Improvement of Neurogenic Bladder Dysfunction Following Combined Cell Therapy with Mesenchymal Stem Cell and Schwann Cell in Spinal Cord Injury: A Randomized, Open-Label, Phase II Clinical Trial

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OBJECTIVE: **To investigate the efficacy of intrathecal combined administration of autologous bone marrow-derived mesenchymal stem cells (BMSCs) and Schwann cells (SCs) in urinary function improvement in complete spinal cord injury (SCI) patients for the first time.**

METHODS: **This study was a randomized phase II clinical trial, including treatment and control arms. Patients with traumatic complete SCI-induced neurogenic bladder were included. The treatment group received a single intrathecal combined injection of autologous BMSCs and SCs. The control group underwent no additional intervention. The outcome measures of the study were urodynamic study parameters, number of incontinence and urinary tract infection episodes, incontinence quality of life questionnaire, functional status, and sensorimotor improvements.**

RESULTS: **Among a total of 32 recruited patients, 13 and 16 were completely followed up in the treatment and control group, respectively. Changes in bladder compliance ($P = 0.032$), maximum pressure of detrusor during the filling phase ($P = 0.013$), maximum pressure of detrusor at the maximum urinary flow rate ($P = 0.020$), maximum urinary flow rate ($P = 0.001$), and postvoid residual volume ($P = 0.001$) after 6 months were significantly different between the 2 groups. The number of urinary incontinence episodes ($P = 0.022$) significantly reduced in the treatment group after 6 months compared with the baseline. The incontinence quality of life total and domain scores significantly improved in the treatment group compared with the control group after 6 months.**

CONCLUSIONS: **The combined intrathecal administration of BMSCs and SCs significantly improved the urodynamic study parameters, urinary incontinence rate, and incontinence quality of life in complete SCI-induced neurogenic bladder.**

INTRODUCTION

Spinal cord injury (SCI) could negatively affect various aspects of the patient's quality of life (QoL), such as lower urinary tract (LUT) function, which is common among SCI patients.[1,2] Recent advances in SCI care have significantly reduced

Key words
- Mesenchymal stem cell
- Neurogenic bladder dysfunction
- Schwann cell
- Spinal cord injury
- Stem cell therapy

Abbreviations and Acronyms
AEs: Adverse events
BDNF: Brain-derived neurotrophic factor
BMSCs: Bone marrow-derived mesenchymal stem cells
I-QOL: Incontinence quality of life
IQR: Interquartile range
LUT: Lower urinary tract
NGB: Neurogenic bladder
P_{detmax}: Maximum pressure of detrusor during the filling phase
$P_{detQmax}$: Maximum pressure of detrusor at the maximum urinary flow rate
PVR: Postvoid residual
Q_{max}: Maximum urinary flow rate
QoL: Quality of life
SCI: Spinal cord injury
SCs: Schwann cells
UDS: Urodynamic study
UTI: Urinary tract infection

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the rate of mortality due to urinary tract infection (UTI) and sepsis, previously known as leading causes of death in this patient population. Nevertheless, urological complications remain a notable source of morbidity in SCI patients. Current therapeutic approaches to SCI-related neurogenic bladder (NGB) mostly target symptoms at the bladder level focusing on preventing upper urinary tract injury and improving urinary continence.[3-6] Therefore, there is a need for novel treatment strategies aiming to reverse the neurologic injury in SCI-induced NGB at a higher level.

The restoration of clinical and functional abilities following SCI is heavily reliant on overcoming the challenges associated with axonal regeneration. These challenges are attributed to a range of factors, such as neuronal and axonal degeneration, scar tissue formation, and the presence of inhibitory molecules, among others. Effectively addressing these obstacles requires a broad, multifactorial strategy. Despite years of research into various molecular therapies, many have struggled to achieve meaningful success in clinical trials.[7-9] This discrepancy between experimental research and clinical application may be due to a narrow focus on individual pathologic mechanisms, while SCI involves a complex combination of interrelated mechanisms and sequential pathologic processes. To enhance recovery outcomes in clinical settings, treatments must target several aspects of SCI's intricate pathology simultaneously.[9]

A range of therapeutic strategies has been investigated to promote functional recovery following SCI, with stem cell therapy emerging as a leading option. Previous research has assessed various cell types to identify those most effective for treating SCI. Mesenchymal stem cells (MSCs), in particular, have attracted significant attention due to their advantageous qualities for SCI treatment.[10,11] These qualities include the release of neuroprotective and immunomodulatory factors, along with a low risk of immune rejection.[12,13] Additionally, some studies have emphasized the potential of Schwann cells (SCs) for SCI therapy, highlighting their ability to facilitate neural regeneration. SCs, originating from the neural crest, are valuable candidates when used in combination with MSCs for transplantation. They secrete essential growth factors like brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor, nerve growth factor, and neurotrophin-3, all of which stimulate nerve growth and regeneration. Moreover, SCs play a pivotal role in the creation of the myelin sheath in the peripheral nervous system, which enhances the speed and efficiency of electrical signal conduction along axons, aiding in functional recovery.[14] Based on past findings, using SCs and MSCs together could offer superior regenerative effects and improved recovery outcomes after SCI compared to using either cell type individually.[15,16] This combination works synergistically by leveraging the immunomodulatory, anti-inflammatory, and neurotrophic properties of MSCs, which are mediated through their secretome,[17,18] alongside the endogenous neuroregenerative capacity of SCs, which contribute to remyelination and secrete neurotrophic factors.[19] Together, this dual approach holds great promise for advancing SCI treatment.[15,20]

Previous preclinical studies have demonstrated significant improvements in urodynamic study (UDS) parameters following stem cell therapy in animal models of SCI-induced NGB.[21] Various mechanisms underlying this effect have been suggested, such as partial sparing of descending pathways and synaptic reorganization in the dorsal gray commissure with the generation of new reflex pathways. Other mechanisms, such as increased neurogenesis and local anti-inflammatory effects for improvements in UDS parameters following stem cell therapy have also been reported.[22-26] All these mechanisms lead to improvements in bladder and urethral sphincter dysfunctions and enhance UDS parameters.

To the best of our knowledge, no prior phase II clinical trial has investigated the efficacy of combined cell therapy on SCI-induced NGB specifically. Therefore, this randomized phase II clinical trial was designed to evaluate changes in UDS parameters, LUT function, and patients' QoL after combined cell therapy in patients with complete SCI-induced NGB.

MATERIALS AND METHODS

Study Design and Participants

This research was conducted as a randomized, open-label, parallel-group, active-controlled phase II clinical trial, adhering strictly to the ethical principles outlined in the Declaration of Helsinki. The study received approval from the ethics committee of Shahid Beheshti University of Medical Sciences and the institutional review board of Shohada Tajrish Hospital. The trial was registered with the Iranian Registry of Clinical Trials (IRCT20200502047277N4). Prior to participation, all eligible individuals were thoroughly briefed on the study's objectives, procedures, and potential risks, including adverse events (AEs). Written informed consent was obtained from all participants to ensure a clear understanding of their involvement in the study.

Eligible participants were adults aged 18 years or older who had sustained a complete traumatic cervical or thoracic spinal cord injury (SCI), classified as grade A on the American Spinal Injury Association Impairment Scale (AIS). The study included individuals with either subacute SCI (defined as an injury occurring between 14 days and 6 months prior to enrollment) or chronic SCI (injury occurring more than 6 months prior). Additionally, participants were required to have a diagnosis of neurogenic bladder (NGB) secondary to SCI, as confirmed by urodynamic testing and based on the International Continence Society guidelines.

Exclusion criteria included the presence of urethral strictures or anatomical abnormalities of the bladder, a history of major pelvic surgeries such as prostatectomy, sphincterotomy, augmentation cystoplasty, or colectomy, and any complications affecting the upper urinary tract. Moreover, participants with co-existing neurologic or psychiatric conditions, pre-existing comorbidities such as diabetes mellitus or cardiovascular disease, a history of malignancy within the past 10 years, or urinary tract infections (UTIs) confirmed through urinalysis or urine culture were excluded from the study. Inability to undergo urodynamic studies (UDS) for any reason also served as an exclusion criterion.

Between November 2020 and August 2021, all patients with complete SCI who presented at the neurosurgery department of Shohada Tajrish Hospital were screened for eligibility. Those meeting the inclusion criteria were randomized into 2 groups (1:1 ratio) using a computer-generated block randomization method. Participants were assigned to either the treatment or control group. Due to the ethical considerations and the nature of the

intervention, the study did not employ placebo control or blinding. To ensure comprehensive and transparent reporting of the trial's outcomes, the study adhered to the Consolidated Standards of Reporting Trials guidelines.

Cell Isolation, Characterization, and Preparation

SC isolation was performed by surgically excising a 12 cm segment of the sural nerve from the calf under general anesthesia. The nerve was immediately placed in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA) and transported under sterile, temperature-controlled conditions to the laboratory. After the removal of residual soft tissue, the nerve was sectioned into 1–2 mm fragments and enzymatically digested with collagenase (1.4 U/mL; Sigma, St. Louis, MO, USA) and Dispase (2.4 U/mL; Sigma) for 3 hours at 37°C. The resulting cell suspension was washed, filtered, and incubated in DMEM/F12 without fetal bovine serum (FBS; Gibco) for 5 days at 37°C in 5% CO2, followed by a gradual increase of FBS to 10% over the next week. Schwann cell identification was confirmed via immunocytochemical analysis using anti-S100 and anti-glial fibrillary acidic protein (GFAP) antibodies (Santa Cruz Biotechnology, CA, USA), followed by staining with a horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich) and chromogenic detection using 3,3′-diaminobenzidine (Sigma-Aldrich). Hematoxylin (Sigma-Aldrich) was used for nuclear counterstaining, and the cells were visualized under a light microscope to confirm SC identity and morphology.

Bone marrow aspirates (100–150 mL) were collected from the iliac bone and diluted in Hanks' Balanced Salt Solution (HBSS; Sigma) at a 1:1 ratio to isolate mesenchymal stem cells (MSCs). The diluted samples were processed using a Ficoll density gradient (1.077 g/L; Sigma) at a 1:3 ratio, followed by centrifugation at 400g for 40 minutes to recover the mononuclear cell layer. To further purify the MSCs and remove platelets, additional centrifugation steps were performed. The differentiation potential of the isolated MSCs was tested by inducing osteogenic differentiation in a medium containing 100 nmol/L dexamethasone (Sigma), 50 mg/mL ascorbic acid 2-phosphate (Sigma), and 10 mmol/L β-glycerophosphate (Merck, Rahway, NY, USA) for 21 days, with Alizarin Red staining (Sigma) confirming calcium mineralization. For adipogenic differentiation, MSCs were cultured for 3 weeks with 0.5 mmol/L hydrocortisone (Sigma), 0.5 mmol/L isobutyl methylxanthine (Sigma), and 60 mmol/L indomethacin (Gibco), and differentiation was confirmed by Oil-Red O staining. Flow cytometry analysis was performed on 1×10^5 cells suspended in 100 μL phosphate-buffered saline (PBS; Sigma) and stained with monoclonal antibodies against CD34, CD45, CD73, CD90, and CD105 (all PE-conjugated; Abcam). After washing with PBS, the cells were fixed with 1% paraformaldehyde and analyzed using a FACS Calibur cytometer (Becton Dickinson) and CellQuest software (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), with histograms generated via WinMDI 2.8 software (Scripps Research Institute, La Jolla, California, USA).

Intervention

Finally, a combination of SCs and MSCs (a concentration of 5×10^6 cells per mL for each cell type), suspended in 6 mL saline was used for intrathecal injection. At the operating room, lumbar puncture was carried out using a 24G needle at L4/L5 levels. Slow intrathecal injection of the cellular mixture was performed after the needle insertion into the arachnoid space was confirmed. The cerebrospinal fluid leakage was prevented by keeping the needle in place for 1 minute. Patients were discharged 1 hour after the injection. Patients in the control group merely received standard rehabilitation therapy with no additional intervention.

Follow-Up and Outcome Measures

All outcome measures were evaluated at baseline and 6 months after the injection. Primary outcome measures of the study were UDS parameters, including postvoid residual (PVR), bladder capacity, bladder compliance, maximum pressure of detrusor during the filling phase (P_{detmax}), maximum pressure of detrusor at the maximum urinary flow rate ($P_{detQmax}$), maximum urinary flow rate (Q_{max}), and bladder filling sensation. One examiner performed all urodynamic examinations using a computer-assisted urodynamic unit (Medical Measurement Systems, Germany). Bladder compliance of more than 30 mL/cm H2O was considered normal. Secondary outcome measures were urinary incontinence, urinary tract infection, and incontinence quality of life (I-QOL) questionnaire to evaluate LUT and its impact on patient's QoL before and after injection.[27-29] Patients were also monitored for potential treatment-related AEs during the study period using Common Terminology Criteria for Adverse Events Version 5.0. Urinalysis with renal and bladder ultrasounds were also performed at each visit.

Statistical Analysis

Based on a pilot population of patients with SCI-induced NGB in our department, by considering a mean PVR of 302.4 mL and a standard deviation of 81.1 mL, a minimum of 24 patients (12 in each group) would be required to observe a minimum change of 100 mL in the mean PVR with a two-sided significance of 0.05 and a power of 0.80. A dropout rate of 25.0% was also anticipated and a sample size of 32 (16 in each group) was finally estimated for this study. A per-protocol approach was used for data analysis. All the quantitative data were presented as mean ± standard deviation, and all the qualitative data were expressed as the frequency with percentage. All continuous outcome measures were compared between the baseline and 6-month postoperatively using the Wilcoxon signed-rank test. For univariate analysis, differences between the 2 study groups in continuous variables and changes (6-month value − baseline value) in variables over the study period were assessed using the Mann–Whitney U test. The same was performed for categorical variables using Chi-Square and Fisher's Exact tests. To compare the categorical outcome measures between the baseline and 6-month postoperatively, McNemar's test was utilized. Outcome measures that were significantly different between the 2 study groups in univariate analysis were also included in multivariate analysis to control for potential confounders. Regarding this, multiple linear regression analysis was performed to evaluate the effect of study intervention on significant changes in outcome measures via a backward stepwise method while controlling for different potential risk factors. The change (6-month value − baseline value) in the outcome measure was the dependent variable and independent variables included age, sex, level of injury, time elapsed since the onset of injury, and the study group (treatment vs. control). The model was controlled

for the effect of baseline values of outcome measures. The level of significance in this study was $P < 0.05$. All statistical analyses were performed using STATA 15 (Stata Corp. LLC, College Station, Texas, USA).

RESULTS

Patients' Characteristics

A total of 54 patients were screened for eligibility, and a total of 32 patients were recruited in this study. In the treatment group, 3 patients were lost to follow-up. Therefore, a total of 29 patients with SCI-induced NGB were followed up, including 13 and 16 in treatment and control groups, respectively (Figure 1). Most participants were male, including 10 (76.9%) and 10 (62.5%) in treatment and control groups, respectively. The mean age of participants was 35.0 ± 8.8 (32.5 [interquartile range (IQR), 28.3, 39.5]) and 32.9 ± 7.6 (38.0 [IQR, 26.0, 43.0]) years in treatment and control groups, respectively. In terms of level of injury, 4 (30.8%) and 5 (31.2%) patients had cervical SCI in treatment and control groups, respectively. Others had a thoracic level of injury. No significant difference was found between the 2 groups in baseline, demographic, and clinical characteristics (Table 1).

AEs

AEs observed in this study included neuropathic pain, fatigue, paresthesia, and spasticity. Among the reported AEs, only paresthesia, reported by 3 patients, was probably related to the intervention. In terms of Common Terminology Criteria for Adverse Events grading, 9 (64.3%) AEs were grade I, and 5 (35.7%) were grade II. All grade II AEs were treated successfully using standard treatment (Table 2). Cases with neuropathic pain or paresthesia were medically managed using gabapentin, and 2 cases with spasticity were managed using oral baclofen and botulinum toxin injection (Table 2).

Urodynamic Study Parameters

Significant increases were found in $P_{detQmax}$ (baseline: 33.1 ± 23.9 (31.0 [IQR, 9.0, 55.0]) cmH2O; 6-month: 45.2 ± 20.7 (40.0 [IQR, 29.0, 65.0]) cmH2O; $P = 0.041$) and Q_{max} (baseline: 2.9 ± 2.4 (4.0

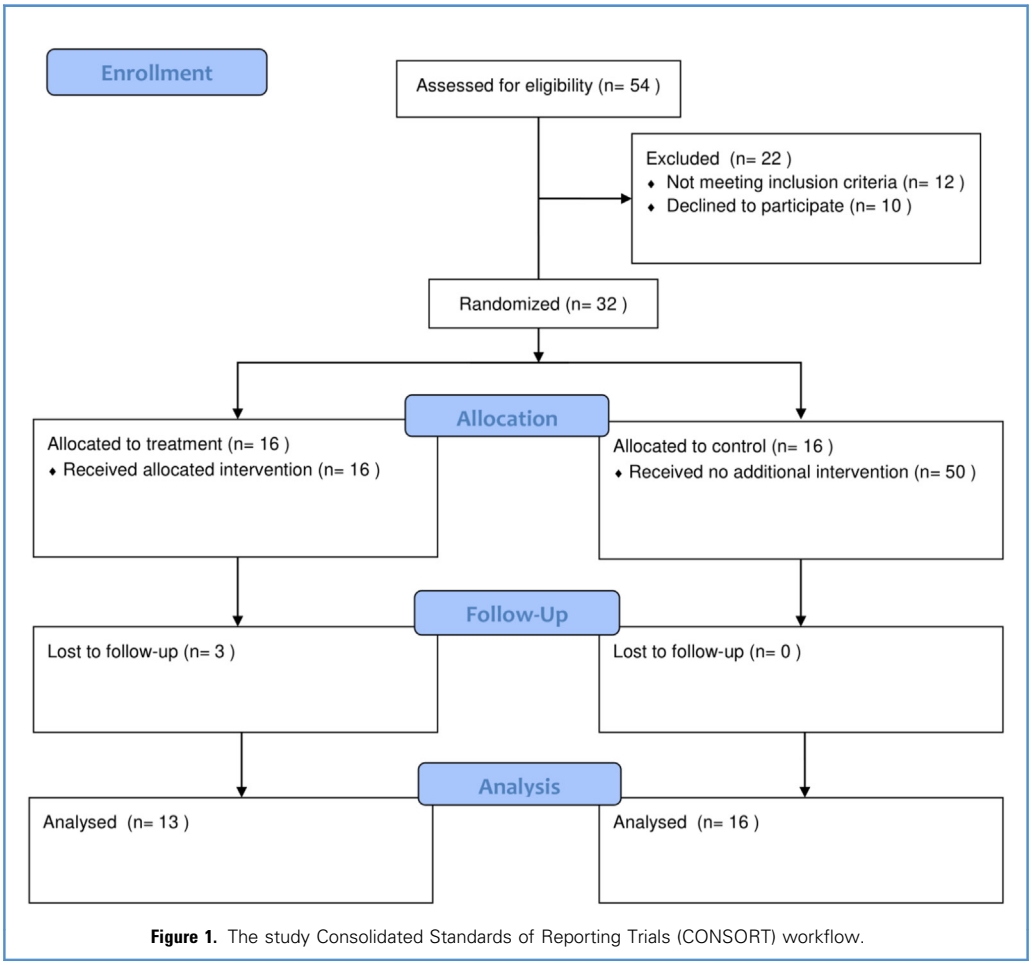

Figure 1. The study Consolidated Standards of Reporting Trials (CONSORT) workflow.

Table 1. Demographic and Clinical Characteristics of the Patients

Characteristic	Treatment Group	Control Group	*P*-Value
Sex — no. (%)			
Female	3 (23.1%)	6 (37.5%)	0.404
Male	10 (76.9%)	10 (62.5%)	
Age (years)	35.0 (±8.8)	32.9 (±7.6)	0.423
Cause of injury — no. (%)			
Accident	10 (76.9%)	12 (75%)	0.965
Falling	2 (15.4%)	3 (18.8%)	
Others	1 (7.7%)	1 (6.2%)	
Level of injury — no. (%)			
Cervical	4 (30.8%)	5 (31.2%)	0.978
Thoracic	9 (69.2%)	11 (68.8%)	
Time elapsed since injury (months)	15.69 (±15.53)	14.69 (±7.72)	0.398
Chronicity — no. (%)			
Subacute	2 (15.4%)	2 (12.5%)	0.823
Chronic	11 (84.6%)	14 (87.5%)	

[IQR, 0.0, 5.0]) mL/s; 6-month: 5.5 ± 4.7 (1.5; IQR, 5.0–8.3) mL/s; $P = 0.018$) in the treatment group 6 months after the injection compared with the baseline. Significant reductions were also noted in PVR (baseline: 329.3 ± 101.5 (360.0 [IQR, 275.0, 410.0]) mL; 6-month: 204.7 ± 141.6 (180.0 [IQR, 83.0, 375.0]) mL; $P = 0.006$) and P_{detmax} (baseline: 44.5 ± 26.3 (52.0 [IQR, 19.5, 67.5]) cmH2O; 6-month: 31.4 ± 19.3 (31.0 [IQR, 15.0, 50.0]) cmH2O; $P = 0.012$) in the treatment group 6 months after the injection compared with the baseline. Changes in bladder compliance (treatment: −0.8 ± 31.6 (4.2 [IQR, 0.1, 9.1]) mL/cmH2O; control: −2.1 ± 6.7 (−0.5 [IQR, −2.0, 0.4]) mL/cmH2O; $P = 0.015$), PVR (treatment: −124.6±119.9 (−90.0 [IQR, −250.0, −10.0]) mL; control: 8.9 ± 15.9 (5.0 [IQR, 0.0, 13.8]) mL; $P < 0.001$), P_{detmax} (treatment: −13.1 ± 15.0 (−11.0 [IQR, −25.0, −3.5]) cmH2O;

Table 2. Adverse Event Grading According to Common Terminology Criteria for Adverse Events

Patient	Adverse Event	No. (%)	Causal Link with Intervention	Degree	Management
2	Pain	1 (7.1%)	Not related	Grade II	Gabapentin
	Fatigue	1 (7.1%)	Not related	Grade I	Self-limiting condition
3	Paresthesia	1 (7.1%)	Probable	Grade I	Gabapentin
4	Paresthesia	1 (7.1%)	Probable	Grade I	Gabapentin
5	Pain	1 (7.1%)	Unlikely	Grade II	Gabapentin
	Spasticity	1 (7.1%)	Not related	Grade II	Oral baclofen and botulinum toxin injection
6	Fever	1 (7.1%)	Not related	Grade I	Paracetamol
	Fatigue	1 (7.1%)	Not related	Grade I	Self-limiting condition
	Spasticity	1 (7.1%)	Not related	Grade II	Oral baclofen
7	Paresthesia	1 (7.1%)	Unlikely	Grade I	Gabapentin
8	Pain	1 (7.1%)	Unlikely	Grade I	Gabapentin
10	Pain in extremity	1 (7.1%)	Not related	Grade I	Gabapentin
11	Paresthesia	1 (7.1%)	Probable	Grade I	Gabapentin
12	Pain	1 (7.1%)	Unlikely	Grade II	Gabapentin

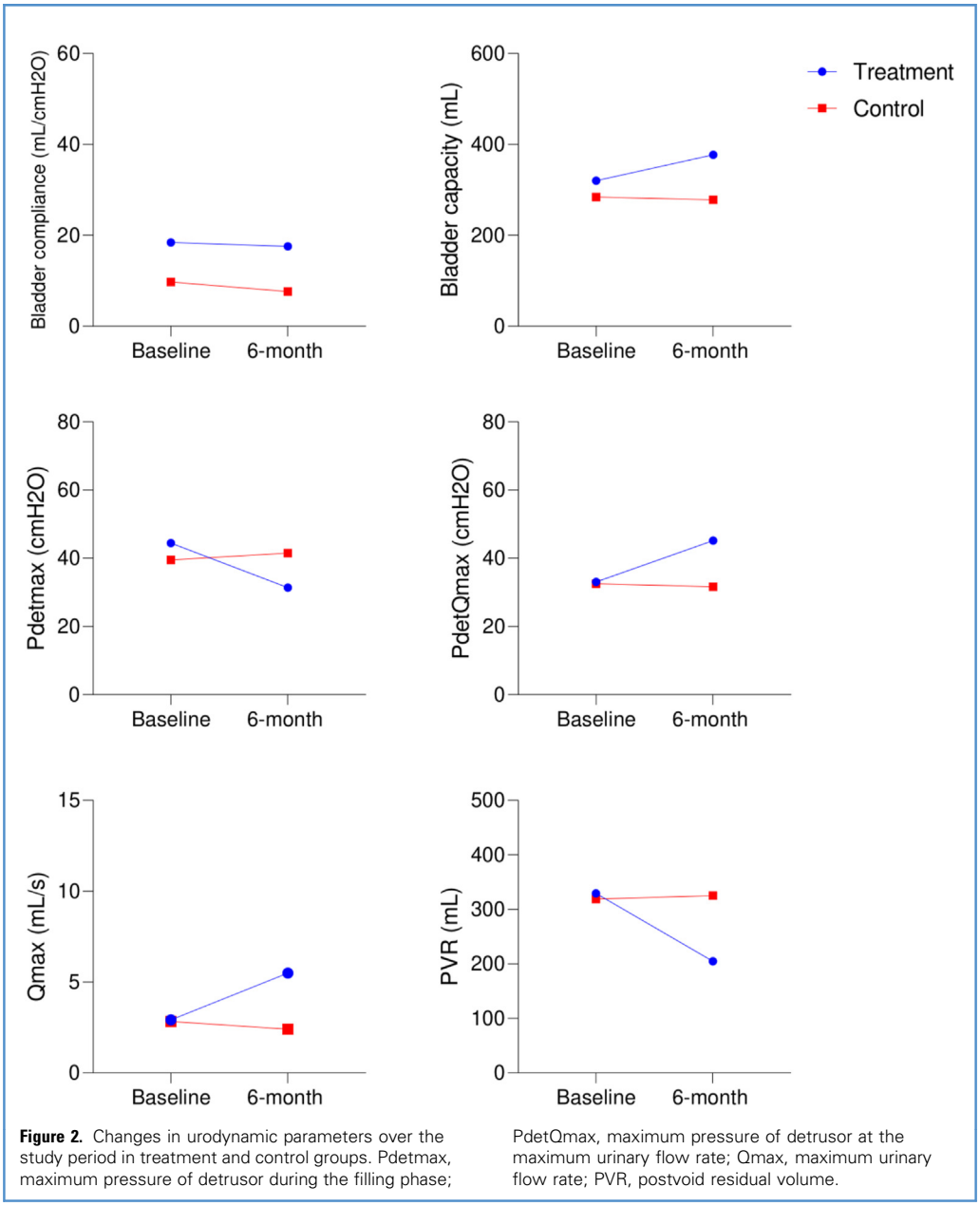

Figure 2. Changes in urodynamic parameters over the study period in treatment and control groups. Pdetmax, maximum pressure of detrusor during the filling phase; PdetQmax, maximum pressure of detrusor at the maximum urinary flow rate; Qmax, maximum urinary flow rate; PVR, postvoid residual volume.

control: 2.0 ± 9.6 (4.5 [IQR, 0.0, 8.8]) cmH2O; P = 0.013), $P_{detQmax}$ (treatment: −12.1 ± 19.4 (−14.4 [IQR, −26.0, 0.0]) cmH2O; control: −0.9 ± 3.2 (0.0 [IQR, −3.5, 0.0]) cmH2O; P = 0.020), and Q_{max} (treatment: −2.6 ± 3.2 (−2.0 [IQR, −4.5, 0.0]) mL/s; control: −0.4 ± 0.9 (0.0 [IQR, 0.0, 1.2]) mL/s; P = 0.001) after 6 months were significantly different between treatment and control groups. Bladder capacity also increased after 6 months, yet its change (treatment: 57.2 ± 128.9 (27.0 [IQR, −56.5, 167.0]) mL; control: −5.9 ± 35.5 (−5.0 [IQR, −20.0, 0.0]) mL; P = 0.232) was not significantly different between the 2 groups. Three patients in the treatment group (23.1%) had improvements in bladder sensation, yet no patient reported such changes in the control group. Moreover, among 3 patients with an areflexic type of NGB in the treatment group, 2 (66.7%) developed overactive detrusor activity after 6 months. No such change was observed in patients with areflexic NGB in the control group (n = 3). Changes in UDS parameters in the 2 study groups are depicted in Figure 2 and Table 3.

Urinary Incontinence and Infection

The treatment group also showed a significant reduction in the mean number of urinary incontinence episodes per day compared with the control group (treatment: −2.2 ± 2.1 (−3.0 [IQR, −4.0,

Table 3. Outcome Measures at Baseline and 6-Month After the Injection

Outcomes	Treatment Group	Control Group	*P*-Value†
Bladder capacity (mL)			
Baseline	320.00 ± 139.96	284.38 ± 79.50	-
6-month	377.15 ± 142.80	278.44 ± 61.69	-
Change	57.15 ± 128.86	−5.94 ± 35.46	0.232
P-value*	0.182	0.247	-
Bladder compliance (mL/cmH2O)			
Baseline	18.41 ± 30.39	9.74 ± 8.23	-
6-month	17.57 ± 14.91	7.6 ± 3.2	-
Change	−0.83 ± 31.60	−2.1 ± 6.7	0.015‡
P-value	0.152	0.179	-
P_{detmax} (cmH2O)			
Baseline	44.46 ± 26.26	39.50 ± 17.4	-
6-month	31.38 ± 19.31	41.50 ± 16.79	-
Change	−13.08 ± 15.04	2.00 ± 9.62	0.013‡
P-value	0.012‡	0.479	-
$P_{detQmax}$ (cmH2O)			
Baseline	33.06 ± 23.94	32.50 ± 19.48	-
6-month	45.18 ± 20.70	31.63 ± 19.13	-
Change	−12.12 ± 19.44	−0.87 ± 3.24	0.020‡
P-value	0.041‡	0.311	-
Q_{max} (mL/s)			
Baseline	2.92 ± 2.44	2.82 ± 2.61	-
6-month	5.49 ± 4.69	2.41 ± 2.31	-
Change	−2.57 ± 3.23	−0.42 ± 0.91	0.001‡
P-value	0.018‡	0.092	-
PVR (mL)			
Baseline	329.31 ± 101.54	318.75 ± 79.32	-
6-month	204.69 ± 141.61	325.00 ± 72.39	-
Change	−124.62 ± 119.86	8.75 ± 15.86	<0.001‡
P-value	0.006‡	0.151	-
Mean no. of urinary incontinence (per day)			
Baseline	5.62 ± 1.98	5.31 ± 1.74	-
6-month	3.46 ± 1.27	4.81 ± 0.98	-
Change	−2.15 ± 2.08	−0.50 ± 1.15	0.022‡
P-value*	0.008‡	0.114	-
Mean no. of UTI (over the prior 6 months)			
Baseline	1.85 ± 0.90	1.87 ± 0.81	-
6-month	1.46 ± 0.97	2.63 ± 1.89	-
Change	−0.38 ± 1.12	0.75 ± 1.91	0.101
P-value	0.194	0.101	-

PVR, postvoid residual volume; UTI, urinary tract infection.
*Wilcoxon signed-rank test was performed to compare variables at the 6-month follow-up with baseline.
†Mann–Whitney U test was performed to compare changes in variables between the 2 study groups.
‡Indicates statistically significant.

0.0]); control: −0.5 ± 1.2 (0.0 [IQR, −1.8, 0.0]); P = 0.022). Nevertheless, changes in the mean number of UTI episodes over the prior 6 months were not significantly different between the treatment and control groups after 6 months (treatment: −0.4 ± 1.1 (0.0 [IQR, −1.5, 0.5]); control: 0.8 ± 1.9 (0.0 [IQR, 0.0, 1.0]); P = 0.101) (Table 3).

I-QOL Questionnaire

The changes in the I-QOL total score were significantly different between the treatment and control groups after 6 months (treatment: 12.6 ± 14.9 (10.0 [IQR, 2.0, 15.5]); control: −6.1 ± 10.6 (−8.5 [IQR, −12.0, 3.0]); P < 0.001). Further, all 3 domains of the I-QOL questionnaire, including avoidance behaviors (treatment: 5.0 ± 7.0; control (5.0 [IQR, 0.0, 8.0]): −2.4 ± 4.6 (−3.0 [IQR, −4.0, 0.8]); P = 0.002), psychosocial impacts (treatment: 4.9 ± 6.3 (3.0 [IQR, 0.5, 5.5]); control: −0.8 ± 3.3 (−2.0 [IQR, −3.0, 2.3]); P = 0.001), and social embarrassments (treatment: 2.6 ± 3.0 (3.0 [IQR, 0.0, 5.0]); control: −2.3 ± 3.3 (−2.5 [IQR, −5.0, 0.8]); P = 0.001) significantly improved in the treatment group at 6-month after the injection compared with the control group. Changes in I-QOL total score and domain scores are illustrated in Figure 3.

Multiple Regression Analysis

Multiple linear regression analysis controlled for the potential effect of age, sex, level of injury, and time elapsed since the injury onset was performed to evaluate the effect of study intervention on changes in outcome measures. In this regard, multivariate analysis demonstrated a significant association between study intervention and changes in bladder compliance (B = 10.1 [95% CI, 2.3–17.9], P = 0.013), PVR (B=−133.6 [95% CI, −193.0 – −74.3], P < 0.001), P_{detmax} (B=−13.5 [95% CI, −21.5 – −5.5], P = 0.002), $P_{detQmax}$ (B=−11.4 [95% CI, −20.9 – −1.9], P = 0.021), and Q_{max} (B=−3.0 [95% CI, −4.7 – −1.3], P = 0.001). Similarly, the change in the mean number of urinary incontinence over the prior 6 months was significantly associated with the study intervention (B=−1.3 [95% CI, −2.1 – −0.6], P = 0.001). The changes in I-QOL total score (B = 16.6 [95% CI, 9.1–24.1], P < 0.001), as well as scores of avoidance behaviors (B = 8.8 [95% CI, 5.9–11.7], P < 0.001), psychosocial impacts (B = 5.7 [95% CI, 2.0–9.5], P = 0.004), social embarrassments (B = 4.4 [95% CI, 2.2–6.5], P < 0.001)

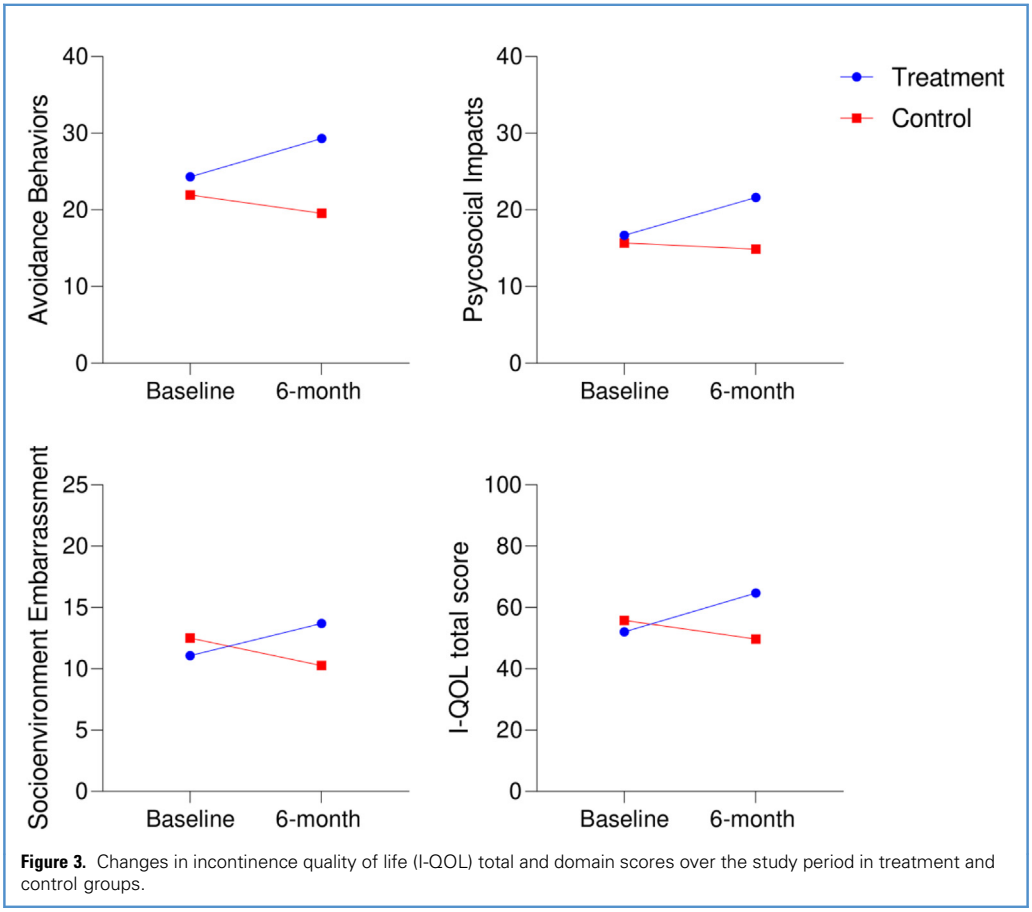

Figure 3. Changes in incontinence quality of life (I-QOL) total and domain scores over the study period in treatment and control groups.

were significantly associated with the study intervention according to the multivariate analysis.

DISCUSSION

To the best of our knowledge, this study represents one of the first clinical trial to specifically evaluate the effects of combined cell therapy on urological changes in patients with SCI-induced NGB. Based on both univariate and multivariate analyses, combined cell therapy using both MSCs and SCs significantly increased various UDS parameters, including bladder compliance, P_{detmax}, $P_{detQmax}$, Q_{max}, and PVR in patients with complete SCI. Additionally, this combined approach led to a significant reduction in mean number of urinary incontinence episodes in this group of patients. Notably, significant improvements in incontinence-related QoL were also observed following combined cell therapy compared with the control group.

The present findings were in line with previous preclinical studies suggesting the efficacy of combined cell therapy using both MSCs and SCs.[30] Ban et al. reported that the SC-MSC co-graft group had more regenerative axons of the corticospinal tract passing through the damaged cavity to the caudal cord compared with the SC graft group, MSC graft group, or placebo group. Moreover, the SC-MSC group showed complete myelin sheaths and organelles under electron microscopy.[30] However, a few clinical investigations on the efficacy of this specific combination in SCI patients have been conducted. In our prior phase I clinical trial, we demonstrated the safety of combined cell therapy using MSCs and SCs in patients with chronic and subacute SCI.[31] Nevertheless, the present phase II trial is the first to assess the efficacy of this treatment on SCI-induced NGB specifically, with adequate power and a control arm.

Numerous mechanisms for the positive effects of stem cell therapy on UDS parameters in SCI-induced NGB have been proposed based on preclinical reports.[21-23,25,26] Hu et al. found a significant reduction in voiding pressure, nonvoiding contractions, and PVR in rats that received intravenous bone marrow-derived MSCs (BMSCs) compared with the control group.[22] According to their immunohistochemistry study, the labeled BMSCs were discovered in the dorsal gray commissure of L3-4, which suggests the synaptic reorganization of the voiding reflex without any involvement in partial descending pathways' sparing. Erdogan et al. assessed the effects of direct administration of fetal allogeneic umbilical cord-derived MSCs on rats with incomplete SCI.[21] They showed that due to decreased inflammation, lamina muscularis thickness and bladder wall fibrosis were significantly reduced in rats that received MSCs in comparison with the control group. Park et al. also reported a similar effect for MSC transplantation in reducing inflammation, yet it could not improve the bladder function significantly.[24] Temeltas et al. found significant improvements in baseline pressure, maximum capacity, voiding pressure, PVR, and mean uninhibited contraction amplitude in rats that underwent cell transplantation using neuronal-glial restricted precursors or BMSCs compared with the control group.[26] They suggested neurogenesis by the neural progenitor cells as the possible explanation for their finding. Other mechanisms such as sparing, sprouting, or modification of descending pathways have also been proposed in prior preclinical studies.[25]

Mesenchymal stem cells (MSCs) facilitate spinal cord injury (SCI) recovery through several mechanisms. They release a variety of immunomodulatory factors, such as interleukin-10, transforming growth factor-β, and prostaglandin E2, which suppress the activity of immune cells, including T-cells and macrophages. Moreover, MSCs downregulate the production of pro-inflammatory cytokines, notably tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β).[16] MSCs also safeguard neurons and axons from secondary degeneration post-SCI by promoting the secretion of neurotrophic factors, such as BDNF and glial cell line-derived neurotrophic factor, which enhance the survival and differentiation of neural progenitor cells.[32] Additionally, MSCs mitigate the formation of glial scars, which impede axonal regeneration, by stimulating the activity of matrix metalloproteinases, enzymes that degrade the extracellular matrix.[33]

Schwann cells, a specialized glial cell type, play an indispensable role in nerve regeneration. In the event of SCI, these cells migrate to the lesion site, where they support axonal regeneration by secreting various neurotrophic factors and extracellular matrix proteins.[34] These neurotrophic factors include nerve growth factor, BDNF, neurotrophin-3, fibroblast growth factor, ciliary neurotrophic factor, and glial cell line-derived neurotrophic factor.[14] The release of these factors promotes neuronal survival, axonal extension, and synaptogenesis. Additionally, Schwann cells produce extracellular matrix proteins, such as laminin and fibronectin, which provide a scaffold conducive to axonal growth.[14] Schwann cells further contribute to remyelination by migrating to the damaged site, aligning axons, and promoting the restoration of myelin sheaths.[35,36]

In clinical setting our study demonstrated significant improvements in various UDS parameters were found 6 months after the intrathecal cell therapy in this study (bladder compliance, P_{detmax}, $P_{detQmax}$, Q_{max}, and PVR). Similarly, Cheng et al. evaluated patients' UDS parameters before and 6 months after intraspinal transplantation of umbilical cord-derived MSCs in 10 patients with chronic thoracolumbar SCI. Their results demonstrated a significant increase in the bladder capacity and a nonsignificant improvement in Q_{max} and PVR values.[37] Mendonça et al. also found an increase in bladder capacity at 6 months postoperatively in 14 patients with chronic SCI who underwent intralesional administration of BMSCs, yet it was not statistically significant.[38] They also observed a significant increase in bladder compliance at 6 months postoperatively compared with the baseline. Similar to our study, they showed variable findings regarding the changes in detrusor overactivity after cell therapy. Moreover, in their study, all patients remained in need of CIC, and no change was found in urinary incontinence. Vaquero et al., in a phase 2 clinical trial, evaluated the safety and efficacy of intrathecal administration of autologous BMSCs through 3 injections in 11 patients with chronic SCI.[39] They found a decrease in PVR and an increase in bladder compliance at filling in 66.6% of patients 10 months after the first intrathecal injection. The variations in the results could be attributed to several factors: the different sample sizes and heterogeneity in the study populations, the lack of a control group in some

studies, differences in the methods of cell administration, and inconsistencies in the types or dosages of cells used. These factors likely contributed to the differences observed in outcomes across the studies. Additionally, other studies evaluated bladder function as a secondary outcome, which may have also contributed to the variability in the results.

Bladder dysfunction has been shown to significantly impact the perceived quality of life (QoL) in individuals with SCI.[40,41] Complications such as incontinence and UTIs can strain relationships with family, friends, and intimate partners, often leading to embarrassment and social withdrawal. As a result, individuals may avoid activities they once enjoyed. The method chosen for bladder management can further exacerbate these physical, psychological, and social challenges, making proper bladder function crucial to overall comfort and QoL.[42,43] Given the profound effects of incontinence, recurrent UTIs, and bladder management on patient-reported outcomes, urinary issues remain a top health priority for individuals with SCI, often surpassing concerns related to mobility impairment and loss of independence. Therefore, the evaluation and management of neurogenic lower urinary tract dysfunction are essential to reduce morbidity and mortality in this population.

This study aimed to improve QoL by addressing bladder and urinary tract dysfunction. Notably, it represents the first investigation to evaluate the impact of cell therapy on incontinence-specific QoL (I-QOL), the mean number of UTIs, and episodes of urinary incontinence in individuals with SCI-induced neurogenic bladder (NGB). Our results demonstrated a significant increase in both the total and domain-specific I-QOL scores six months after the administration of combined intrathecal cell therapy. The observed improvement in QoL may be attributed to enhanced detrusor muscle contractility, leading to a reduction in PVR and its associated complications. Given the limited evidence currently available, future studies examining NGB treatment approaches, such as stem cell therapy, should incorporate patient-reported outcome measures to explore the potential correlation between changes in UDS parameters and I-QOL scores.[44-46]

This study had several limitations. First, the follow-up period was limited to 6 months, indicating the need for future studies with longer follow-up durations to assess the long-term effectiveness of combined cell therapy. Second, there was variation in patient baseline characteristics, such as the time since injury and the level of injury, which differed across participants. Nevertheless, multivariate analysis was employed to adjust for potential confounding factors. Third, due to ethical concerns and the invasive nature of the intervention, it was not feasible to implement blinding or use a placebo. Additionally, as this was a single-center study, further research conducted in multiple centers is necessary to confirm these findings in diverse clinical settings.

This study also lacked a placebo control and blinding, which was inherent to the study's design. Cells were obtained from autologous sources, requiring patients to undergo procedures for bone marrow aspiration and sural nerve harvesting. As a result, patients were fully aware of the sampling procedures. Conducting such invasive interventions, including bone marrow aspiration and sural nerve biopsy, without a clear medical indication would have been ethically unjustifiable.

CONCLUSIONS

The combined intrathecal administration of MSCs and SCs was significantly effective in improving the UDS parameters in patients with traumatic SCI-induced NGB. Additionally, this combined cell therapy strategy could remarkably improve the QoL and reduce the number of urinary incontinence episodes in patients. While this study indicated the efficacy of combined stem cell therapy in SCI-induced NGB, future investigations are necessary to address the differences between various subtypes of NGBs and explore the use of combinatorial approaches in addition to cell therapy in this group of patients.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Mohammadhosein Akhlaghpasand: Methodology, Investigation, Conceptualization. **Roozbeh Tavanaei:** Writing — review & editing, Methodology, Conceptualization. **Farzad Allameh:** Investigation. **Maede Hosseinpoor:** Writing — original draft, Investigation. **Hossein Toreyhi:** Investigation. **Maryam Golmohammadi:** Investigation. **Atieh Hajarizadeh:** Investigation. **Alireza Alikhani:** Writing — original draft. **Maryam Hafizi:** Formal analysis. **Maryam Oraee-Yazdani:** Data curation. **Alireza Zali:** Investigation. **Saeed Oraee-Yazdani:** Supervision, Project administration.

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