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The Efficacy of Intralesional Transplantation of Autologous Bone Marrow Mesenchymal Stem Cells in Patients with Traumatic Complete Spinal Cord Injury at Dorsal Spine

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ABSTRACT

Background: Spinal cord injury (SCI) constitutes an individual, social, and economic devastating problem.

Objective: This investigation aimed to assess the safety and prospective benefits of intralesional transplantation of autologous bone marrow mesenchymal stem cells (MSCs) as a treatment option for patients with traumatic complete SCI at the dorsal spine, where spontaneous recovery has been unsuccessful.

Patients and methods: 45 patients who had complete traumatic SCI at the dorsal spine were hospitalized at Al-Azhar University Hospital. They underwent intralesional transplantation of autologous MSCs after failure of natural repair as part of this prospective interventional trial. A complete neurological examination and routine laboratory and radiological investigations, including plain X-rays, CT, MRI of dorsal spines, EMGs, and NCVs, were performed for all patients to assess the level and completeness of the injury. ASIA impairment scale was used to evaluate the neurological state.

Results: All cases were initially classified as ASIA grade A, indicating complete spinal cord injury. The average injury duration was 16.04 ± 8.3 months. Mild neurological improvement was noticed in 4.5% who progressed to grade C, 33.3% to grade B, while 62.2% remained at grade A. Complications were minimal, with 8.9% experiencing allergic skin reactions. A significant inverse correlation was found between injury duration and clinical outcome, suggesting better recovery in patients with shorter injury durations.

Conclusion: Clinical outcome improvement was noticed in using intralesional transplantation of autologous MSCs as early as possible after failure of natural repair and before the occurrence of adhesive gliosis, which prevents the growth of axons and regeneration.

Keywords: Mesenchymal stem cells, Spinal cord injury, Transplantation of MSCs.

INTRODUCTION

Traumatic SCI is a sudden, unanticipated catastrophe that is considered a disastrous event, leading to a high disability rate. In the Middle East and North Africa, there are 23–27 cases of SCI for every million people. Patients with SCI frequently experience disruptions in their sensory, motor, and autonomic systems, which have disastrous consequences for the economy, society, and person. Therefore, developing a treatment that works for these people is essential [(1)].

Traumatic SCI is characterized by direct mechanical injury with shattered, displaced bone pieces and discs around the spinal cord. Neural apoptosis, spinal cord edema, oxidative stress, inflammation, and electrolyte imbalance are examples of secondary injuries [(2)]. Devastating tissue damage, axonotmesis, demyelination, Wallerian degeneration, syringomyelia, and glial scar formation can result from primary or secondary traumas [(3)].

Numerous therapies, including surgery and medication, have been used to treat traumatic SCI, however none of them are especially successful [(4)]. Most recently, there has been discussion on the potential of a wide variety of stem cell types for transplantation, including neural stem cells [(5)], Schwann cells, embryonic stem cells, mesenchymal stem cells (MSCs), and induced pluripotent stem cells [(6)], which reported an efficient treatment for traumatic SCI in animal models. MSCs possess the capacity to secrete neuroprotective cytokines, such as vascular endothelial growth factor (VEGF), glial cell line-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF), in addition to the ability to differentiate and replace damaged cells. These cytokines facilitate neural regeneration, strengthen axon growth, and regenerate damaged neurons [(7)].

Currently, animal models of SCI have validated the safety and effectiveness of MSC transplantation, which may be as effective in treating SCI in humans as they are in animals. Through meta-analysis, the effectiveness and safety of MSCs in treating individuals with SCI have been comprehensively evaluated. However, cytotherapy is still in its infancy due to the many differences and uncertainties that exist in clinical trial protocols concerning subject selection, cellular type, transplantation time, administration dose, and delivery method. Therefore, to develop a safe and effective treatment for SCI, well-designed and standardized clinical research must be conducted [(8)].

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MATERIALS & METHODS

This prospective interventional trial was conducted on 45 patients.

Inclusion criteria: Older than 18 years of any sex, had an ASIA impairment scale grade of A at the dorsal spine, and had failed spontaneous repair after six months of injury.

Exclusion criteria: Open wounds, active infectious diseases, pregnant women, neurodegenerative diseases, chronic illnesses, medical conditions that preclude surgery, such as severe respiratory complications, coagulopathies, hepatic dysfunction, and patients who were terminally ill.

Between June 2022 and June 2023, these patients were admitted to Al-Azhar University Hospitals and treated with intralesional transplantation of autologous bone marrow MSCs.

Preoperative evaluation: All patients underwent a thorough history taking, general, and neurological examination to assess the level and completeness of the injury. Every patient underwent standard laboratory testing as well as a comprehensive radiographic evaluation that included plain X-rays, CT scans, MRI dorsal spine. EMG and NCV were performed for each patient. The neurological condition of each subject was assessed using the ASIA score (Figure 1).

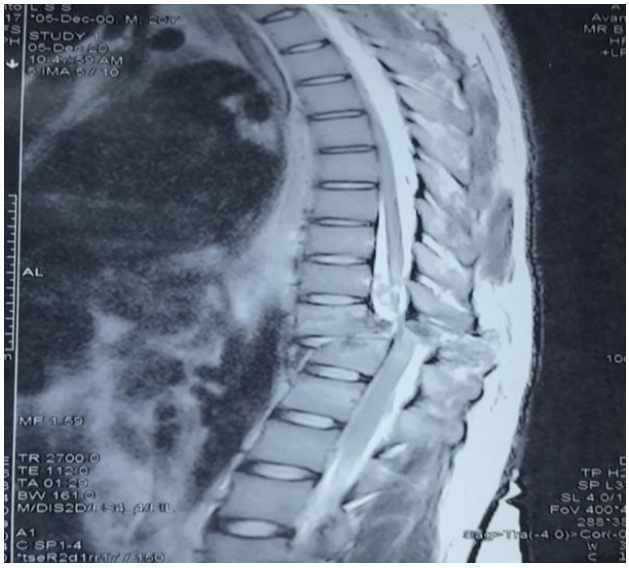

Figure (1): MRI dorsal spine complete cord injury.

Preparation of mesenchymal stem cells: Under complete aseptic technique, 150 ml of blood is aspirated from the iliac bone marrow with the aid of heparin and diluted with sterile phosphate buffer saline (PBS). Separation of mononuclear cells (MNC) using Ficoll-Hypaque. MNC undergoes immunologic separation using mesenchymal (CD105). Labelling the cells using FcR blocking reagent and microbead reagents by using the cell separator apparatus (Mini MACS). The cells were counted by flow cytometry (FAM). Separated MSCs were resuspended in 4 ml of fresh platelet-rich plasma (FRP). The stem cells were cultured to increase their numbers. The viability of stem cells was checked regularly. The final volume (4 – 6 ml) is handled in a cold atmosphere in the operating room. The viability of stem cells was checked regularly by turban blue and cells purification was done by magnetic separation (Figure 2, 3, 4).

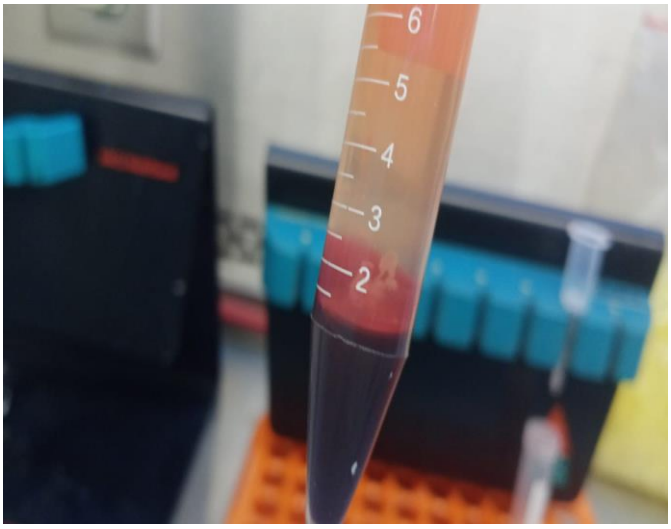

Figure (2): Stem cells examination using inverted microscope.

Figure (3): Stem cells under inverted microscope.

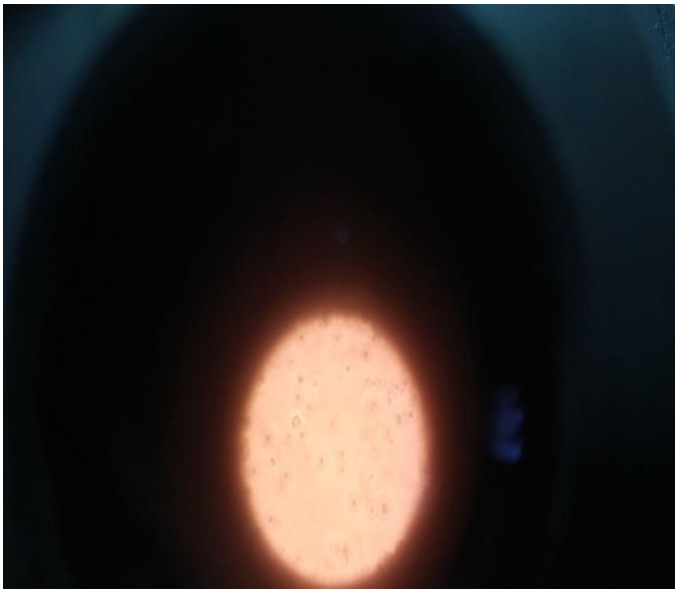

Figure (4): Milky white ring of mononuclear cells.

Operative technique: The patient underwent surgery under general anesthesia. Transpedicular fixation of the dorsal spine was done routinely in the fractured spine to achieve alignment. The dorsal spinal cord was exposed at the level of injury through a laminectomy at the selected level. Opening of the dura, identification of the injured cord, visualization of the cut ends, release of gliotic tissue, and adhesive scar around the injured area. The stem cells were laid down in place through gentle direct injection at the identified site of injury and stabilized in place by gel foam. Tight closure of the dure and wound in layer at the end (Figure 5).

Figure 5: Intraoperative exploration of injured cord.

Post-operative care and follow-up: Following surgery, the patients were evaluated clinically for motor, sensory, and sphincteric control as a functional improvement using the ASIA impairment scale at regular intervals of every two months for one year. By the end of six months, routine EMG and NCV, as well as MRI dorsal spine, were performed. The primary results of MSC transplantation were safety and effectiveness. The potential mild side effects, like allergic skin reaction, fever, headache, back pain, numbness, abdominal distention, as well as serious side effects like tumor or immune reaction, were monitored during follow-up. Standardized rehabilitation was carried out before and after stem cell transplantation.

Ethical approval: On May 31, 2022, The Research Ethics Committee at Damietta Faculty of Medicine, Al-Azhar University Hospitals gave its approval (Approval Code: IRB 00012367-22-05-003). Every patient gave their written and verbal agreement. The study adhered to the Helsinki Declaration throughout its execution.

Statistical analysis

For the statistical analysis, SPSS version 26 (IBM Inc., Chicago, IL, USA) was used. The mean and standard deviation (SD) of each quantitative measure were used as representations. Qualitative factors were represented by frequency and percentage (%). To ascertain the correlation between different variables in non-normal/non-linear monotonic relationships, the Spearman rank correlation equation was utilized. A two tailed P value < 0.05 was considered statistically significant.

RESULTS

With a mean age of 31.6 ± 9.5 years, table (1) revealed that 77.8% of the subjects were men and 22.2% were women. In 62.3% cases, poly trauma was the mechanism of injury, while single spinal trauma accounted for 37.7%. In 80% of patients, motor car accidents (MCA) were the primary cause of injury, followed by falls in 17.8% and motorcycle crashes (MCC) in 2.2%.

Table (1): Demographic and characteristic data among the participants

Age		31.6±9.5
Sex	**Female**	10(22.2%)
	Male	35(77.8%)
Mechanism of injury	**Isolated spinal trauma**	17(37.7%)
	Poly trauma	28(62.3%)
Cause	**MCS**	1(2.2%)
	Falling	8(17.8%)
	MCA	36(80%)

Data are presented as mean ± SD, or frequency (percentage), MCS: motorcycle crash, and MCA: motor car accident.

The average length of injury was 16.04±8.3 months, according to Table 2.

Table (2): Spinal cord injury characteristics among the participants

	All (N=45)
ASIA grade (A: Complete injury)	45(100%)
level of injury (DORSAL)	45(100%)
Duration of injury (months)	16.04±8.3

Data are presented as mean ± SD, or frequency (percentage), ASIA: American Spinal Injury Association and grade A: Complete injury: No motor or sensory function is preserved in the sacral segment.

Table (3) showed that in terms of complications, allergic skin reactions occurred in 8.9% of cases.

Table (3): Complications of the stem cells transplantation among the participants

		All (N=45)
Complications	**Allergic skin reaction**	4(8.9%)
	No	41(91.1%)

Data are presented as frequency (percentage).

In terms of the clinical outcome, the neurological evaluation showed that, as indicated in table (4), there was a slight improvement in the ASIA grade, 4.5% of cases were grade C, 33.3% of cases were grade B, and the majority of participants (62.2%) showed no change at all.

Table (4): Clinical outcome of the stem cells transplantation among the participants

		All (N=45)
Clinical outcome	**Motor incomplete (C)**	2(4.5%)
	Sensory incomplete (B)	15(33.3%)
	Complete injury (A)	28(62.2%)

Data are presented as frequency (percentage), grade A: Complete injury: No motor or sensory function is preserved in the sacral segment, grade B: Sensory incomplete: Sensory but not motor function is preserved below the level of injury including the sacral segment and grade C: Motor incomplete: Motor function is preserved below the level of injury, and more than half of the key muscles tested below the level of injury have a muscle grade less than 3.

As seen in table (5), there was no statistically significant link with either age or sex, but there was an inverse significant correlation between the clinical outcome and the length of damage in the Spearman coefficient.

Table (5): Correlation between clinical outcome and patients' characteristics

	Age	Sex	Duration of injury
Clinical outcome	r=-.257	r=.109	r=-.650
	P=.088	P=.476	P<.001

r: Spearman correlation.

DISCUSSION

The present study revealed that the mean duration of injury among participants was 16.04 ± 8.3 months. In terms of complications, 8.9% of patients developed allergic skin reactions. Neurological assessment showed mild improvement in the ASIA impairment scale: 4.5% of participants improved to grade C, 33.3% to grade B, while 62.2% remained at grade A, indicating no improvement. A significant inverse correlation was identified between injury duration and clinical prognosis (Spearman coefficient), suggesting that patients with shorter durations of injury had better neurological outcomes. However, age and sex showed no statistically significant association with clinical outcome.

One of the key strengths of this study is the direct intralesional application of autologous bone marrow-derived mesenchymal stem cells (BM-MSCs), which allowed for targeted delivery at the site of injury after removal of scar tissue. This method potentially enhances the effectiveness of cell transplantation. In contrast, systemic delivery methods such as intravenous infusion often result in most cells being trapped in the lungs, liver, and spleen, significantly reducing their availability at the injury site. Additionally, intra-arterial infusion has been associated with microvascular occlusion, posing further risks [(9)].

In this study, the BM-MSC dosage used was 6–7 million cells/cm³, which is within the range used in previous research (10–100 million cells) [(10)]. Future research should aim to standardize cell dosing protocols to determine whether higher doses lead to more favorable outcomes.

Current therapies for chronic spinal cord injury (SCI) primarily aimed to manage symptoms such as neuropathic pain and muscle spasticity, typically through pharmacological or rehabilitative approaches. At present, no intervention has demonstrated consistent clinical efficacy in restoring motor or sensory function in chronic SCI patients [(11)]. Over the past three decades, a wide body of preclinical research has investigated stem cell therapy for SCI. These studies categorized cellular interventions based on the key pathophysiological features of SCI: (1) loss of neurons at the injury site, (2) demyelination of descending axons impairing signal transmission, and (3)

chronic inflammation and reduced neurotrophic factor production [12].

The findings of this study confirmed that BM-MSC transplantation is both safe and feasible, with modest improvements in neurological function. Specifically, 4.5% of patients improved to ASIA grade C and 33.3% to grade B, indicating that BM-MSCs may offer therapeutic potential for chronic SCI. However, the majority of patients (62.2%) remained at ASIA grade A, likely due to the chronic nature or severity of their injuries. Previous studies emphasized that early intervention post-injury leads to more favorable outcomes, whereas the efficacy diminishes with longer injury durations [10]. Our data also supported the hypothesis that shorter injury duration correlates with better outcomes, possibly due to less developed scar tissue and more active chemotactic signaling in the subacute phase. Although, the average injury duration in this study was over 16 months, future investigations should consider including patients in the acute or subacute stages to better evaluate the benefits of early intervention [13].

The current findings are consistent with previous reports that support the safety and feasibility of BM-MSC use in SCI patients [10]. MSCs exert therapeutic effects through immunomodulation and the release of growth factors such as transforming growth factor beta 1 (TGF-β1), interleukin 10 (IL-10), and hepatocyte growth factor 1 (HGF-1) [14].

Yoon *et al.* [15] demonstrated that approximately 33.3% of patients treated in the acute or subacute phase experienced neurological improvement, compared to only 5.2% in the chronic phase. This difference is attributed to glial scarring in chronic injuries, which hinders axon regeneration. Although acute-phase transplantation exposes cells to a hostile inflammatory environment, the subacute phase presents a more favorable therapeutic window due to lower inflammatory activity and incomplete scar formation. In a study by **Dai *et al.*** [16], autologous BM-MSCs were transplanted in patients with chronic SCI, resulting in sensory and motor score improvements after six months. Ten of the twenty patients in the treatment group exhibited improvement, while the control group showed negligible changes. These improvements may be attributed to the high cell dose used. Encouraged by these findings, researchers have explored various cell types, including olfactory ensheathing cells, Schwann cells, and mesenchymal stem cells in SCI therapy [17]. Experimental evidence suggests that MSCs promote regeneration through neural repair, angiogenesis, immunosuppression, remyelination, and inhibition of gliosis and apoptosis [18-21].

In the current study, 8.9% of patients developed cutaneous allergic reactions, aligning with earlier studies that reported similar, though sometimes higher complication rates [22]. Generally, MSC therapy has shown a low risk of adverse events [23-25]. Minor side effects such as fever, GI disturbances, headaches, and urinary infections have been documented [26]. **Kishk *et al.*** [27] reported neuropathic pain in 24 SCI patients post-transplantation, potentially linked to frequent dosing, as those patients received MSCs monthly. Most studies, in contrast, employed single-dose administration, and the relationship between dosing frequency and adverse effects remains unclear.

Despite some functional improvements reported in clinical trials, such as enhanced muscle reinnervation in 44.4% of patients in one study [28], the mechanisms and long-term efficacy of MSC therapy are still being understood. **Park *et al.*** [29] observed functional improvement in only 30% of their cohort, suggesting variability in patient responses.

Our study further supports an inverse relationship between injury duration and prognosis, emphasizing the potential benefit of early BM-MSC transplantation [30].

LIMITATIONS

This study had several limitations. One notable constraint is the lack of evidence confirming the differentiation of transplanted cells into neuronal or glial lineages.

CONCLUSION AND RECOMMENDATION

Clinical outcome is noticed in using stem cell transplantation in the first nine months after injury, before adhesive gliosis that prevents the growth of axons and regeneration. Future research should explore the use of pre-differentiated neural stem cells and incorporate advanced imaging and molecular techniques to verify cellular integration and lineage commitment.

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Clinical trial number: not applicable

Consent to participate: All the patients who participated in the study gave their written consents.

Availability of data and material: An excel data sheet is available upon request.

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