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Effects of Human Erythropoietin on Functional Outcome of Patients with Traumatic Cervical Cord Injury; A Pilot Randomized Clinical Trial

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ABSTRACT

Objective: To determine the effects of recombinant human erythropoietin (rhEPO) on functional outcome and disability of patients with traumatic cervical spinal cord injury (SCI).
Methods: This was a randomized, double blind, placebo controlled clinical trial being performed in Nemazee and Shahid Rajaei hospitals of Shiraz during a 3-year period from 2011 to 2014. A total number of 20 patients with acute traumatic cervical SCI less than 8 hours after injury were included. We excluded those with anatomic cord dissection, penetrating cord injury and significant concomitant injury. Patients were randomly assigned to receive rhEPO in 500IU/mL dosage immediately and 24-hour later (n=11) or placebo (n=9). All the patient received standard regimen of methylprednisolone. Neurological function was assessed on admission, 1, 6 and 12 months after the injury according to the American Spinal Cord Injury Association (ASIA).
Results: Overall we include a total number of 20 patients. The mean age of the patients was found to be 40.1±9.5 (ranging from 19 to 59) years. There were 18 (90.0%) men and 2 (10.0%) women among the patients. There was no significant difference between two study groups regarding the baseline characteristics. The baseline ASIA score was comparable between two study groups. The motor and sensory ASIA scores were comparable between two study groups after 1, 6 and 12 months follow-ups. We also found that there was no significant difference between two study groups regarding the motor and sensory outcome in complete cord injury and incomplete cord injury subgroups.
Conclusion: Administration of rhEPO does not improve the functional outcome of patients with traumatic cervical SCI.
Clinical trial registration: The study has been registered with Iranian Registry for Clinical Trials (www.irct.ir; IRCT2014122920471N1)

Keywords: Erythropoietin; Cervical cord injury; Functional outcome; Disability; Neuroprotective effect.

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Introduction

Traumatic spinal cord injury (SCI) is a catastrophic sudden and devastating event associated with high mortality and morbidity and high social and economic burden. In developing countries the age range of traumatic SCI is 18-32 years while in developed countries the age is over 65 years because of aging population [1]. A global-incident rate (2007) is estimated at 23 SCI cases per million (179 312 cases per annum). Regional data are available from North America (40 per million), Western Europe (16 per million) and Australia (15 per million). Extrapolated regional data are available for Asia-Central (25 per million), Asia-South (21 per million), Caribbean (19 per million), Latin America, Andean (19 per million), Latin America, Central (24 per million), Latin America-Southern (25 per million), Sub-Saharan Africa-Central (29 per million), Sub-Saharan Africa-East (21 per million) [1,2].About 80.7% of all spinal cord injuries are reported in men (2), but elder women with osteoporosis have a propensity for vertebral fractures from falls with associated SCI [3-5].

Currently there no appropriate treatment available for SCI. The SCI treatment begins with restraining the spine and lessening the inflammation to restrain further damage. The neuroprotective drugs act to reverse specific secondary injuries and to avoid neural damage. These agents are supposed to stimulate axonal regeneration. The sole standard therapeutic intervention for patients with SCI is the administration of high dose methylprednisolone sodium succinate (MPSS) within eight hours of injury which has been shown to be associated with better motor and sensory function [6-11]. Several neuroprotective agents have been applied for treatment of SCI including naloxone which blocks the neurotoxic effects of the endogenous opioid dynorphin A [12,13]. Tirilazad, a potent lipid preoxidation inhibitor, that inhibit the peroxidation of neuronal membranes [14], and nimodipine that prevents calcium-dependent activation of destructive cellular enzymes and presynaptic glutamate release [15]. Minocycline has reported to be neuroprotective in animal injury models and cause to improve motor recovery at one year [16]. Injection of basic fibroblast growth factor (bFGF) has been reported to recover functional and respiratory parameters in animal injury models, probably by decreasing glutamate-mediated excitotoxicity [17].

Erythropoietin (EPO) is a tissue protective agent which acts via modulating of numerous signaling pathways of inflammation, apoptosis cell death and vascular integrity. Interestingly, the protective effects have been reported with both endogenous and exogenously administered EPO [7]. It protects endothelial, neural, cardiac, and other cell types against cytotoxic damage [18,19]. Recently, it has been demonstrated that rhEPO receptors are present in capillary cells of the subcortical white matter as well as on bodies and proximal dendrites of motor neurons of the ventral horn enclosed by a dense plexus of EPO-immunoreactive fibers [5,7,14,20]. Recombinant human erythropoietin (rhEPO)is currently administrated broadly with an outstanding safety profile, clinical trials seeking its potential to inhibit motor neuron apoptosis and the neurological shortages [19,21,22]. The aim of the current study is to determine the neuroprotective effects of rhEPO along with MPSS in patients with traumatic cervical SCI.

Materials and Methods

Study Population

This was randomized, placebo controlled, randomized clinical trial being performed in Nemazee and Shahid Rajaei hospirals, both tertiary healthcare centers affiliated with Shiraz University of Medical Sciences during a 3-year period from 2011 to 2014. We included a total number of 30 adult (>18 years) patients with acute traumatic cervical SCI who arrived at the emergency room not later than 8 hours after the injury. We excluded the patients with anatomic cord dissection, penetrating cord injury, injury beyond cervical cord, significant concomitant injury, contraindication for methylprednisolone, contraindication for erythropoietin. The study protocol was either approved by institutional review board (IRB) and medical ethics committee of Shiraz University of Medical Sciences, All the patients provided their informed written consents. The study protocol was also registered with Iranian registry for clinical trials (IRCT2014122920471N1; www.irct.ir).

Study Protocol

A complete neurologic examination including motor and sensory evaluation, deep tendon reflex recording and plantar reflex were performed by the neurosurgery resident. All the patients underwent cervical radiography, CT-Scan and MRI. Patients were randomly assigned to two study groups using a computer-based random digit generator based on the admission numbers. All the patients received Methylprednisolone intravenously (30mg/kg as bolus doseand 5.4 mg/kg/hr for 23 hour as maintenance). Those assigned to first group received rhEPO (PDpoetin, Pooyesh Darou, Tehran, Iran)in a dosage of 500 unit/kg as bolus dose followed by another 500 unit/kg administered 24-hour after the first dose. The second group received injection of placebo (normal saline) in two same intervals.

The rehabilitation started after 24 hours of admission. Patients were visited 1, 6 and 12 months after the injury and were assessed regarding neurologic function. Neurologic function was assessed using the American Spinal Injury Association (ASIA) score rating for sensory and motor function. According to ASIA score, the

motor score is based on the examination of 10 key-muscles on each side. For each movement, force is measured and assigned a coefficient from 0 (absence of muscle contraction) to 5 when contraction creates a movement in all the joint amplitude against a complete resistance. The maximal total score is so 100 (50 on the Right and 50 on the Left). The sensory score is established after studying tact and prick sensitivity on a key point in each of 28 dermatomes on each side. Absence of sensitivity is quoted: 0, the hypo or the hyperesthesia: 1 and normal sensitivity: 2. The sensory evaluation was started by testing the light touch and the lower part of the body. The physician recording the outcome was blinded toward the study groups. All the patients and those visiting the patients postoperatively were blind to the study groups. Only the statisticians were aware of the study groups.

Statistical Analysis

The power of the study was 80% and the α-coefficient was considered 0.05. Thus the number of required patients was 15 in each group. All the statistical analyses were performed with Statistical Package for Social Sciences (SPSS Inc., Chicago, USA). Data are presented as mean±SD and proportions as appropriate. Independent t-test was applied for comparing the parametric data between groups and paired t-test was used to compare such data within the groups. Proportions were compared with chi-square test. A two-tailed *p*-value of less than 0.05 was considered statistically significant.

Results

We screened 30 patients for eligibility out of whom 27 full filled the inclusion criteria and were randomized to two study group. During the study 7 patients passed away and thus were excluded from the study. Thus the total number of patients included in the final analysis was 20 (Figure 1). The mean age of the patients was found to be 40.1±9.5 (ranging from 19 to 59) years. There were 18 (90.0%) men and 2 (10.0%) women among the patients. C5fracture was found to be the most common site of fracture being recorded in 11 (55.0%) patients being followed C6 in 3 (15.0%) and C7in 3 (15.0%). The most common type of fracture was A3 in 12 (60.0%) patients. Surgery was performed in 14 (70.0%) patients while 6 (30.0%) patients were managed conservatively. Twelve (60.0%) patients were complete cord on admission while 8 (40.0%) patients were diagnosed to be incomplete cord injury. Baseline and demographic information of 20 patients with cervical cord injury is summarized in Table 1.

The baseline ASIA score was comparable between two study groups (Figure 1). Baseline motor ASIA scores for right upper (p=0.496), right lower (p=0.055), left upper (p=0.855) and left lower (p=0.306) were comparable between two study groups. The motor assessment in 1 month follow-up revealed the same results; i.e. the motor powers of right upper (p=0.579), right lower (p=0.275), left upper (p=0.956) and left lower (p=0.536) were comparable between two study groups. In the same

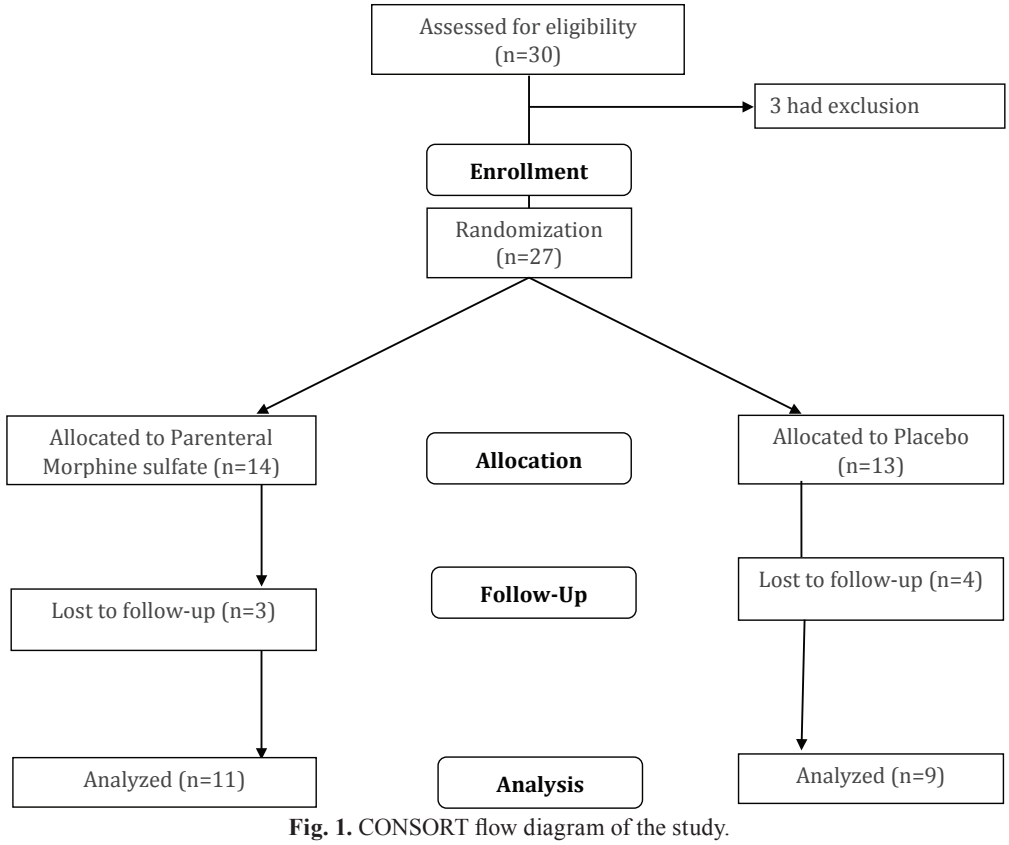

Fig. 1. CONSORT flow diagram of the study.

Table 1. Baseline characteristics of 20 patients with traumatic cervical SCI treated with recombinant human erythropoietin or placebo.

Variable	Erythropoietin (n=11)	Placebo (n=9)	*p* value
Age (years)	37.1±3.4	43.3±5.8	0.417
Gender			
Men (%)	9 (90.9%)	9 (88.9%)	0.711
Women (%)	1 (9.1%)	1 (11.1%)	
Site of injury (n)			
C3 (%)	1 (9.1%)	0 (0.0%)	0.286
C4 (%)	1 (9.1%)	1 (11.1%)	
C5 (%)	7 (63.6%)	4 (44.4%)	
C6 (%)	0 (0.0%)	3 (33.3%)	
C7 (%)	2 (18.1%)	1 (11.1%)	
Type of injury (n)			
A1 (%)	0 (0.0%)	1 (11.1%)	0.477
A2 (%)	1 (9.1%)	2 (22.2%)	
A3 (%)	7 (63.6%)	5 (55.5%)	
B2 (%)	3 (27.3%)	1 (11.1%)	
Treatment			
Surgery (%)	9 (90.9%)	5 (55.5%)	0.336
Conservative (%)	2 (18.1%)	4 (44.4%)	

way the motor ASIA scores was comparable between two study groups after 6 and 12 months follow-ups (Figure 2).The sensory evaluation included the assessment of both pinprick and light-touch sense in upper and lower trunk. The baseline sensory ASIA scores for left light touch (p=0.305), right light touch (p=0.187), left pinprick (p=0.136) and right pinprick (p=0.099) were comparable between two study groups. The sensory assessment in 1-, 6- and 12-month follow-up revealed the same results; i.e. the sensory ASIA scores were comparable between groups (Figure 3).

Over all 12 patients were diagnosed to suffer from complete cord injury while 8 patients had incomplete

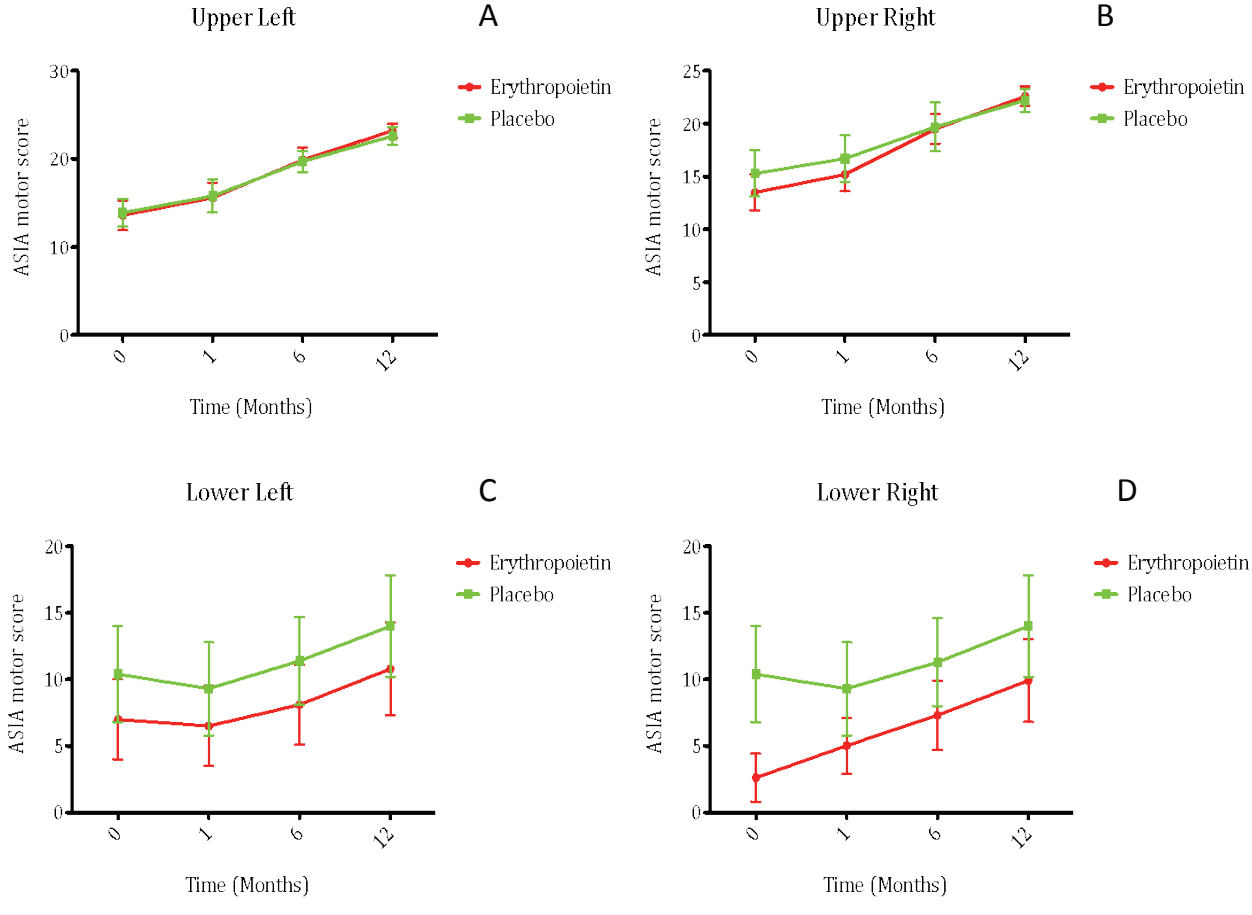

Fig. 2. ASIA motor score in erythropoietin and placebo groups. Graphic depiction of ASIA motor score in all patients. Treatment of erythropoietin did not show any significant change in ASIA motor score of the patients.

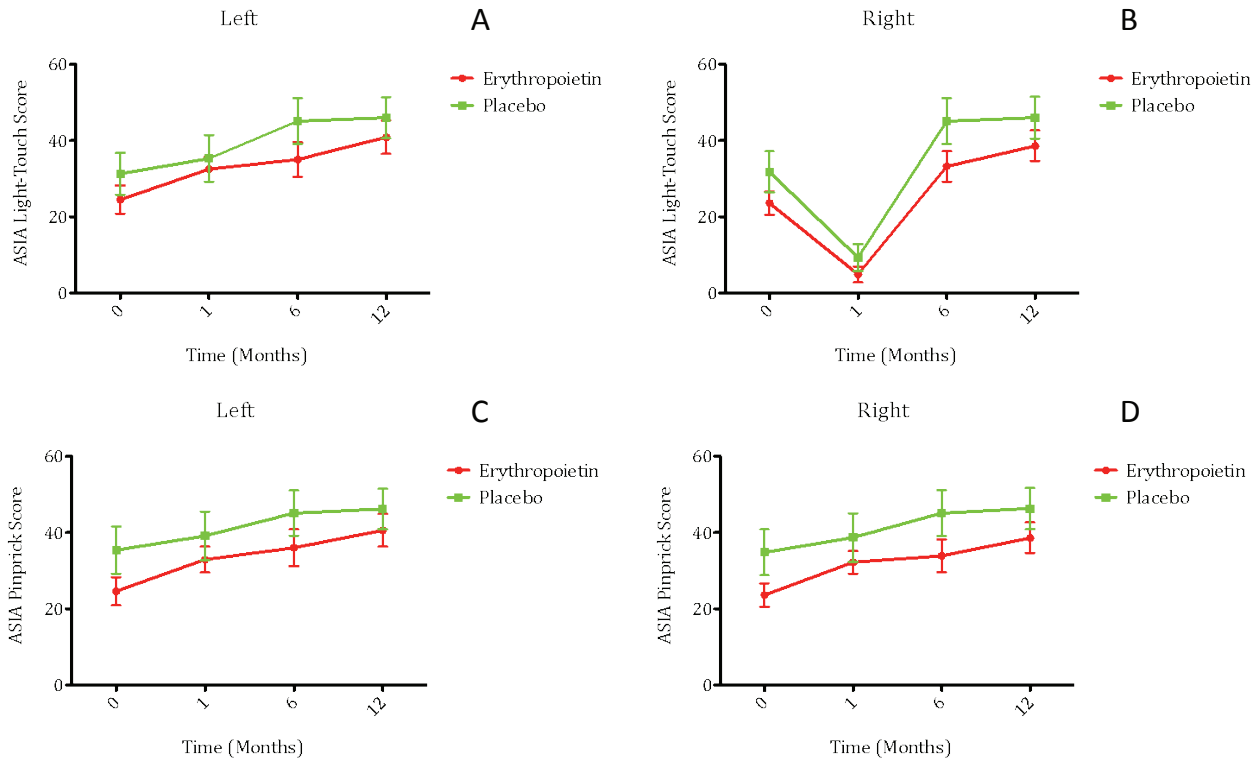

Fig. 3. ASIA sensory score in erythropoietin and placebo groups. Graphic depiction of ASIA sensory Score in all patients regarding Light-Touch (A, B) and Pinprick (C, D). Treatment of erythropoietin did not show any significant change in ASIA sensory score.

cord injury. We compared the motor and sensory outcome between those who received erythropoietin and those who received placebo according to cord status. We found that there was no significant difference between two study groups regarding the motor and sensory outcome in complete cord injury subgroup (Table 2). We also found that there was no significant difference between two study groups regarding the motor and sensory outcome in incomplete cord injury subgroup (Table 3).

Discussion

As the safety and efficacy profile of rhEPO is investigated and proved in diverse neurological diseases and injuries, we aimed to investigate the role of this agent in traumatic cervical cord injury. We found that administration of rhEPO along with methylprednisolone within 8 hours of injury was not associated with improved functional outcome (both motor and sensory) according to ASIA score. This

Table 2. The sensory and motor outcome of 12 patients with complete cervical cord injury receiving erythropoietin or placebo.

Variable	Erythropoietin (n=8)	Placebo (n=4)	*p* value
Age (years)	33.25±11.7	34.25±13.5	0.896
Left upper motor on admission	12.5±6.14	12.5±5.44	0.999
Right upper motor on admission	12.5±6.14	12.5±5.44	0.999
Left lower motor on admission	2.87±8.13	0.0	0.506
Right lower motor on admission	0.0	0.0	---
Left light touch on admission	19.25 ±7.9	18.75±11.78	0.931
Right light touch on admission	19.25±7.9	18.78±11.78	0.93
Left pinprick on admission	19.25±7.9	18.75±11.78	0.931
Right pinprick on admission	19.25±7.9	18.75±11.78	0.931
Left upper motor after 12 months	22.75±3.15	20.25±2.98	0.946
Right upper motor after 12 months	22.0±3.07	20.0±3.46	0.822
Left lower motor after 12 months	5.75±9.11	3.0±4.76	0.625
Right lower motor after 12 months	5.12±7.66	30±4.76	0.889
Left light touch after 12 months	34.87±12.3	37.7±21.54	0.375
Right light touch after 12 months	34.25±12.2	37.5±21.91	0.693
Left pinprick after 12 months	34.87±21.32	38.25±21.17	0.482
Right pinprick after 12 months	34.25±12.2	38.0±21.55	0.659

Table 3. The sensory and motor outcome of 8 patients with incomplete cervical cord injury receiving erythropoietin or placebo.

Variable	Erythropoietin (n=3)	Placebo (n=5)	*p* value
Age (years)	47.33±23.6	50.6±18.6	0.896
Left upper motor on admission	16.0±2.64	15.0±4.52	0.999
Right upper motor on admission	16.0±2.64	17.6±7.05	0.999
Left lower motor on admission	18.0±6.24	18.80±6.18	0.506
Right lower motor on admission	9.66±8.50	18.80±6.18	---
Left light touch on admission	38.66±11.54	41.4±12.46	0.931
Right light touch on admission	35.33±57.77	42.2±10.37	0.93
Left pinprick on admission	38.66±11.54	48.8±9.85	0.931
Right pinprick on admission	35.33±5.77	47.6±9.09	0.931
Left upper motor after 12 months	24.33±1.15	24.4 ±1.34	0.946
Right upper motor after 12 months	24.33±1.15	24.0±2.23	0.822
Left lower motor after 12 months	24.33±1.15	22.8±4.91	0.625
Right lower motor after 12 months	22.33±3.05	22.8±4.91	0.889
Left light touch after 12 months	57.0±1.73	52.6±7.6	0.375
Right light touch after 12 months	50.33±9.81	52.8±7.15	0.693
Left pinprick after 12 months	56.0±0.0	52.6±7.6	0.482
Right pinprick after 12 months	50.33±9.81	53.0±6.7	0.659

questions the clinical role of rhEPO in treatment of patients with traumatic cervical SCI. As the main biological basis ofrhEPO neuroprotection is the inhibition of neuronal apoptosis, which is a very early event post injury within potentially viable site of cord injury [23], the 8-hr time window is likely of critical relevance for rhEPO therapy in spinal cord injury and we used this time frame in our study, as delayed administration of rhEPO is shown to be out of clinical benefit in human brain injury [24]. Erythropoietin did not change ASIA motor scores and sensory examination significantly in upper or lower extremities of patients compared to placebo and also there was no change with regard to thesite, type, kind of their injury and sex of patients.

Our finding of ineffectiveness of early treatment of recombinant erythropoietin to improve neurological function of patients with cervical SCI brings some point of views. The first point is that the present study is a leading study sought to indicate the beneficial effect of rhEPO in human SCI setting. The small size of sample study is a common and inevitable problem in such pilot and preliminary human clinical trials. Although we first included 27 patients, 7 patients were eliminated from the trial due to their death during the study. Because of sample size issue, we just employed simple dosage of rhEPO, 500 unit/kg while the best biochemical results was obtained with 5,000 IU/kg of rhEPO. Conducting additional clinical trials with higher dosages of rhEPO might shed more light on dose dependency of rhEPO therapy in SCI.

The main idea of present study was based on animal model of SCI. However, time course and sequence of pathophysiologic events contributing to the evolution of spinal cord injury may be different in humans from mouse and rat models, so it is a distinct possibility that human patients administered rhEPO would experience no desired outcome than observed in the previous animal model of study.Concomitant administration of rhEPO with methylprednisolone is another challenging issue. A critical aspect in all bio-specific therapy is the expression of receptor in target cells to obtain willing results. rhEPO receptor expression is found to be associated with inflammatory condition [25] andEPO-R play important roles in modulating the inflammatory response to injury. Whether methylprednisolone, a potent anti-inflammatory agent, affect the expression ofrhEPO receptor in human spinal cord or not may influence clinical outcome of rhEPO therapy. Availability of this large (34 kD) molecule in the site of spinal cord after intravenous infusion is a critical matter to discuss.

The rout of administration may affect the clinical outcome of rhEPO therapy in different diseases. Both intravenous and subcutaneous administrations are usually employed to deliver rhEPO to patients with renal failure. There are a few pharmacokinetic and pharmacodynamics advantages to the subcutaneous route over intravenous administration in clinical studies [26]. In most of animal model studies of SCI, rhEPO was administrated intraperitoneally [21]. One difference between animal model of SCI and present study may be a different rate of absorption of rhEPO from the I.P. or I.V. injection site and mobilization into the injured region of the spinal cord as a result of the profound hemodynamic changes that occur in traumatic SCI. A complementary set of experiment is required to confirm that intravenous administration of a total of 100,000 IU of rhEPO lead to an increase in CSF rhEPO levels.

As a first and unique report in human subject, the results of rhEPO therapy in a small scale study of traumatic cervical SCI were not consistent with the efficacy of rhEPO observed in animal models of traumatic spinal cord injury. The comprehensive results obtained using a large group of patients

with different characteristics may enlighten the exact efficacy of rhEPO in human SCI. Although intravenous high-dose rhEPO is well tolerated in traumatic cervical SCI but is not associated with an improvement in clinical outcome at 1, 6 and 12 months. However, a larger scale clinical trial is need to seek possible beneficial effect regarding different patient characteristics. This prospective is undertaking in our group.

Conflict of interest: None declared.

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