

Research Article



Evaluation of Bone marrow Erythropoietin Receptor in Trauma Hemorrhagic Shock patients

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Abstract

Background: Altered hematopoietic stem cells (HSCs) have been observed in severe trauma and hemorrhagic shock (HS). Modulation of erythropoietin receptor (EpoR) can be the one of the reason of HSCs dysfunction. Therefore, purpose of the study was to evaluating the expression of the bone marrow (BM) erythropoietin receptor (EpoR) in trauma hemorrhagic shock (T/HS). **Objective:** Therefore, our aim of the study was to evaluate the expression of BM- EpoR in T/HS patients. **Methods:** We conducted a prospective cohort study. 70 with T/HS and 30 controls were recruited. Peripheral blood sample (PBS) was collected for measurement of serum EPO levels on admission. Those patients survived on day 3, collected BM sample (n=14) for expression of EpoR on BM erythroid cells and was evaluated by flow cytometry. Clinical parameters were prospectively collected on admission. **Results:** Significantly decreased the expression of EpoR (<0.05) on bone marrow in the T/HS patients when compared with control group. A serum level of EPO was elevated in T/HS patients on admission. **Conclusions:** Our studies suggest, T/HS patients display defective erythropoiesis. Expression of EpoR on BM may be used predictor of delay recovery of hematopoietic dysfunction.

Keywords: Erythropoietin-receptor (EpoR), bone marrow (BM), Trauma hemorrhagic shock (T/HS).

Introduction

Hemorrhagic shock (HS) and its sequelae of multi-organ dysfunction (MOD) and sepsis are the major leading cause of death after trauma [1]. Cytokine storm dysregulates balance of pro-inflammatory and anti-inflammatory cytokines which leads to clinically fatal outcome [2]. The role of bone marrow (BM) dysfunction in trauma hemorrhagic shock (T/HS) has been poorly understood. BM dysfunction in T/HS

leads to persistent anemia and increased the susceptibility to infection and sepsis, mainly due to dyserythropoiesis and myelopoiesis. BM dysfunction is a multifactorial process. Excessive pro-inflammatory cytokine milieu and elevated levels of circulating catecholamines change the behavior BM microenvironment in a T/HS [3-4]. Erythropoietin receptor (EpoR) may be one of the parameter of BM erythropoiesis. Therefore, the aim of the study to probe to mechanism underlying alternation of HSCs in T/HS by evaluating parameter of BM-EpoR.

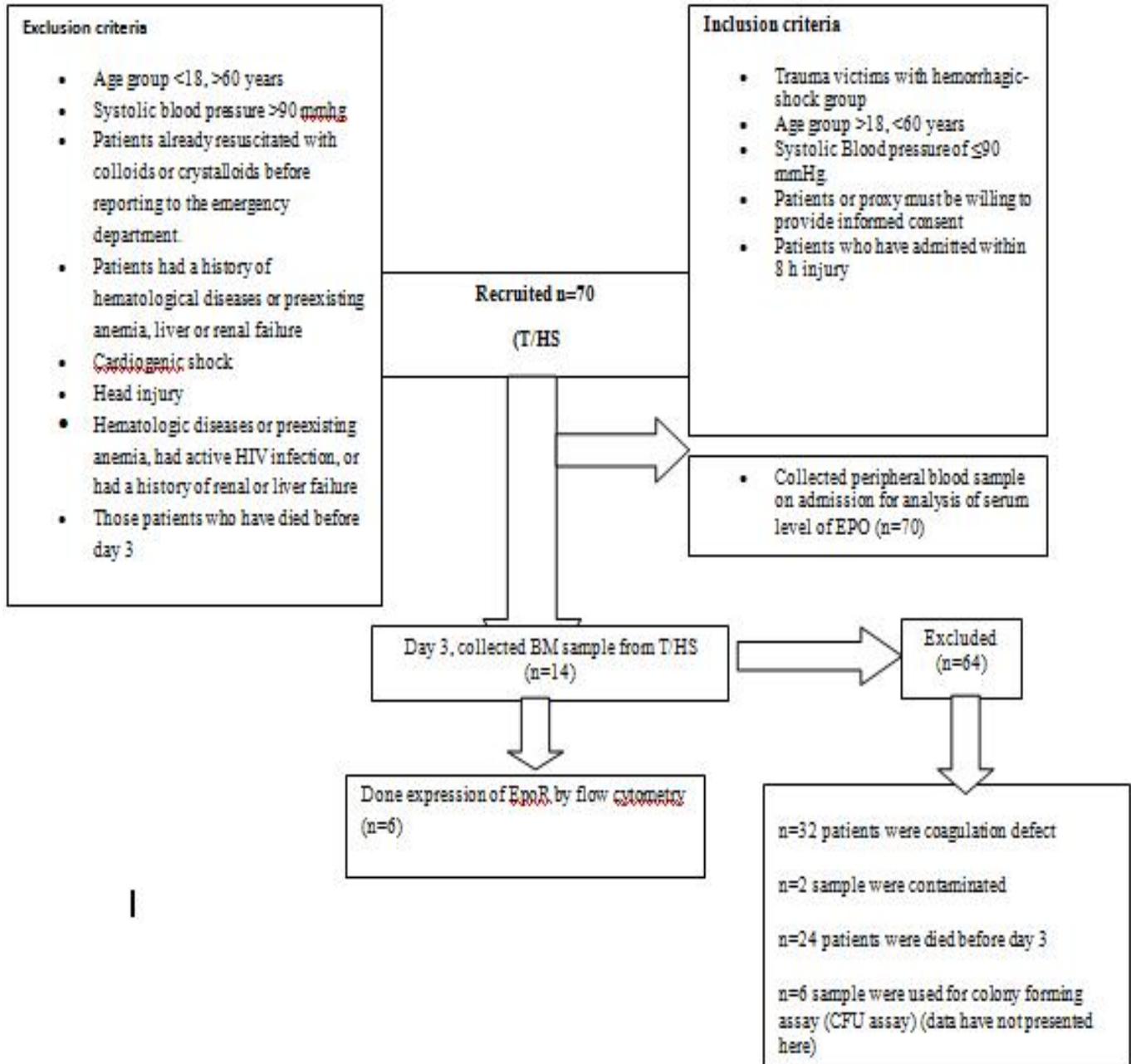
Materials and Methods

Patients

70 T/HS patients and 30 control group were recruited from October 2011 to November 2014. Our ethics committee approved (Ref. no. IEC/NP-278/2010) the

present study, and signed informed consent was obtained from patients and patients’ relatives. Peripheral blood samples (PBS) were collected on admission for determination of serum EPO level. BM samples were prospectively collected from those patients survived on day 3(n=14) from admitted to the ICU or ward. Six BM samples were excluded.

Figure: 1 Consort diagram



Inclusion criteria

Trauma victims with hemorrhagic-shock
Age group >18, <60 years
Systolic blood pressure of ≥ 90 mmHg.
Patients or proxy must be willing to provide informed consent
Patients who have admitted within 8 h injury

Exclusion criteria

Age group <18, >60 years
Systolic blood pressure >90 mmhg
Patients already resuscitated with colloids or crystalloids before reporting to the emergency department.
Patients had a history of hematological diseases or preexisting anemia, liver or renal failure
Cardiogenic shock
Head injury
Hematologic diseases or preexisting anemia, had active HIV infection, or had a history of renal or liver failure
Those patients who have died before day 3

Sample collection

PBS was collected from T/HS patients, those admitted within 8h of injury. Blood was put on incubator for 2h at 37°C . Serum was collected by centrifugation at 1800g for 20 min. at room temperature, aliquated & stored at -80°C until analysis. The results of clinical examination were recorded on admission.

Measurement of serum level of EPO

Serum level of EPO was determined using a commercially available EPO kit, (stem cell technologies) following the manufacturer's instructions.

Determination of bone marrow Erythropoietin receptor by flow cytometry

Single color immunofluorescent staining was used to analyze the phenotypic characteristics of mononuclear cells (MNCs). MNCs were resuspended in PBS buffer. MNCs were blocked with 2% BSA, After blocking, 10 μL of conjugated antibody Erythropoietin R/Epo R-Phycoerythrin (EpoR-PE, R& D system,) was added to up to 1×10^6 cells and incubated for 30 minutes at

room temperature. Unbound antibody was removed by washing the cells twice in flow cytometry staining buffer. The data were analyzed with BD software.

Statistical analysis

Data analysis

Categorical data are expressed in frequency (%) and continuous data are in Mean \pm SD or Median (Minimum, Maximum). The associations between two categorical variables were seen by using Chi-square/Fisher's exact test. For normally distributed continuous variables, the mean differences were compared by using Students's t-test for two independent groups and One-way analysis of variance for more than two groups. For skewed data (non-normal distribution), the differences were seen by using Mann-Whitney test between two independent groups and the difference among more than two independent groups were seen by using Kruskal-wallis test. We considered p-values < 0.05 to be significant. Statistical analysis was done by using statistical software Stata 11.2.

Results

Demographics

Peripheral blood was collected from 70 patients between 18 years and 60 years of age, with 60 males (86%) and 10 females (14%) (Table1).The results of clinical examination were recorded on admission (Table 1). Registries of the patients were shown in Figure 1.

Serum levels of EPO concentration

On admission, serum levels of EPO were elevated in the T/HS patients when compared to control group (10.4 vs. 7.8) (Table 2).

Determination of bone marrow erythropoietin receptor by flow cytometry

On day 3, we analysed bone marrow EpoR expression by using flow cytometric analysis. We found significantly decreased the expression of EpoR in T/HS when compared to the control group (Fig. 2). As shown in Table 3, down regulate the expression of EpoR was shown in T/HS patients when compared with control group.

Table 1: Patient Characteristics

Characteristics	Trauma hemorrhagic shock (T/HS) Patients (n=70)	Control group (n=30)
Demographics	n(%)	n(%)
Age (in years) *	34.9±12.0	35.9±13.1
Sex		
Male	60(86)	29(97)
Female	10 (14)	1(3)
Mode of injury		
RTC	36(51)	
FALL	18(26)	
RTI	5(7)	
Other	11(16)	
Mechanism of injury		
Blunt trauma	53(76)	
Penetrating	4(6)	
Combined	13(19)	
APACHE II **	13 (5, 34)	
Injury severity score (ISS) **	16(4,50)	

Road traffic crash (RTC), railway track injury (RTI), *Mean ±SD, **Median (Min., Max.) Injury Severity Score as calculated by AIS–90

Table 2: Erythropoietin Concentration in serum

S. no	Variable	Trauma hemorrhagic shock group (T/HS, mU/ml (n=70)	Control group mU/ml (n=30)	p- value
1	EPO	10.4 (0.4, 651.3)	7.8 (3.67, 18.8)	1.69

Data are expressed median (min, max),*p value <0.05, Mann-Whitney

Table 3: Expression of bone marrow erythropoietin receptor

Variable	T/HS group (n=6)	Control group (n=13)	p-value
EpoR	2.4 (1,6.8)n=6	22 (6.1,44.4)	0.01

Data are expressed median (min, max) in all groups, Mann-Whitney Test

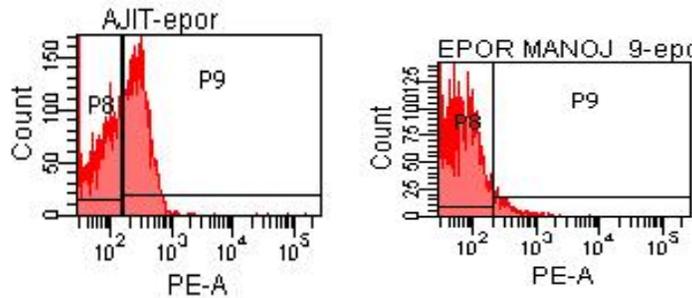


Figure 2. Plots showing EPO-R-(PE) in BM control (left) and Hemorrhagic shock (right).

Discussion

Present study observed elevated serum level of EPO in T/HS patients when compared with control group (Table 2). Endogenously elevated EPO unable to reactivate BM dysfunction. However, we measured expression of BM-EpoR, and found EpoR expression was significantly decreased in patient in T/HS when compared to control group (Table 3) Figure 2. Dysfunction of HSCs has been observed in severe trauma and HS. BM dysfunction in T/HS leads to persistent anemia and increased the susceptibility to infection and sepsis, mainly due to dyserythropoiesis and myelopoiesis. BM dysfunction is a multifactorial process. Excessive pro-inflammatory cytokine milieu and elevated levels of circulating catecholamines change the behavior BM microenvironment in a T/HS [3-4]. Previous studied demonstrated that blunted EPO response to low haemoglobin concentrations, inflammatory mediators, and a hypoferremic state results anaemia in severe trauma [5]. Recent evidence has demonstrated a blunted EPO response as a factor contributing to anaemia of critical illness in specific subsets of patients [6]. HPCs dysfunction is one of the facets of this condition. Alternation of HPCs in T/HS by evaluating parameter of BM-EpoR has not been previously reported in humans. Therefore, we evaluated the serum level of EPO and BM expression of EpoR in T/HS patients and found elevated serum level of EPO on admission, and day 3 significantly decreased bone marrow expression of EpoR in T/HS when compared with control group. Based on finding, T/HS induces modulation of EpoR result alternation of HSCs failure. Our studies that shown, Impaired BM erythropoiesis in T/HS patients, result of downregulation of EpoR expression on erythroid cells.

EpoR is a protein EpoR pre-exists as dimers [7] which upon binding of a 34 kDa ligand EPO, changes its homodimerized state. EPO binds to an erythroid progenitor cell surface receptor to regulate bone marrow erythroid cell proliferation, differentiation, and survival. Function of EpoR is to rescue erythroid (red blood cell) progenitors from apoptosis [7]. In general, the defects in the erythropoietin receptor may produce erythroleukemia and familial erythrocytosis. Mutations in Jak2 kinases associated with EpoR can also lead to polycythemia vera [8-10]. EPO receptor signaling exerts direct cardioprotection in an animal model of renal dysfunction-associated heart failure, probably by mitigating degenerative, pro-fibrosis,

inflammatory, and oxidative processes but not through relief of anemia [11].

Leist *et al.* suggested that EPO mediates tissue-protection through a receptor (tissue-protective receptor) that is pharmacologically distinct from the classic EPO receptor that is known to mediate erythropoiesis [12]. The tissue-protective receptor exhibits a lower affinity for EPO, forms distinct molecular species in cross-linking experiments [13-14]. Rarely, seemingly beneficial mutations in the EpoR may arise, where increased red blood cell number allows for improved oxygen delivery in athletic endurance events with no apparent adverse effects upon the athlete's health. Maintain endothelial cells and to promote tumor angiogenesis by erythropoietin, hence the dysregulation of EpoR may affect the growth of certain tumors. EpoR signaling prevents neuronal death and ischemic injury [15-17]. In our studies, elevated EPO response, and down-regulation of EpoR on erythroid cells. This could be changed the expression of EpoR.

Limitations of our data include the small sample size with expression of BM-EpoR. There are several factors, number of blood transfusions, gender, genetic polymorphisms that influencing inflammatory cytokine levels that cannot be controlled due to the design of the study [18-21]. In this study serum level of EPO and BM-EpoR assessed only one time-point; therefore the correlation of BM-EpoR with BM dysfunction was not feasible. Further investigate in this area must determine the expression of EpoR mRNA on BM by RT-PCR at different time point and more studies to be needed exogenously effect of EPO on hematopoietic stem cells dysfunction in T/HS and alternative approaches that stimulate EpoR.

Based on the findings of this study, significantly decreased BM- EpoR in T/HS patients. It is suggested that decreased the expression of BM-EpoR may be used as a potential biomarker for HSCs dysfunction following T/HS. More research is needed to determine the expression of EpoR mRNA on BM by RT-PCR and alternative approaches that stimulate expression of bone marrow EpoR.

List of abbreviations

T/HS: Trauma hemorrhagic shock, HPCs: hematopoietic progenitor cells, BM: bone marrow,

EPO: Erythropoietin, EpoR: Erythropoietin receptor, ARDS: adult respiratory distress syndrome; ISS: injury severity score; ml: millilitre; MOF: multi organ failure; MODS: multi organ dysfunction syndrome, APACHEII: acute physiology of chronic health evaluation.

Conflict of interests

The authors declare that they have no competing interests.

Acknowledgments

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