was reduced by 5.3% in patients (Absolute values: 44.52 pu [SD 6.56 pu] vs. 47.02 pu [SD 4.48 pu]; p = 0.3976). Grey matter (mean) MTR was reduced by 7.2% in patients (Absolute values: 35.20 pu [SD 7.34 pu] vs. 37.95 pu [SD 5.14 pu]; p = 0.4061). White matter (mean) MTR was reduced by 4.6% in patients (Absolute values: 46.37 pu [SD 6.92 pu] vs. 48.63 pu [SD 4.49 pu]; p = 0.4623) (Fig. 3).

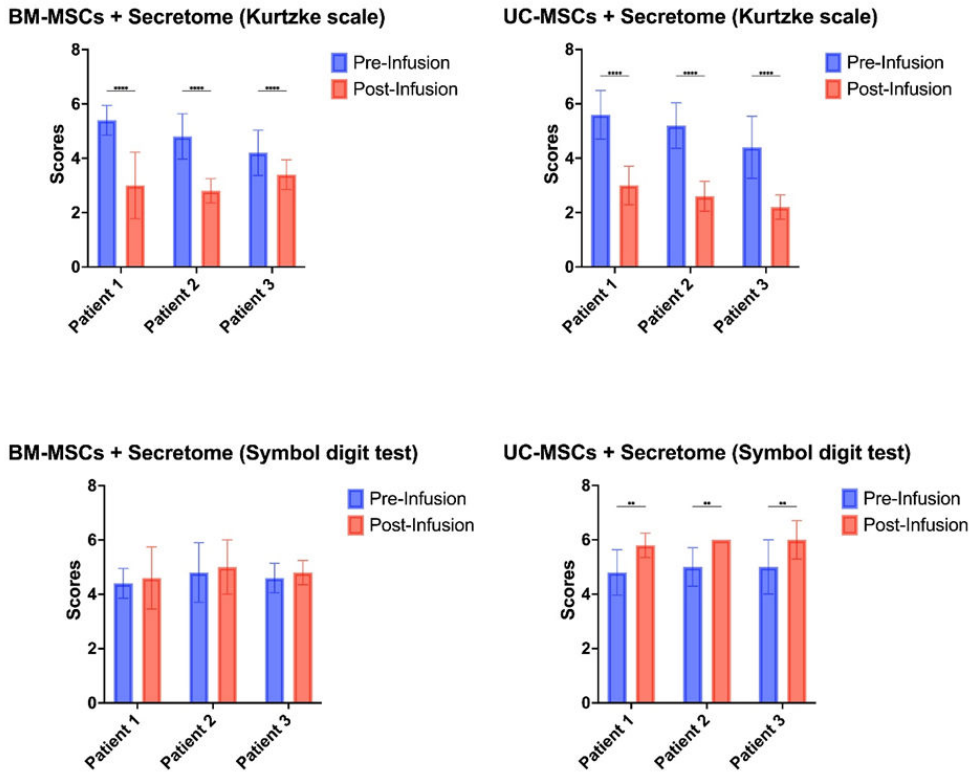



**Figure 3:** The Disability Status Scale demonstrates a marked improvement following injection of mesenchymal stem cells (MSCs) and their secretome derived from bone marrow (BM) and umbilical cord (UC) sources. Additionally, the Symbol Digit Modalities Test (SDMT) indicates enhanced processing speed and sustained attention, primarily by assessing complex visual scanning and tracking abilities.

**Discussion**

This study explored the safety and tolerability of administering Bone Marrow-Derived (BM-MSCs) and umbilical cord-derived mesenchymal stem cells (UC-MSCs), along with their secretome, in patients diagnosed with Multiple Sclerosis (MS). Throughout the follow-up period and one year post-treatment, no serious adverse events were reported.

Our findings, in alignment with prior research, indicate potential clinical benefits such as stabilization of Expanded Disability Status Scale (EDSS) scores, symptom relief, reduced neurological dysfunction and lowered systemic inflammation. These outcomes may reflect a reparative effect of MSC therapy in MS patients [10,11] . Additionally, we observed encouraging trends in cognitive and psychological parameters, as well as moderate improvements in neurological assessments, functional disability and unaided mobility. Enhanced brain connectivity was also noted, alongside reduced dependency in daily activities such as eating, with patients maintaining clear communication.

Despite these promising results, the small sample size limits the generalizability of our conclusions. Further investigations with larger cohorts are essential to validate these observations and better understand the therapeutic potential of MSCs in MS.

Previous clinical trials have consistently demonstrated the safety profile of MSC therapy in MS [11,12]. For example, Riordan, et al., reported that repeated UC-MSC infusions led to improved EDSS scores, reduced neurological impairment and inactive MRI lesions after one year [10]. Similarly, Lublin, et al., administered placental-derived MSCs (PDA-001) to 16 MS patients, observing stable or improved EDSS scores and no progression in lesion count during the follow-up period [13].

Our data also suggest that MSCs and their secretome, derived from BM and UC sources, may offer therapeutic benefits for cognitive and psychological symptoms in MS-a domain with limited treatment options. Cognitive dysfunction affects 50–70% of MS patients and significantly impairs quality of life. Its prevalence varies depending on disease stage and study design [14]. Moreover, newly diagnosed individuals are at increased risk of psychological disturbances [15]. A large-scale survey by Jones et al. involving 4,178 MS patients revealed that those with Secondary Progressive MS (SPMS) were more likely to experience depression compared to other MS subtypes [9]. Given the absence of definitive treatments for cognitive and psychological impairments in MS, exploring novel interventions like MSC therapy is of high clinical relevance. To our knowledge, no prior studies have specifically examined the impact of MSCs on these domains in MS patients, although animal studies have shown memory improvements following MSC administration [11,16].

One year post-treatment, Diffusion Tensor Imaging (DTI) analysis of Normal-Appearing White Matter (NAWM) revealed reduced Radial Diffusivity (RD) in the left hemisphere. Changes in DTI metrics within lesional areas may reflect resolution of inflammation and edema, as well as varying degrees of tissue repair, influencing RD and Axial Diffusivity (AD) values [12]. Amanat, et al., demonstrated that UC-MSC therapy significantly enhanced white matter integrity in children with cerebral palsy over a one-year period [17]. Fernandez, et al., reported clinical and radiological improvements in MS patients treated with autologous adipose-derived MSCs [18]. In contrast, Yamout, et al., observed clinical benefits but no radiological changes following BM-MSC therapy in MS patients [19,20].

Functional MRI (fMRI) in our study showed increased connectivity among networks involved in memory, spatial processing and the Default Mode Network (DMN), correlating with improved cognitive performance and memory scores. These results suggest that UC-MSC infusion may promote neural repair and regeneration, contributing to measurable improvements in cognition, disability status and performance on the Symbol Digit Modalities Test. This aligns with emerging evidence supporting MSCs' role in enhancing neurogenesis and synaptic plasticity [21].

Our findings add to the growing body of literature on stem cell therapy in MS, emphasizing the need for larger-scale studies to confirm its neurological benefits and long-term efficacy. The DMN includes regions such as the medial Prefrontal Cortex (mPFC), hippocampus, Posterior Cingulate Cortex (PCC), Lateral Parietal Cortex (LPC) and precuneus, which exhibit synchronized activity during internally focused tasks like self-reflection and autobiographical memory retrieval. Abnormal DMN activity has been documented in MS patients [22]. Petrou, et al., conducted a study involving 48 patients with progressive MS, where intrathecal MSC administration led to reduced relapse rates, improved MRI lesion profiles and enhanced motor network activity, along with positive outcomes in cognitive assessments and fMRI scans [23].

The therapeutic effects of MSCs may arise from a combination of systemic immunomodulation and localized action at sites of neural damage [24,25]. Although MSCs may not persist long-term *in-vivo*, their beneficial effects are likely mediated through the secretion of paracrine factors that regulate inflammation and tissue homeostasis [25].

In our study, we observed a reduction in CD20/CD19 expression on B lymphocytes following cell therapy, indicating a decrease in B cell populations. This is noteworthy given the role of B cells in MS pathogenesis. There is growing interest in B cell-targeted therapies, including MSCs, which can suppress B cell activation and promote regulatory B cells (Bregs) [26]. However, Rituximab (RTX) was administered one month prior to MSC infusion and its long-lasting pharmacological effects typically extending beyond six months make it unethical to discontinue. Therefore, the observed B cell depletion is likely attributable to RTX rather than MSC therapy [27]. We chose Intravenous (IV) administration for its safety, minimal invasiveness compared to intrathecal delivery and ability to facilitate systemic distribution of anti-inflammatory and anti-fibrotic factors. IV infusion also allows for repeated dosing within a short treatment window [17,28].

## Conclusion

This trial suggests that BM-MSCs and UC-MSCs, along with their secretome, may offer short-term neuroprotective and clinical benefits in MS patients. However, due to the limited sample size and follow-up duration, further studies involving larger cohorts, repeated dosing and objective biomarkers are essential to confirm these findings. We propose that the timing of MSC administration is a critical factor in optimizing therapeutic outcomes. Administering high-dose MSCs during the early inflammatory phase of MS may enhance their effectiveness as a novel treatment strategy.

## Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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## Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Ethical Statement

The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore was exempt.

## Informed Consent Statement

Informed consent was obtained from all participants included in the study.

## Authors' Contributions

All authors contributed equally to this paper.

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