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Research Article

Free Mesenchymal Stem Cell-Associated Exosomes Induce Better Neuroregeneration than Mesenchymal Stem Cells and Neural Differentiated Mesenchymal Stem Cells in Canine Model of Spinal Cord Injury

Hala Gabr[1], Wael A. Elkheir[2], Amal E. Fares[3*], Haithem AM. Farghali[4], Bassem E. Mahmoud[5], Mostafa A. Madbouly[5], Nehal Gamal[6], Ali M. Hamaad[6], Abo A. Elkheir[6], Rokia M. Hassan[3]

[1]Department of Clinical Pathology, Cairo University, Old Cairo, Giza Governorate, Egypt;[2]Department of Military Medical Academy, Military Medicine, Cairo, Egypt;[3]Department of Medical Histology and Cell Biology, Cairo University Old Cairo, Giza Governorate, Egypt;[4]Department of Surgery, Anesthesiology and Radiology, Cairo University, Old Cairo, Giza Governorate, Egypt;[5]Department of Siparadigm Diagnostics, New Jersey, USA;[6]Department of Kasr Alainy School of Medicine, Cairo University, Old Cairo, Giza Governorate, Egypt

ABSTRACT

Aim: The aim of this study is to compare spinal cord regeneration following mesenchymal stem cell injection, neural-differentiated mesenchymal stem cells injection with that following cell free exosome injection.

Method: 20 dogs were randomly divided into Sham group (dorsal laminectomy only) and experimental group which were subjected to a clipping contusion of the spinal cord. One week after SCI, GFP labeled BMSCs, NSCs and MSCs-Exo were transplanted intrathecally to investigate the safety and efficacy of each one in the therapy of SCI. The effects of the transplanted cells in dogs with SCI were determined using functional neurological scoring, histopathological and immunohistochemical methods.

Results: Our data demonstrate different therapeutic approaches for SCI as BMSCS, NSCs and MSCs-Exo enhanced remyelination and augmented neural regeneration, resulting in improved neurological functions. Special attention is paid to MSCs-Exo as they showed the marked improvement in the grey and white matter structure.

Conclusion: MSCs-Exosomes can be successfully used as a promising treatment for spinal cord regeneration.

Keywords: Mesenmchymal stem cells; Bone marrow; Cytoplasmic; Exosomes

Abbreviations: SCI: Spinal Cord Injury; MSCs: Mesenchymal Stem Cells; NSCs: Neural Differentiated Stem Cells; GFAP: Glial Fibrillary Acidic Protein

INTRODUCTION

Spinal Cord Injury (SCI) is one of the main causes of human disability worldwide. According to the WHO, up to 500,000 people present with SCI annually. Statistics show that road traffic accidents represent (38%), falls (22.2%), sports injuries and accidents (22.5%) of the total SCI.

Spinal cord injuries present clinically with impaired sensory and autonomic functions, motor activity deficit and neuropathic pain [1].

Traumatic SCI is the main neurological condition to treat difficulty in the clinic. After the primary injury, structural damage occurs, a series of secondary injuries, including hemorrhage, edema, demyelination, and axonal and neuronal necrosis, are involved in the pathogenesis of SCI. After that, infiltration with inflammatory cells like microglia, fibroblasts, and reactive astrocytes occurred, leading to a fibrous glial scar formation which prevents regeneration of the axon across the lesion [2].

Interventions to improve SCI outcomes are in use today, involving drug treatments, surgeries, and rehabilitation therapy, provide poor outcomes. Therefore, it is fundamental to find safe and

Correspondence to: Amal E. Fares, Department of Medical Histology and Cell Biology, Cairo University, Old Cairo, Giza Governorate, Egypt, Tel: 201118776556, E-mail: amalfares@ymail.com

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effective treatment that can improve SCI outcomes, a goal that is still far away. Stem cells are used recently in SCI treatment as a possible applicable therapy. Stem cell transplantation is used for regeneration of the injured neurons especially Neuronal Stem Cells (NSCs) and Mesenchymal Stem Cells (MSCs). Many studies of SCI, conducted on animal models, stem cell therapy proves enhanced motor activity and neurological functions [3]. However, direct transplantation of MSCs to target tissues remains challenging, as low survival rates, cell dedifferentiation, immune rejection, and tumor formation can all compromise the efficacy of this therapy [4].

Exogenous NSCs transplantation is known as an effective therapy for CNS diseases as NSCs could regenerate the damaged tissues [5]. However, the transplanted NSCs into the damaged tissue of spinal cord differentiate mostly into astrocytes, which can only achieve limited functional recovery [6].

Exosomes are membranous lipid vesicles (diameters of 40-100 nm) which contain functional proteins, mRNA, microRNA, and substances that are involved in the transfer of information between cells. Recent studies showed that exosomes derived from MSCs (MSCs-Exo) promote functional recovery after SCI by attenuating apoptosis and inflammation. They also promote angiogenesis, suppress glial scar formation, attenuate lesion size and promote axonal regeneration [4].

The aim of the present experiment is to compare spinal cord regeneration following mesenchymal stem cell injection, neural-differentiated mesenchymal stem cells injection with that following cell-free exosome injection.

MATERIALS AND METHODS

Methods

Mesenchymal stem cells isolation and culture: A bone marrow aspirate of 10 to 20 ml was obtained from 4 human donors after their informed consent. The aspirates were aspirated from the posterior iliac spine under aseptic conditions, after sterilization and applying of local anesthesia using 1% xylocaine [7,8].

MSCs were isolated from bone marrow aspirates by centrifugation with ficoll/paque at 1500 RPM for 20 mins at 37°C. Isolated MSCs were resuspended in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) low glucose supplemented with 10% FBS, 2 mg/ml l glutamine (Gibco, Grand Island, NY, USA), and 0.3% penicillin streptomycin (Gibco) at 37°C and 5% CO_2 concentration. Culture medium was changed every 3 days with microscopic examination of each flask until 80%-90% confluence and then was passaged. Cells were released with 0.5 ml of 0.25% trypsin/1 mM Ethylenediaminetetraacetic acid (EDTA) (GIBCO, USA) for 5 mins at room temperature.

Neural differentiation of MSCs

Neural differentiation of the cultured MSCs was induced at passage III using 20 mg/ml nerve growth factor in complete high glucose DMEM. Cells differentiation was followed morphologically every 3 days for 2 weeks [7,8].

Exosomes isolation

Culture medium of MSCs was changed and replaced with new clear medium and the flasks were incubated in the 5% CO_2 incubator at 37°C. After 24 hrs; the supernatant was placed into a falcon tube and centrifuged at 1500 RPM for 10 mins then at 3000 RPM for 30 mins. The supernatant was discarded, and the resultant pellet was suspended in saline and prepared for injection.

GFP labeling

In vitro GFP labeling was done by adding pCMV-AcGFP plasmid mixed with lipofectamine at a 2:1 ratio to each plate and incubating at 37°C for 6 hrs before injection.

Cell viability analysis

Cell viability analysis of MSCs and the neural-differentiated MSCs was done by adding equal volumes of the cell suspension to 0.4% trypan blue dye (1:1). Then they were counted using hemocytometer. Blue staining of cells after mixing was considered as an indicator of cell death.

Experimental design

This study was conducted on 20 adult mixed-breed male dogs that weighed (3.77 ± 0.59 kg). All aspects of animal care and treatments were approved by the animal care committee of Cairo University. The dogs were randomly assigned, without bias, into control 4 dogs (that were subjected to Sham operation where animals underwent dorsal laminectomy only) and experimental group [9]. Anesthetized dogs were placed in ventral recumbency on the operating bed and received a spinal cord injury at the L4 level performed by the same veterinary neurosurgeon on experimental 16 dogs. Briefly, after L4 laminectomy, the dura was opened, and the spinal cord was subjected to a guided fixed length clipping contusion to ensure reproducibility of the lesion. Postoperative care included that the dogs were kept warm and given manual bladder evacuation twice per day and prophylactic antibiotics. The dogs had no difficulty in feeding.

The experimental group was further divided into four groups according to treatment after SCI (n=4/group): Group I: left untreated. Group II: received GFP-labelled MSCs in a dose of 2×10^6 by intrathecal injection. Group III: received neural differentiated MSCs in a dose of 2×10^6 by intrathecal injection. Group IV: received cell free exosomes in a dose of 100 ug/kg body weight by intrathecal injection.Anesthesia was done with sodium pentobarbital, 40 mg/kg, University Pharmacy.

Intrathecal injection

It was performed one week after the SCI into the CSF by lumbar puncture using a 22-gauge spinal needle.

Clinical scoring (Initial assessment and follow up)

All dogs were subjected to neurological assessment by a neurologist to exclude any motor or sensory deficits before the experiment, the gait of each animal in the different groups was assessed and videotaped before and after surgery, assessment was done using Olby score (a 14-point functional scoring system for observational gait

analysis) and revised modified Tarlov scale. Both were established and commonly used to evaluate functional differences in dogs with acute spinal cord injury by examining the pain sensation and motor function including, tail movement, weight bearing and movement of limbs (Table 1).

Table 1: Comparison of olby score and revised modified tarlov scale.

Olby Score			Revised Modified Tarlov Scale	
No pelvic limb movement	No deep pain sensation.	0	1	Flaccid hind limbs
	With deep pain sensation.	1		
	But voluntary tail movement.	2		
Non-weight-bearing protraction of pelvic limb with more than one joint involved	Minimal movement of one joint.	3	2	Tone in hind limb
	Less than 50% of the time.	4	3	Purposeful hind limb motion
	More than 50% of the time.	5		
Weight-bearing protraction of pelvic limb	Less than 10% of the time.	6	4	Stands with assistance
	10-50% of the time.	7		
	More than 50% of the time.	8		
Weight-bearing protraction 100% of time with reduced strength of pelvic limb.	Mistake>90% of the rime.	9	5	Stands unassisted
	Mistake 50%-90% of the rime.	10	6	Limited ambulation
	Mistake<50% of the rime.	11		
Ataxic pelvic limb gait with normal strength,	But mistakes made>50% of time.	12	7	Full ambulation
	But mistakes made<50% of time.	13	8	Climbs a 20-incline ramp half-way
	Normal pelvic limb gait.	14	9	Climbs 20 incline ramp

Two different independent observers blinded to cases and control rated the animals by reviewing the videotapes of different groups. Assessment was done weekly for 4 weeks [10].

The animals were euthanatized by injection of 20% solution of pentobarbital sodium and full saturated solution of potassium chloride intravenous [11]. Spinal cord specimens were fixed in 10% formol saline for 24 hrs. Paraffin blocks were prepared and 5 µm thick serial sections were subjected to the following studies in the Histology Department, Faculty of Medicine, Cairo University.

HISTOLOGICAL STUDY

Hematoxylin and Eosin (H&E)

Immunohistochemical study: CD44 (IW-PA1021) for endogenous mesenchymal stem cells. A 0.1 ml primary antibody rabbit polyclonal antibody was applied to sections for 60 mins [12,13]. Tonsil sections were considered +ve control and the reaction are membranous and Glial Fibrillary Acidic Protein (GFAP) is a marker for brain astrocytes. It was diluted using 1: 100 IHC Tek antibody diluent; Incubated for 60 mins room temperature. Antigen retrieval in 10 ml citrate buffer, pH 6.0 for 10-20 mins was required. Staining Pattern: Cytoplasmic (glial cells such as astrocytes and ependymal cells) [14].

Morphometric study

Computer assisted image analysis was performed using Olympus camera connected to Olympus microscope, assessment of the numbers of neurons in the transverse sections of the grey matter in H&E stained sections. Assessment of area percent of both CD44 and GFAP +ve cells were measured. Using interactive measurements menu, the parameters were assessed in 10 high power fields.

Statistical methods

Statistical analysis and calculations were performed using Statistical Package for the Social Sciences (SPSS) version 16. The comparison between the different groups was analyzed using ANOVA test, followed by Bonferroni post-hoc test to detect which pairs of groups caused the significant difference. $P<0.05$ were considered statistically significant [15].

RESULTS

In the sham control group, neuronal morphology was normal; the general structure and structural integrity were preserved.

Clinical results

All experimental dogs (N=16) were subjected to neurological assessment by a neurologist using Olby score (a 14-point functional

scoring system for observational gait analysis) and revised modified Tarlov Scale (Table 2).

Table 2: Olby score/revised modified tarlov scale.

	1st week	2nd week	3rd week	4thweek
Group I (1st animal)	01-Jan	01-Jan	01-Jan	01-Jan
Group I (2nd animal)	0/1	01-Jan	01-Jan	0/1
Group I (3rd animal)	0/1	01-Jan	01-Jan	0/1
Group I (4th animal)	01-Jan	01-Jan	01-Jan	01-Jan
Group II (1st animal)	01-Jan	02-Jan	03-Feb	06-Apr
Group II (2nd animal)	01-Jan	02-Jan	03-Feb	05-Mar
Group II (3rd animal)	01-Jan	02-Jan	03-Feb	05-Apr
Group II (4th animal)	01-Jan	02-Jan	03-Mar	06-Apr
Group III (1st animal)	01-Jan	02-Feb	05-Mar	08-May
Group III (2nd animal)	01-Jan	02-Jan	05-Feb	07-May
Group III (3rd animal)	01-Jan	02-Feb	04-Mar	08-May
Group III (4th animal)	01-Jan	02-Jan	04-Mar	07-Apr
Group IV (1st animal)	01-Jan	03-Jan	05-Apr	09-May
Group IV (2ndanimal)	01-Jan	03-Feb	05-Apr	09-May
Group IV (3rd animal)	01-Jan	02-Feb	04-Mar	08-Apr
Group IV (4th animal)	01-Jan	04-Feb	05-Apr	09-May

The group I which did not received any treatment did not show any clinical or statistically significant difference on both scales, the mean difference (MD) on Olby's score was 0.5 with 95% CI (0.42-1.42) and P=0.18 while on the revised modified Tarlove scale the MD was zero.

In Group II which received intrathecal MSCs achieved a notable significant improvement with MD 4.5 with 95% CI (3.58-5.41) and P=0.0005 on Olby's score, while on the revised modified Tarlove scale the MD was 2.75 with 95% CI (1.95-3.55) and P=0.001.

While in group III, the dogs received intrathecal neural differentiated MSCs showed significant difference with MD 6.5 with 95% CI (5.58-7.41) and P=0.0001 on Olby's score, while on the revised modified Tarlove scale the MD was 3.75 with 95% CI (2.95-4.55) and P=0.0006.

Butin group IV which received cell free exosomes showed the highest significant difference in comparison with other groups on Olby's score with MD 7.75 with 95% CI (6.95-8.55) and P=0.00007, but on the revised modified Tarlove scale was the same like group III with MD 3.75 with 95% CI (2.95-4.55) and P=0.00006.

Histological results

Sections stained with H&E of the dog spinal cord in the control group showed parts of the grey matter with multiple neuronal cell bodies with processes. The neurons were scattered inside eosinophilic neuropil matrix with apparently normal blood vessels. In Group I grey matter appeared disrupted with small shrunken neurons and congested blood vessels. In Groups II and III small spindle neurons were seen surrounded by a hollow and apparent some congested blood vessel in group III. While in Group IV large, regenerated neurons with multiple processes scattered inside eosinophilic neuropil were detected (Figure 1).

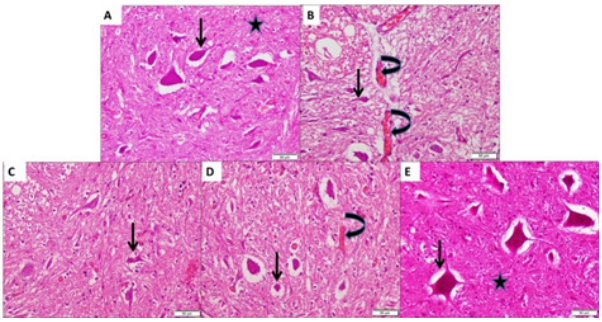

Figure 1: Photomicrographs of the dog spinal cord transverse sections (H&E x200) showing parts of the grey matter in Control Group. (A): with multiple neuronal cell bodies showing processes (arrow). The neurons were scattered inside eosinophilic neuropil matrix with apparently normal blood vessels (star). (B): In Group I (animals) grey matter appeared disrupted with small shrunken neurons (arrow) and congested blood vessels (arrow). (C): In Group II (animals) small spindle neurons surrounded by a hollow (arrow). (D): In Group III (animals) regenerated neuron surrounded by a hollow (arrow) and apparent some congested blood vessels were seen. (E): In Group IV (animals) large regenerated neuron with multiple processes (arrow) scattered inside eosinophilic neuropil were detected (star).

Transverse sections of the white matter showed in Control Group multiple nerve fibers with an axon. There were multiple empty vacuoles without axons in Group I. While in Group II and III some vacuolated nerve fibers were seen. In Group IV many nerve fibers

contained axons were detected (Figure 2).

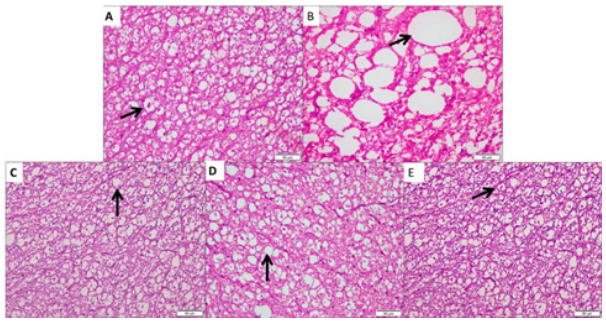

Figure 2: Photomicrographs of the dog spinal cord transverse sections of the white matter (H&E x200) showing in Control Group. (A): multiple nerve fibers with an axon (arrow). (B): In Group I (animals) multiple empty vacuoles without axons (arrow). (C and D): In Group II (animals) and in Group III (animals) some vacuolated nerve fibers (arrow). (E): In Group IV (animals) many nerve fibers containing axon (arrows).

In CD44 stained sections, the control group showed negative immune reaction to CD44. The neurons of the group I showed few positive immune reactions to CD44 which was indicated by dark brown staining. While in group II and III some +ve cells were detected. In group IV many +ve CD44 cells were seen (Figure 3).

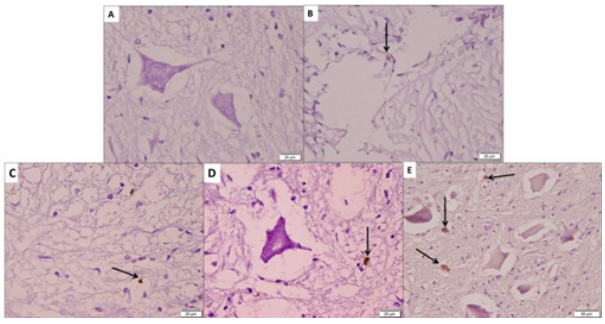

Figure 3: Photomicrographs of the dog spinal cord sections (CD44 immunostaining x400) showing in: Control Group (A): -ve immune reaction. (B): Group I (animals) few positive immune reactions. (C) and (D): Group II (animals) and Group III (animals) some +ve cells among the nerve fibers (arrow). (E): Group IV (animals) many +ve spindle cells among the nerve fibers (arrow).

In GFAP-stained sections, the control group showed few astrocytes with short processes with GFAP immunostaining. While Group I showed many +ve GFAP cells. Group II and III showed some +ve GFAP cells while group IV showed few and small +ve GFAP cells with short processes (Figure 4).

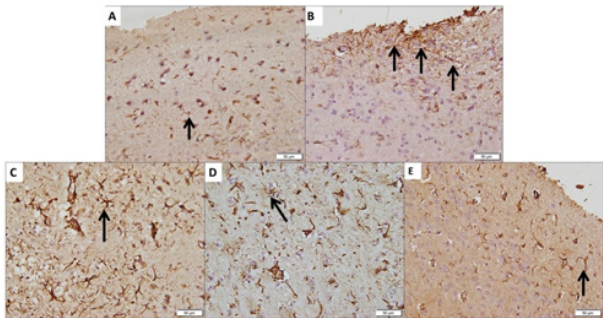

Figure 4: Photomicrographs of the dog spinal cord sections (GFAP immunostaining x200) showing in: Control Group (A): Few astrocytes with short processes. (B): Group I (animals) many +ve cells. (C) and (D): Group II (animals) and Group III (animals) some +ve cells (arrows) and (E): Group IV (animals) few and small +ve cells with short processes.

Morphometric results

Group I (untreated group) showed a significant decrease in the mean number of neurons when compared to control group and other experimental groups.

There was a significant increase in CD44 +ve cells in groups II, III and IV compared to group I, it also showed significant increase in group IV compared to groups II and III.

There was a significant increase in GFAP +ve cells in group I compared to control group and other experimental groups. A significant decrease in the mean number of GFAP +ve was found in group IV compared to groups II and III (Table 3).

Table 3: Mean count of neurons, mean number of CD44 +ve cells and mean number of GFAP +ve cells in control and experimental groups ± SD and (P-value). *: Significant decrease compared to other groups; $^{\#}$: Significant increase compared to groups I; $^{\square}$: Significant inecrease compared to groups II and III; $^{\infty}$: Significant increase compared to other groups; $^{\Delta}$: Significant decrease compared to groups II and III.

	Count of neurons	Number of CD44 +ve cells	Nmber of GFAP +ve cells
Control group	14.5 ± 2.01	-	3.7 ± 1.53
Group I	3.4 ± 2.24*	1.1 ± 0.42	31.7 ± 2.64$^{\infty}$
Group II	11.6 ± 3.82	3.8 ± 1.97$^{\#}$	15.5 ± 3.48
Group III	10.8 ± 3.64	4.3 ± 2.76$^{\#}$	11.9 ± 4.63
Group IV	12.2 ± 2.78	10.8 ± 2.83$^{\#\square}$	5.2 ± 3.72$^{\Delta}$
		(P-value=0.01)	(P-value=0.01)

DISCUSSION

Despite technological advances and some clinical trials, the therapeutic possibilities for SCI still remain reduced and represent a great challenge for clinicians and neuroscientists so this study was designed to compare spinal cord regeneration after induction of spinal cord injury followed by mesenchymal stem cell injection, neural-differentiated mesenchymal stem cells injection with that followed by cell free exosome injection. Spinal cord injury in this work was assessed clinically by Olby score and modified Tarlov scale [16].

In the current study, the control group revealed normal histological organization of the spinal cord structure in the grey and white matter, while the neurodegenerative changes were observed in H&E stained sections of Group I in the form of grey matter with small shrunken neurons with congested blood vessels while the white matter showed vacuolation with absence of the axons. A significant decrease in the number of neurons was detected as compared to other groups. These findings were explained by Xu et al. who stated that, traumatic SCI results in destruction of capillary network, activation of inflammation, loss of neural connectivity and formation of glial scar in the injured spinal cord with neurodegeneration and dysfunction [17]. Moreover, Guan et al. mentioned that, the primary injury which occurs directly after SCI will be easily irreversibly transformed into the secondary injury which damages spinal cord at the molecular level through inflammation, oxidative stress and apoptosis [18].

As during the primary stage, a global reduction of blood flow is observed, as a result of vasospasm, together with focal micro hemorrhages or thrombosis, causing a global dysfunction of the blood-spinal cord barrier. The cascade of events also affects electrolytic homeostasis around cellular membranes leading to the blockage of neuronal transmission [19]. The apoptotic changes and cell death were explained by that, the influx of water caused by acidosis promotes cytotoxic edema followed by cellular death [20]. The inflammatory process involves an immune response mediated by cellular invasion after disruption of the blood-spinal cord barrier with the production of cytokines promoting neurodegeneration [21].

Repair following SCI is defective due to both cell intrinsic factors and the extrinsic injury environment. Neurons of the mammalian CNS have low intrinsic regenerative ability due to a lack of growth driving signals and suboptimal availability or arrangement of subcellular machinery to enable growth cone reformation and axonal elongation [22].

Bradbury and Burnside explained the formed glial scar limits the functional recovery after SCI. As a healing response, the scar acts to spatially contain and isolate damaged area [23]. However, reactive injury response fails to restore spinal cord structure, pathological changes progress and the tissue within and around the scar remains dysfunctional.

In this work, group I exhibited a significant decrease in CD44 +ve immunostained cells and a significant increase in GFAP +ve immunostained cells compared to other groups. CD44 results in this group were explained by Drapeau et al. who stated that, migration of endogenous stem cells, seeking sites in need of repair, is crucial for the processes involved in ongoing normal maintenance and rejuvenation of healthy tissue, as well as for specific repair and healing of the injured tissue [24]. Although Endogenous Neural Stem Cells (NSCs) are found in the ependymal zone around the central canal of the spinal cord, neuronal dysfunction, and degeneration progress. As the releasing inflammatory factors and astrocytosis reaction, harm the NSCs niche [6].

Cheng et al. also added, an inflammatory response occurs after SCI. This response affects endogenous NSCs survival, self-renewal, migration, and differentiation [25].

GFAP, a cytoskeletal protein, is the major component of glial cells. GFAP expression is up regulated in SCI early stage, and some cytokines are secreted to promote axonal regeneration. However, in SCI late stage, many hyperplastic glial scars limit the growth of axons [26].

GFAP results were explained by Nathan and Li who stated that, reactive astrocytosis is a pathological process involved in excessive generation of astrocytes in response to CNS damages following trauma as destruction to spinal cord-blood barrier, leads to leakage of serum and plasma, increasing inflammatory reactions and enhancing activation of transforming growth factor β (TGF-β) which induce proliferation and migration of astrocytes at and around injury site and thus an increase in their number [27].

These results were also in accordance with Bradbury and Burnside who mentioned that, the scar that formed after SCI is formed of two distinct components: the lesion core, which is primarily composed of stromal derived fibroblasts and inflammatory immune cells, and the lesion border which surrounds the core and is primarily composed of hypertrophic astrocytes [23]. It is becoming increasingly clear that an inhibitory microenvironment composed of scar tissue and myelin proteins preventing nerve regeneration and neuronal differentiation after SCI [28].

Histological examination H&E stained sections of groups II and III showed moderate improvement in spinal cord structure in the form of grey matter with some small neurons surrounded with hollow and congested blood vessels. White matter appeared with vacuolated nerve fibers. Some CD44 +ve and GFAP +ve immunostained cells were observed with these groups. The improvement of MSC-treated group (group II) is attributed to immunomodulatory effect of MSC, as these cells secrete many cytokines and trophic factors such as TGF-α, IL-6, VEGF. These neurotrophins can offer trophic and structural support promoting an adequate environment for the survival of neurons and regrowth of axons after SCI [29]. These findings were in accordance with Rosado-de-Castro et al. who reported BMSC transplantation has neuroprotective effects, which might act on neuroinflammation, reducing cell death and diminishing the secondary tissue damage [30].

The transplantation of BMSCs is known as an effective therapy for SCI by attenuating inflammation and apoptosis, promoting angiogenesis and axonal regeneration, reducing astrocyte scars and syringomyelia, and promoting motor recovery [31]. However, the clinical application of BMSCs transplantation is very limited due to low survival rate and differentiation rate *in vivo* [32].

The improvement of NSCs-treated group (group III) was explained by Qian et al. who stated that, transplanted NSCs can survive, proliferate and differentiate in the injured spinal cord with functional recovery which is a result of transplanted NSCs which can sustain the survival of host cells and support local

axonal sprouting by release of trophic factors and cytokines with neuroprotective and immunomodulatory effects [33]. Exogenous NSCs transplantation creates a paracrine activity modulating the post-SCI inflammatory response and feeds the injured area with growth factors and rendering additional neurotrophic support which could also have a positive influence on the endogenous ependymal stem cells [34].

In this study, the marked improvement was observed with group IV as H&E stained sections showed large, regenerated neurons with multiple processes in the grey matter and apparent normal white matter with many nerve fibers. A significant increase in CD44 +ve immunostained cells and a significant decrease in GFAP +ve immunostained cells as compared to groups II and III. Exosomes have the capacity to carry intracellular sorted cargoes, including various proteins, lipids, mRNA, and microRNA, and further target select cells in various ways including delivering functional substances [35].

Several studies have demonstrated that exosomes participate in therapeutic effects, such as wound regeneration and reduction of neuronal cell death following cerebral ischemia [36].

In 2010, Lai et al. demonstrated for the first time that neurotrophic factors and nerve growth factors secreted by MSCs are exosomes. Teng et al. confirmed that exosomes secreted by MSCs promote angiogenesis and attenuate inflammation in myocardial ischemic injury [37,38].

Huang et al. explained our results with MSCs derived exosomes (MSCs-Exo) group by its effect in relieving apoptosis, the inflammatory response and stimulating angiogenesis. Khoshsirat et al. attributed MSCs-Exo effect to its ability in neural repair by reduction of inflammation, angiogenesis, and neurogenesis [39,40]. They also mentioned, the use of exosomes in the treatment of brain injury is more beneficial than employing MSCs themselves. Unlike MSCs, exosomes are easy to transport and maintain without differentiation.

The anti-inflammatory effect of MSCs-exosomes is achieved through inhibition of complements mRNA synthesis and release, and inhibition of NF-κB activation by binding to microglial cells. MSCs-exosomes also attenuate apoptosis, glial scar formation and promote axonal regeneration [4].

In 2019 Lu et al. demonstrated MSC-Exo treatment achieved better functional recovery after SCI by and explained as SCI causes detachment of pericytes from the vascular wall, leading to disruption of microvascular stability and increase permeability in Blood Spinal Cord Barrier (BSCB). Exosomes can relieve SCI by regulating the GFAP expression and suppressing glial scar formation [26,41]. MSC-Exo exerts obvious neuroprotective effects on SCI by reducing SCI-induced astrocytosis and inhibiting inflammation [42].

CONCLUSION

It can be concluded that MSCs-Exosomes can be successfully used as a promising treatment for spinal cord regeneration.The research has been given ethical approval.Potential conflict of interest. The authors have no conflicting financial interest.

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