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PROCESSING OF BONE MARROW MONONUCLEAR STEM CELLS IN ACUTE SPINAL CORD INJURY

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ABSTRACT

The present study was conducted to evaluate the concentration of MNCs and its CD 34 positive cell fraction in the infusion sample of the autologous bone marrow concentrate used in the SCI patients. Bone marrow was the first source of mononuclear stem cells for experimental and later for clinical use. For experimental use, it is still probably the best and most accessible. 44 patients with loss of motor and sensory functions due to injury between T6 and L3 skeletal levels were selected. Finding shows that middle age groups [Mean ± SD (Range) =32.70 ± 9.80] are more prone to the SCI. among all the mode of injuries, the direct fall from height is the most common one (57 %) and L1, L2 and T12 are three main levels of motor loss due to SCI. There was a significant positive correlation of the total volume of bone marrow aspirated with bone marrow derived mononuclear cells ($p<0.01$, $r=0.92$). The volume of bone marrow aspirated had a significant positive correlation with the number of bone marrow derived CD34+ cells ($p<0.01$, $r=0.81$). The total number of mononuclear cells had significant positive correlation with bone marrow derived CD34+ cells ($p<0.01$, $r=0.78$). No significant association was found between mononuclear cells and $CD34^+$ cells count with age and sex of patients. We found that the number of mononuclear cells and $CD34^+$ cells were higher if greater amount of volumes of bone marrow was aspirated hence improving the chances of recovery.

KEYWORDS: SCI, CD34+, MNCs, BMC, BM.

INTRODUCTION

With technical advancement, huge advances have been made worldwide in the adult stem cell field. Several donor sites can be used for obtaining mononuclear stem cells of bone marrow. Bone marrow was the first source of mononuclear stem cells for experimental and later for clinical use. For experimental use, it is still probably the best and most accessible, as small amounts of bone blood can be easily aspirated in local analgesia. Site of aspiration is usually from the posterior iliac crests.

High velocity injuries have severely exposed the limitations of the conventional managements of severely injured patients and morbidity of the patients has increased greatly. Spinal injury associated with complete cord (neurological) involvement has been recorded for hundreds of years.[1] The hopelessness and helplessness experienced by both patients and treating doctors is responsible for the use of stem cells in the field of spinal cord injury management. The incidence of spinal cord injury is estimated at between 35 and 80 per million of population per year in most nations and as yet shows no sign of reduction.[2, 3, 4]

It has long been believed that intrinsic repair is quite restricted after spinal cord injury because neurogenesis rarely occurs in the central nervous system (CNS). As a result stem cell transplantation has become a promising therapeutic option for spinal cord injury (SCI) patients. A stem cell has the capability of forming various tissues under specific signals received from the body and the surroundings. The aim of stem cell therapy is to repair a damaged tissue that has lost the property to heal itself. In order to accomplish this goal, stem cells have been transplanted into the damaged area, directing them to grow a new, healthy tissue. The use of autologous bone marrow cells (BMCs) for stem cell therapy in SCI patients has more advantages. First one can avoid all problems associated with the immunological rejection or graft-versus-host reactions [5] which are frequently caused in allogenic cell transplantation. Secondly autologous BMC therapy is considered safe by not being associated with carcinogenesis.[6]

Some studies show that the total no of mononuclear cells obtained for transplantation was 4×10^8 cells and the $CD34^+$ cells in them was 90×10^6 cells.[7]Mononuclear

stem cells are contained in bone marrow (BM) aspirates in a concentration of approximately 10-100 MSC per 1×10^6 BM cells.[8,9,10,11,12] In this study we have found that in 10^8 bone marrow cells, around 1 to 3×10^7 $CD34^+$ cells are present.

The present study was conducted to evaluate the concentration of MNCs and its CD 34 positive cell fraction in the infusion sample of the autologous bone marrow concentrate used in the SCI patients.

MATERIAL AND METHODS

A prospective study was conducted in the Department of Transfusion Medicine in collaboration with Department of Orthopaedic Surgery, King George's Medical University Lucknow. Patients of acute spinal cord injury were taken from the Orthopedics Department KGMU, Lucknow. Patients with loss of motor and sensory functions due to injury between T6 and L3 skeletal levels were selected. Time elapsed since injury was noted. Complete history, neurological examination of the patient with all the necessary investigations was done.

Procedure: 70-180 ml bone marrow was aspirated from the posterior iliac crests under general anaesthesia (GA). Bone marrow aspirate- was collected in CPDA blood bags and processed by sedimentation and centrifugation in a closed sterile system following good laboratory practices in Component Laboratory of Transfusion Medicine Department, KGMU. Mononuclear stem cells were isolated and quantified for automated counts as well as CD34 counts.

After processing infusion of volume approx 8-10 ml was re-infused in the patient at the time of surgery at the site of injury into spinal cord.

The patient was monitored for recovery of motor and sensory loss till 6 weeks and then called for regular follow up after every one month.

Monitoring of recovery was done by ASIA method.

Investigation and Techniques used

Cell counts**:** It was done by automated cell count and confirmed by Neubauer's chamber.

CD34+ Count**:** It was done by immunoflowcytometer for bone marrow mononuclear cells.

RESULTS

As shown in Table 1 that 44 cases of spinal cord injury were included in our study. Out of them 36 (81.8%) were males and 8(18.2%) were females. Age ranged from 14yrs to 51 yrs. In which 4(9%) patients had age below 20 yrs and 6(14%) patients had age above 40 yrs. The most prevalent age group at injury was both 20-30 yrs and 31-40yrs group with equal distribution (39% each). The mean age at the time of injury was 32.70 ± 9.80 yrs. The most common cause of injury was fall from height (n=25; 57 %). The level of Motor Loss is shown in Table 2 for 44 patients. Majority of cases showed complete paraplegia below L1 i.e. 18 (41%) followed by L2-. 11 (25%), T 12- 8 (18%). Other levels have percentage less than 10. The sensory status of the patients was also assessed in all the cases and it is found that the anaesthesia levels were related almost as per motor level. Majority of cases showed (Table 3) anaesthesia below L1 i.e. in 24 (54.54%) cases, followed by L2 in 12 (24.27%), L3 in 1 (2.27%), T10 in 3 (6.81%) and below T12 in 4 (9.09%) cases.

Table 1: Age and Sex distribution of the SCI patients

Age Group (in years) ↓	Sex →	Male	Female	Total	Percentage
		36	**8**	**44**	**100 %**
< 20		3	1	4	9 %
20-30		16	1	17	39 %
31-40		14	3	17	39 %
>40		3	3	6	14 %

Table 2: Relation between mode of injury and motor loss

Mode of injury ↓	Levels of motor loss							Total cases in different types of mode of injury	%
	*T6	*T8	*T10	*T12	*L1	*L2	*L3		
Direct fall from height	0	0	2	7	8	7	1	**25**	**57**
Indirect fall from height	0	0	0	0	3	0	0	**3**	**7**
Fall of weight on body	0	0	1	0	4	3	0	**8**	**18**
Motor vehicle accidents	1	1	0	1	3	1	1	**8**	**18**
Total cases in different levels	**1**	**1**	**3**	**8**	**18**	**11**	**2**	**Total cases=44**	
%	**2**	**2**	**7**	**18**	**41**	**25**	**5**		

*** Complete paraplegia below___**

Table 3: Type and Level of Sensory loss

Diagnosis	No.(n=44)	%
Anaesthesia below L1	24	54.54
Anaesthesia below L2	12	27.27
Anaesthesia below T10	3	6.81
Anaesthesia below T12	4	9.09
Anaesthesia below L3	1	2.27

Association of sex and age with count of CD34+ cells: No significance difference was found with the count of CD34+ cells with the sex of the patients (Table 4). Maximum mean of CD 34+ count was found in the age group of above 40 years (Table 5).

Table 4: Means of CD 34 count in different Sexes

Sex type	Means of % CD 34 count ± Standard deviation
Male	0.3900 ± 0.2163
Female	0.3531 ± 0.3258
P-value = 0.7622 (insignificant)	

Table 5: Means of CD 34 in different Age groups

Age groups (in years)	Means of % CD 34 count ± Standard deviation
< 20	**0.1850 ± 0.1436**
20- 30	**0.3029 ± 0.2443**
31- 40	**0.4282 ± 0.3990**
> 40	**0.4433 ± 0.1945**

Association between aspirated volumes of bone marrow with bone marrow derived mononuclear cells, (Table-6): The mean of the total number of mononuclear cells was 2.18 ± 1.24×10 ^{6} (median 2.08×10 ^{6}, range 0.09-4.88×10 ^{6}) in the aspirated volume of 70-120 ml bone marrow aspirated. The mean of the total number of mononuclear cells was 6.8±1.48 ×10 ^{6} (median 7.08×10 ^{6}, range 3.90-8.87×10 ^{6}) in the aspirated volume of 130-180 ml bone marrow aspirated. There was a significant positive correlation of the total volume of bone marrow aspirated with bone marrow derived mononuclear cells ($p<0.01$, range=0.92).

Table 6: Comparison of mononuclear cells on the basis of aspirated bone marrow volume (ml)

Characteristics	Total number of Mononuclear cells		
Total volume aspirated (ml) ↓	**Mean ± SD ($\times10^6$)**	**Median ($\times10^6$)**	**Mean range ($\times10^6$)**
70-120	2.18 ± 1.24	2.80	0.09 - 4.88
130-180	6.8 ± 1.48	7.08	3.90 - 8.87
($p<0.01$, r=0.92)			

Association between aspirated volumes of bone marrow with bone marrow derived CD34+ cells (Table 7) The mean of the total number CD34+ cells was 2.41±1.198×10 ^{6} (median 1.98×10 ^{6}, range 1.09-4.94× 10^{6}) in the aspirated volume of 70-120 ml bone marrow aspirated. The mean of the total number CD34+ cells was 7.51± 1.71×10^{6} (median 7.15×10 ^{6}, range 4.11-9.90 ×10^{6}) in the aspirated volume of 130-180 ml bone marrow aspirated. The volume of bone marrow aspirated had a significant positive correlation with the number of bone marrow derived CD34+ cells ($p<0.01$, r=0.81).

So the data shown in table 6 and 7 clearly indicates that volume of aspiration of range 130-180 ml has much more significant count of MNCs and CD 34 positive cells as compared to range 70-120 ml.

Table 7: Comparison of CD34^{+} cells on the basis of aspirated bone marrow volume (ml)

Characteristics	Total number of CD34^{+} cells		
Total volume aspirated (ml) ↓	**Mean ± SD ($\times10^6$)**	**Median ($\times10^6$)**	**Mean range ($\times10^6$)**
70-120	2.41±1.198	1.98	1.09 - 4.94
130-180	7.51± 1.71	7.15	4.11 - 9.90
($p<0.01$, r=0.81)			

Association between total numbers of mononuclear cells with bone marrow derived CD34+ cells; (Table 8): The mean of the total number of CD34+ cells was 2.07±0.82 $\times 10^6$ (median 1.93×10^6, range 1.09-3.90) when the total number of mononuclear cells were 0.79-4.88$\times10^6$. The mean of the total number of CD34+ cells was 6.45±1.84 $\times10^6$ (median 6.25×10^6, range 4.11-9.90$\times10^6$) when the total number of mononuclear cells were 2.09-8.87$\times10^6$. The total number of mononuclear cells had a significant positive correlation with bone marrow derived CD34+ cells ($p<0.01$, range=0.78).

Table 9 has summarized information's showing means of various related variable such as the mean of the total volume aspirated from spinal cord injury patients was 126.70 ± 31.93 ml (mean range =117-136.41 ml) and the mean of the total volume of mononuclear cell suspension infusion was 10.08±2.41 ml (mean range = 9.35-10.81 ml). The mean of the lymphocytes, monocytes and mononuclear cells count before RBC depletion were 2.63±1.18× 10^3/µl (mean range = 2.27-2.99× 10^3/µl), 0.97±0.57× 10^3/µl (mean range =0.79-1.14× 10^3/µl), and 3.61±1.58 × 10^3/µl (mean range =3.13-4.09 × 10^3/µl) respectively. We extracted $CD34^+$ stem cells count from bone marrow aspirate and the mean percentage of $CD34^+$ cells concentration was 0.36±0.37 % (0.25-0.48 %) and the mean of the total number of $CD34^+$ cells infusion in the patients was 3.21± 2.20×10^7 with mean range (2.54-3.88 $\times10^7$).

Table 8: Comparison of total number of CD34 + cells on the basis of the number of mononuclear cells

Mononuclear cells	Total number of CD34+ cells		
Range ($\times10^6$)	Mean ± SD ($\times10^6$)	Median ($\times10^6$)	Mean range ($\times10^6$)
0.79-4.88	2.07 ± 0.82	1.93	1.09 - 3.90
2.09-8.87	6.45 ± 1.84	6.25	4.11 - 9.90
($p<0.01$, r=0.78)			

Table 9: Distribution of Parameters

Parameters	Mean ± SD (95% Confidence interval)	Mean range
Total volume aspirated	126.70 ± 31.93 ml	(117.00 - 136.41)
Lymphocytes count	2.63 ± 1.18 × 10^3/µl	(2.27 - 2.99) × 10^3/µl
Monocytes count	0.97 ± 0.57 × 10^3/µl	(0.79 - 1.14) × 10^3/µl
Mononuclear cells count	3.61 ± 1.58 × 10^3/µl	(3.13 - 4.09) × 10^3/µl
Volume of infusions	10.087 ± 2.41 ml	(9.35 - 10.81) ml
$CD34^+$ (%)	0.36 ± 0.37	(0.25 - 0.48)
Total no. of CD34+ cells infused	3.21± 2.20×10^7	(2.54 - 3.88) $\times10^7$

DISCUSSION

Spinal cord injury is a major neurological problem affecting people at large. Stem cell transplantation in spinal cord injury patients has shown a ray of light. Different sources of stem cells are being exploited for spinal cord injury as well as other neurological disorders.[13]

Most of the studies in medical literature are from advanced countries where the problem and presentations are different with respect to mode of injury, sex, incidence etc. The age distribution of patient is comparable with studies from the other parts of India and world. Most commonly affected age group being 20-29.[14] In our study the most prevalent age group was 20-40 years. In both 20-30 yrs and 31-40yrs group had equal prevalence of SCI of 39%.

Sex distribution has shown a male female ratio in this study. 81.8% (n=36) of spinal cord injury patients were males and 18.2% (n=8) were females, which is comparable to other recent studies.[15] (13.5 Male : 1 Female),[16] (8.98 Male:1 Female),[17] (3.7 Male:1 Female),[18] (3.7Male:1 Female),[19] (2.5Male:1 Female),[20] (4Male:1 Female), [21] (4.3 Male: 1 Female),[14] (2.96 Male:1Female). These studies reflect a changing trend as they cover a period of 15-20 years.

A study found that the most common cause of injury was fall from height including roof, trees, electricity pole (44.5%) followed by motor vehicle accident (34.7%). Falls were more common in second and third decades. Road side accidents were also commoner in third and fourth decades.[14] In our study, 25(57%) spinal cord injuries occurred due to direct fall from height, 3(7%) due to indirect fall from height, 8(18%) due to fall of weight over body and 8(18%) due to motor vehicles accidents.

The anatomical level of injury in the spinal cord is divided into high (cervical) and low injuries (thoracic, lumbar and sacral). The neurological level of injury is defined as the most caudal segment of the spinal cord that has normal function[22,23] The neurological assessment has an importance in making clinical diagnosis, for monitoring and also had prediction of functional outcomes. In 10 – 15 % of the patients with traumatic spinal cord injury there is a difference between

the anatomical and the neurological levels of injury. The reason is probably due to multi-level injuries, vascular pathologies and/or a spinal-cord oedema following injury.[24] A study found that out of 483, 164 patients were tetraplegics and 283 patients were paraplegics, while 36 patients had no neurological deficit. Dorsolumber spine injury was commonest with first lumber being the most common fractured vertebrae followed by twelfth dorsal vertebrae; cervical spine was next with most common site being fifth and sixth cervical vertebrae[14] In our study we found that out of 44 patients of paraplegia 18 (in 41% cases) patients has complete paraplegia below L1, 11 (in 25% cases) with complete paraplegia below L2, 8(in 18% cases) with complete paraplegia below T12 and rest of the cases were found below 10%. Radiologically 18 patients had fracture at T12 vertebrae, 15 patients had fracture at L1 vertebrae and 2 patients had dislocation at T11 over T12 vertebrae. 4, 1 and 4 patients had fracture at L2 vertebrae, L3 vertebrae and T12 vertebrae respectively. Further, we found that out of 44 cases, 24 patients had anaesthesia below L1, 12 below L2, 1 below L3, 3 below T10 and 4 below T12.

CD34 is an antigen expressed on early uncommitted and committed progenitor cells. [25] A study suggested that the infused $CD34^+$ PBPC (peripheral blood progenitor cell) dose could be used as a predictor of hematopoietic recovery after engraftment. Since then, a direct correlation between the numbers of collected or infused $CD34^+$cells/kg of body weight and the recovery of hematologic has been identified by many investigators.[26] The $CD34^+$ cell dose has a more profound effect on recovery of platelets than granulocytes. [25] In this study CD34 is used as a surrogate maker for stem cell infusion. In 10^8 bone marrow cells, around 1 to 3×10^6 CD34 cells are present. In the present study approximately 70-180 ml bone marrow were harvested from iliac crest and mononuclear cells were concentrated to the final volume of approximately (8-10 ml). While in the other study they concentrated final volume of mononuclear cells to 1.8 ml. [27] that was infused into the surrounding area of the injury site. A study showed the mononuclear cells were resuspended in saline and autologous plasma for a total volume of 80 ml. [7]

A study reported that the total volume of cells aspirated from the ileum was 728±72 ml (range 600 to 800) per patient, and the total volume of injected BM-MNCs was 45±7 ml (range 30 to 50) per patient. Total number of injected BM-MNCs was $3.5\pm0.8\times10^9$ (range 2.0 to 4.7×10^9), and that of $CD34^+$ cells was $6.8\pm2.6\times10^7$ (range 2.4 to 9.7×10^7). [28] In our study the mean of the total volume aspirated from spinal cord injury patient was 126.70±31.93 ml (117.00-136.41) and the mean of volume of mononuclear cell suspension infusion was 10.08± 2.41 ml (9.35-10.81).The mean of the mononuclear cells before RBC depletion was 3.61±1.58 × 10^3/µl (3.13-4.09× 10^3/µl).The mean of the mononuclear cells count before RBC depletion, total volume aspirated and volume of infusion were 3.79± 1.59 cells/µl, 125.97± 31.42ml, 10.18 ± 2.45 ml respectively in a male group of spinal cord injured patients. The mean of the mononuclear cells count before RBC depletion, total volume aspirated and volume of infusion were 2.76± 1.27 × 10^3/µl, 130± 36.25 ml, 9.36 ± 2.33 ml respectively in a female group of spinal cord injured patients.

There is a need to define a minimum safety threshold for $CD34^+$ cell dose for haematopoietic cell transplantation. This minimum dose would define a cell number in the graft, below which an unacceptable proportion of patients would be expected to have delayed hematopoietic recovery or failure to engraft. Some studies show that the average total $CD34^+$ obtained for transplantation was 90×10^6 cell. [27] In our study the mean percentage of the $CD34^+$ cells concentration was 0.36±0.37 % (0.25-0.48) while mean of the total number of $CD34^+$ cell infused patients were 3.21± 2.20 ×10^7 (2.54-3.88×10^7). We also found that the mean percentage of $CD34^+$ cells concentration and the total $CD34^+$ cells infused in male's spinal cord injured patients were 0.36± 0.40 %, 3.22± 1.97×10^7 cells respectively. While the mean percentage of $CD34^+$ cells concentration and the total $CD34^+$ cells infused in female's spinal cord injured patients were 0.39± 0.22%, 3.18± 3.23×10^7 cells respectively.

CONCLUSION

As a result of this study we were able to standardize our techniques for collection, processing and infusion of bone marrow mononuclear cells. We found that the number of mononuclear cells and $CD34^+$ cells were higher if greater amount of volumes of bone marrow was aspirated hence improving the chances of recovery. Infusion volume of approximately 9 ml was appropriate for transfusing these cells at the site of injury. Hence concentration and volumes of bone marrow plays a significant role in the further stem cell treatment of spinal cord injury. Commonest population in Indian prone to SCI are males in the age group of 20-40 years. Usually that fall is direct from a height and majority have shown sensory loss below L1.

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