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Original Article

Stem Cell Research in Bangladesh: Neurosurgical Perspective

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Abstract:

Purpose*: Traumatic spinal cord injury (SCI) is a severe neurological disease which causes sensory loss, motor paralysis and autonomic difficulties which is usually treated with physiotherapy. Complete spinal cord injury with ASIA Grade A patients remains untreated and neglected in our country since long back. Here we describe an effective strategy for the treatment of complete spinal cord injury by autologous bone marrow derived stem cell.*

Material and Method: *The study was conducted in the Neurosurgery department, Dhaka Medical College Hospital, Dhaka, Bangladesh from January, 2016 to January 2019. Total 22 Patients with spinal cord injury (ASIA Grade-A) were included in this study. Total 60 ml of autologous bone marrow was aspirated and processed to prepare 6-7 ml of bone marrow aspirate concentrate (BMAC) which was transplanted at the site of cord injury. Bony alignment was done by decompression and stabilization. Post-surgical physiotherapy and regular follow up was given.*

Result*: Surgical outcome was assessed by ASIA Grading. Among 22 patients, 10 patients (45.45%) improved by one grade, 7 patients (31.82%) improved by two grade, 2 patients (9.09%) improved by three grade, 3 patients (13.64%) did not show any improvement of any grade as because their follow-up period was less than 6 months. 19 patients (86.36%) noticed sensory, 16 patients (72.72%) noticed bladder improvement and 19 patients (86.36%) noticed bowel improvement to some extent.*

Conclusion*: Stem cell therapy is safe and effective. Steady and focused progress in stem cell research will open the door for many disable patients in the country like Bangladesh.*

Key words: *Spinal cord injury (SCI), Bone marrow aspirate concentrate (BMAC), American Spinal Injury Association (ASIA)*

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Introduction:

Every year, around the world, between 250 000 and 500 000 people suffer from a spinal cord injury (SCI). The majority of spinal cord injuries are due to preventable causes such as road traffic crashes, falls, violence, etc[1]. Bangladesh is a densely populated developing country in Asia, with 964 persons per square km^2. In Bangladesh, Most common causes of spinal injury were falls (50%), followed by road traffic accidents (RTA) and carrying loads on the head. 74.8% of the injured persons had been rescued from the accident site by local people but only 16.1% had been transported by ambulance. The spine board had never been used. More than half of the injured received initial treatment only at a sub-district or district hospital where none of the requisite facilities were

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available. While being transported from one hospital to the other, 10.7% experienced neurological deterioration to some extent[3]. Studies found that males (84%) are predominantly affected by traumatic SCI. Three-fourths (75%) of the injured were in the economically productive age group of 15-45 year. As a result, SCI has a negative impact on families as well as the national economy of Bangladesh. Both age and gender have nearly the same distribution as seen in other studies on developing and developed countries[4]. Spinal cord injury is certainly a debilitating and devastating condition in terms of its effect on a person's physical, mental, familial as well as social life[5]. Due to its profound impact on a person's overall quality of life and increasingly high incidence, injury to spinal cord due to any pathology is now considered as a morbid condition as well as a threat to both personal and national economy [6,7]. Spinal cord injury itself is a crippling condition, at the same time may lead to a variety of complications which can affect the life of the patient as it increases the treatment cost significantly and accelerate the disease process to early mortality[5,7,8]. The complex pathology of SCI may be divided into primary and secondary injuries. The primary injury is induced by mechanical damage and resultant hemorrhage. Many factors contributing to secondary injury include: excitatory amino acid toxicity, oxidative damage, inflammation and autoimmune response, edema, ischemia. These combined injury mechanisms, leading to glial and neuronal cell death, demyelization and axonal degeneration, are manifested as a severe impairment in neurological function[9]. At the cellular level, mild contusion to the spinal cord causes massive neuronal and glial cell loss, demyelination, cavitation and glial scarring[10]. Resulting in loss of sensory perception, distal motor paralysis and severe functional impairment. Functional improvement depends on the success of a combination of molecular, cellular and rehabilitative physiotherapy. Safe and effective therapies are clearly needed to enhance the quality of life for SCI patients[11]. Stem cell transplantation at the site of injury has emerged as a possible alternative therapy for severe spinal cord injury [12].Human bone marrow-derived mesenchymal stem cells (HBMSCs), identified alongside hematopoietic stem cells and possessing tremendous capacity for self-renewal and differentiation, are a type of adult stem cell that have demonstrated positive effects in the treatment of SCI[13,14].

In the current study, we report on surgical outcome of autologous bone marrow derived stem cell transplantation in 22 patients of SCI treated at Dhaka Medical College Hospital, Dhaka, Bangladesh. In addition to BMAC transplantation in the spinal cord, decompression and fixation of respective fragmented unstable vertebral column was done.

Materials and Methods:

A total of 22 SCI patients were enrolled in our study at Neurosurgery department, Dhaka Medical College Hospital, Dhaka, Bangladesh from January, 2016 to January 2019. There were 16 male patients (72%) and 6 female patients (28%) aged ranged from 11-67 years, with an average age of 33.5 years. According to the American Spinal Injury Association's classification of SCI (ASIA impairment scale) all the 22 cases were of grade A. Among all SCI patients, there were 6 cases involving the cervical region,

13 cases involving the thoracic region, 3 cases involving lumber region. In our study, the etiology of SCI were RTA in 13 cases and fall from height in 9 cases. Informed written consent were obtained from all participants before intervention.

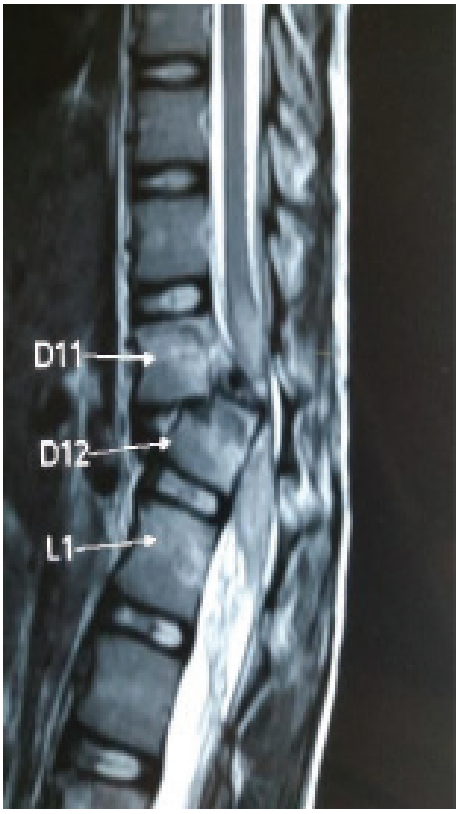


Fig.-1: *Pre operative MRI of SCI*

Transplantation methods:

Preparation of autologous bone marrow aspirate concentrates (BMAC):

Under aseptic conditions, 60 ml of bone marrow was aspirated from the posterior superior iliac crest and placed immediately into a sterile bag containing 4 ml of ACD solution. Then the aspirate was filtered from the bag and placed in a container and centrifuged for 14 min at 4000 rpm at room temperature. The supernatant plasma, Buffy coat were discarded and the nucleated cells which were precipitated are collected in a special syringe and transplanted at the site of injury immediately. Before transplantation in the cord, decompression and bony fusion of the vertebral column was done.

Fig.-2: *BMAC equipment set*

Fig.-3: *Collection procedure of bone marrow*

Fig.-4: *Centrifugation in BMAC centrifuger*

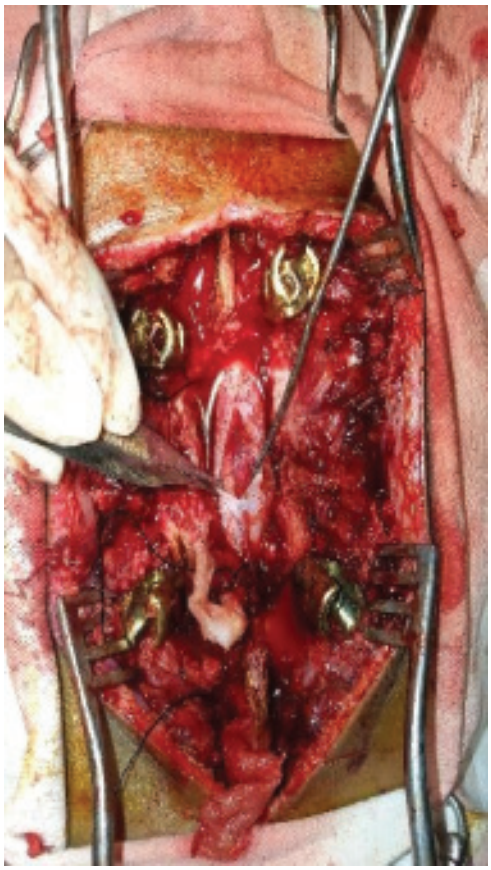


Fig.-5: *Per operative view with durotomy*

Procedure of application of bone marrow aspirate concentrate (BMAC):

Adequate decompression by laminectomy, partial corpectomy and discectomy followed by stabilization with transpedicular screw and rod was done. After durotomy at the site of injured spinal cord, the contused cord elements, fibrin debris, haematoma was sucked out and the site of injured area was prepared for transplantation, then the CSF pathway was made established. Subsequently the centrifuged BMAC stem cell was transplanted at injured site and in the surrounding place. Then water tight closure of dura was done. A total of 6 ml BMACs were injected into each SCI patient.

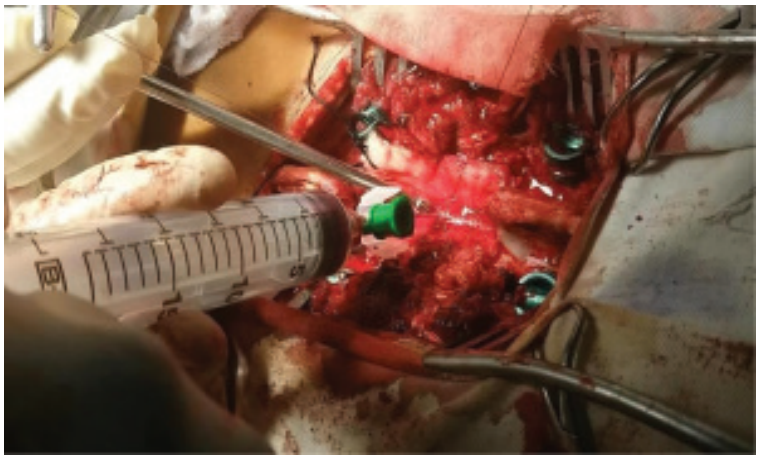
Neurological grading:

Neurological grading was performed using the ASIA impairment scale as follows: Grade A, complete: no motor or sensory function is preserved in the sacral segments S4-S5; grade B, incomplete: sensory but no motor function is preserved below the neurological level and includes the sacral segments S4-S5; grade C, incomplete: motor function is preserved below the neurological level and more than half of key muscles below the neurological level have a muscle grade <3; grade D, incomplete: motor function is preserved below the neurological level and at least half of the key muscles below the neurological level have a muscle grade of ≥3; and grade

E, normal: motor and sensory function is normal.

All the patients were assessed for ASIA grading on the day prior to transplantation, as well as at regular intervals following treatment.

(a)



(b)

Fig.-6: *(a): Application of BMAC at the site of cord injury, (b): Application of BMAC at the site of cord injury*

Urinary and bowel function:

Acute retention is a common issue for SCI patients. Assessments of bowel and bladder function are integral components of the Barthel activities of daily living (ADL) index. In order to better qualify the Barthel ADL index measures, bladder dysfunction was divided into no automatic micturition, incontinence, difficulty in urination, poor of urine control and dribbling urine, bowel dysfunction was divided into constipation, fecal incontinence and dry stools. Result was analyzed as methods above.

Results:

Neurological grading by ASIA impairment scale:

The post interventional result was evaluated after 6 months in the form of motor, sensory and autonomic.

Our analysis of the 22 SCI study patients receiving autologous BMAC transplantation was notable for all patients exhibiting improvement in sensory and motor function. Of the 22 SCI study patients, 19 individuals (86.36%) improved by one or two or three grades as measured by the ASIA

impairment scale where 10 (45.45%) of those 22 patients improving by one grade, 7 (31.82%) of the 22 patients improved by two grade and 2(9.09%) of those 22 patients improved by three grade. 3 (13.64%) patients did not show any improvement (Table II).

The prognosis of SCI patients appears to be dependent on the stage of SCI, the cause(s) of SCI and patient's age. Considering the multiple derived characteristics of our study patients, we compared the therapeutic effects of autologous bone marrow transplantation on SCI patients according to the variables in Table III.

Assessment of urinary and bowel function:

Of our 22 individuals (100% of the total SCI study subjects) suffering from urinary dysfunction in the form of acute retention. Patient's recovery information is shown in Table IV. 22 patients (100%) presented to us with acute urinary retention requiring

Table-I

Basic information of the patients when admitted to hospital.

Number	Gender	Age(years)	Injury site	Cause of injury	ASIA grade
1	F	11	T11-T12	RTA	A
2	M	35	T11- T12	RTA	A
3	M	50	C5-C7	FALL	A
4	M	45	C3-C4	FALL	A
5	F	17	T10-T11	FALL	A
6	M	30	T11-T12	RTA	A
7	M	63	T3-T4	FALL	A
8	M	18	C5-C6	FALL	A
9	F	11	C5-C6	RTA	A
10	M	28	T7-T8	RTA	A
11	M	18	T11-12	FALL	A
12	F	12	L1-2	RTA	A
13	M	30	T9-10	RTA	A
14	M	45	T11-12	FALL	A
15	M	40	T11-12	RTA	A
16	M	12	C5-6	RTA	A
17	F	40	T11-12	RTA	A
18	M	67	L2-3	RTA	A
19	M	44	T5-6	RTA	A
20	M	41	L1-2	FALL	A
21	M	17	T11-12	FALL	A
22	F	15	C6-7	RTA	A

ASIA: American Spinal Injury Association.

Table-II

Different variables impacting the efficacy of stem cell transplantation.

Factors	Initial cases (n=22)	Cases improved 6 months after cell transplantation (n=22)	Improvement rate (%)
Cause of injury			
Trauma RTA	13	11	84.61
FALL	9	8	88.89
Non-trauma	0	0	0.00
Site of injury			
Cervical vertebrae	6	4	66.67
Thoracic vertebrae	13	12	92.31
Lumbar vertebrae	3	3	100.00
Gender			
Male	16	14	87.50
Female	6	5	83.33

Table-III

ASIA grading before and after cell transplantation

ASIA rating before transplantation(n)	Initial cases (n=22)	ASIA grading Improvement 6 months after transplantation					Number of cases presenting improvement
		A	B	C	D	E	
A	22	3	10	7	2	0	19(86.36%)
Total	22	3(13.64%)	10(45.45%)	7(31.82%)	2(9.09%)	0(0%)	19(86.36%)

ASIA: American Spinal Injury Association.

Table-IV

Recovery of urinary function in spinal cord injury (SCI) patients

Types	Initial cases (n=22)	Cases improved 6 months after cell transplantation (n=22)
Acute Urinary retention	22	16

Table-V

Recovery of bowel function in spinal cord injury (SCI) patients

Types	Initial cases (n=22)	Cases improved 6 months after cell transplantation (n=22)
Hard stool	22	19

catheterization. Of the twenty two SCI individuals suffering from urinary dysfunction, 72% (16/22) experienced post-stem cell transplantation improvement in urinary function to varying degrees.

Of the 22 individuals (100% of the total SCI study subjects) suffering from bowel dysfunction, in the form of dry stool. Patient's recovery information is listed in Table V. Of the 22 individuals, 19 cases (86.36%) experienced post-stem cell transplantation improvement in bowel function to varying degrees.

Discussion:

A series of animal experiments[15,16] and clinical trials [17, 18] have previously demonstrated that stem cells have beneficial effects for SCI. Our study demonstrates that motor and sensory dysfunction, urinary and bowel functional disorders may improve significantly following stem cell transplantation.

A study using Human Bone Marrow Mesenchymal Stem Cell (HBMSC) transplantation for the treatment of SCI[17] demonstrated that 29.5% of patients in the acute stage (<2 weeks) experienced an improvement in ASIA impairment rating from grade A to either B or C. Additionally, 33.3% of patients in the subacute stage (2-8 weeks) experienced an improvement in ASIA impairment rating from grade A to either B or C; while no improvement in ASIA impairment scale occurred in the chronic (>8 weeks) group.

In our current study, 86.36% of SCI patients experienced an improvement in ASIA grading, with the majority of these patients receiving stem cell transplantation and adequate decompression after 2 weeks of SCI with the exception of one patient who was in the chronic (after 1 year) SCI stage by laminectomy and removing soft tissue.

Stem Cell therapy appears to have been more beneficial for the patients in the current study than for those in the study by Yoon et al, in theory, the higher the grade, the greater the improvement; however, our data suggests an inconsistency with this theory as because our all samples were complete spinal cord injury with lowest grading. In addition, while our study were followed-up patients for more than six month. Yoon et al continued for 10.4 months. In our study among 22 SCI patient 10 patients improved by one grade, 7 patients improved by two grade and 2 patient improved by 3 grade and 3 patients did not improve yet at all but patient may improve in subsequent follow up.

Normal urinary and bowel function make a significant contribution to quality of life. Of the 22 SCI patients (100% of total) reporting urinary dysfunction, 16 patients (72.73%) experienced improved micturition following stem cell transplantation. In the study by Kishk et al[19] where no patients experienced complete recovery in urinary function, but in our study five patients experienced a return to normal function and other with dribbling urine prior to stem cell therapy. All 22 patients having bowel dysfunction in the form of hard stool, 19 patients (86.36%) experienced an improvement in function following stem cell therapy; this result is commensurate with the results from the study by Kishk et al. In addition to aiding the functional integrity of autonomic nerves for normal urinary and bowel function, the return of intestinal secretions following stem cell transplantation may have ameliorated a significant factor in the bowel dysfunction in certain patients.

Conclusion:

Although limitations exist, bone marrow derived stem cell transplantation has demonstrated its effectiveness for the treatment of SCI. The majority of our patients were benefited clearly from transplantation with notable improvements in sensory, motor and autonomic function. The mechanisms by which stem cells benefit SCI patients, however, are not fully clear. Currently, the mechanisms by which stem cells are believed to repair damaged tissue include the secretion of neurotrophic factors, the ability to re-wrap injured nerve fibers suffering demyelization and the formation of neural circuitry by transplanted cells that are able to differentiate into neurons[20-21]. However, despite steadily accruing evidence in support of the therapeutic benefits of stem cell transplantation, universal consensus regarding the mechanisms of action does not yet exist. Additional studies of autologous bone marrow derived stem cell transplantation for the treatment of SCI remain a critical pursuit.

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