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Research Article

Interleukin (IL)-8 is an early predictor of mortality following trauma hemorrhagic shock

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Abstract

Background: Trauma injury and hemorrhagic shock frequently leads to the imbalance of immune system known as Systemic Inflammatory Response syndrome (SIRS) and is connected to the morbidity or mortality. Pro and Anti-inflammatory, which play a significant role in the development of multiple organ failure (MOF). **Objective:**This study investigates the serum cytokines levels in patients with trauma hemorrhagic shock and the association of these cytokines with clinical outcome. **Design:** Prospective cohort study. **Patients:** A total 70 patients with trauma hemorrhagic shock admitted to the emergency department, level 1trauma centre. **Method:** Peripheral blood samples were collected in each patient for determination of serum cytokines concentration. Samples were obtained within 8 h of post injury with T/HS patients. Standard resuscitation techniques as per Advance Trauma Life Support were used in each patient. Clinical and laboratory data were prospectively collected. **Results**: High concentrations of circulating IL-6, IL-10, IL-8, ($p < 0.05$) were detected in a trauma hemorrhagic shock as compared with healthy control group. At study entry, IL-8 concentrations were higher in non-survivors as compared with survivors T/HS patients but not TNF-α, IL-1 β, IL6, IL10. **Conclusions**: We found increased the serum levels of IL-6, IL-10, and IL-8 are detected in T/HS as compared to normal healthy control. This study suggests a much higher degree of activation of immune-inflammatory in T/HS than in normal healthy control. In a subgroup, increased serum levels IL-8 values were found to be an early predictor of mortality following T/HS.

Keywords: Trauma hemorrhagic shock (T/HS), inflammatory cytokine, SIRS, and outcome.

Introduction

Trauma remains a significant public health issue and is the leading cause of death in persons younger than 40 years. Up to 50% of early deaths are due to massive haemorrhage [1, 2]. The first peak of death after injury represents the immediate effects of trauma with death at the scene (53—72%) or within the first hour [3]. Excessive production of inflammatory cytokines leads to the SIRS and MOF in severe trauma and hemorrhagic shock [4–5].

Cytokines are proteins that are important in cell signaling and produced by innate and adaptive immune systems and they act as effectors or immunomodulators of inflammatory response, which in turn play prominent roles in the development of sepsis or multiorgan failure MOF[7]. An imbalance between the early systemic inflammatory response and the later compensatory anti-inflammatory response may be responsible for organ dysfunction and increased susceptibility to infections [4, 8, 9]. Previous studies demonstrated IL-6 and IL-8 predicting the development and the degree of severity of the systemic inflammatory response (SIRS) in patients with severe injuries [10, 11].

However, some few studies showed cytokines pattern of severe trauma or hemorrhagic shock. But there are no data to support the relationship of pro and anti-inflammatory cytokines and with clinical outcomes in terms of survival or death. The purpose of this study was to evaluate the prognostic value of the serum levels of the pro- and anti-inflammatory cytokines (e.g., (IL-6, IL-8, IL-10, TNF-α, and IL-1 β) and its correlation with clinical outcomes in terms of survival or death T/HS.

Materials and Methods

Patients

Our ethics committee approved the present study, and signed informed consent was obtained from all patients relatives. We prospectively included 70 patients with Trauma hemorrhagic shock, were admitted within 8h of injuries, Department of Emergency Medicine, between October 2011 to November 2013, age 18-60 years, were eligible for inclusion. Exclude patients with neurogenic shock, cardiogenic shock, and had a history of hematologic diseases or preexisting anemia, had active HIV infection, or had a history of renal or liver failure at the JPN Apex Trauma Centre, All India Institute of Medical Sciences, New Delhi, India. Severity of injury was assessed by calculating the injury severity score (ISS) based on abbreviated injury scale. Main outcome measure of interest was 15-day mortality. Early mortality was defined as death occurring the first 48h.

Sample collection

Peripheral blood sample were collected from trauma hemorrhagic shock patients, those who have admitted within 8h of injury. Blood was put on incubator for 2h at 37^0C. Serum was collected by centrifugation at 1800g for 20 min. at room temperature, aliquated & stored at -80 ^{0}C until analysis.

Cytokine analysis

Serum cytokines were determined using a commercially available cytokine kit, ((IL-6, IL-8, IL-10, TNF-α, and IL-1 β, eBioscience: USA) following the manufacturer's instructions. The results of clinical examination and Laboratory parameter (Hemogram, Biochemistry, Coagulation profile) were recorded on admission (Table 2).

Patient management and treatment

After admission, all patients treated with 2L Ringer Lactate as per Advance Trauma Life Support (ATLS. A standardized clinical examination, a focused assessment with sonography for trauma (FAST) and at least chest and pelvic x-rays were performed. After diagnostics in the emergency room, a trauma scan (CTscan of head, cervical spine, chest, abdomen and pelvis) was accomplished. Results were analyzed by an attending radiologist and an attending trauma surgeon. At time of admission to the intensive care unit (ICU), the clinical examination and FAST were repeated.

Subgroup analysis

In a subgroup, Patients were divided into two groups based on outcome in terms of survival or death. Early mortality was defined as death occurring during the first 48 hours.

Statistics

Data analysis

Categorical data are expressed in frequency (%) and continuous data are in Mean ± 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. Receiver operating characteristics (ROC) curve analysis for validation of IL-8 as a predictive marker. We considered p-values < 0.05 to be significant. All the statistical analysis was done by using statistical software Stata 11.2.

Results

Demographics

70 patients were included in this study; 44 (69%) patients survived and 26 (31%) died. Demographic, clinical and laboratory data for the survivors and

nonsurvivors are summarized in Table 1 and Table 2. Patients who died had higher ISS scores as compared with survivors. Additional characteristics are shown in Table 5.

Table 1. Patient Characteristics

Characteristics	Trauma hemorrhagic shock (T/HS) Patients (n=70)	Healthy volunteer (n=30)
Demographics	n(%)	n(%)
Age (in years) *	34.9±12.0	35.9±13.1
Sex		
Male	60(86)	29
Female	10 (14)	1
Mode of injury RTA FALL RTI Other	 36(51) 18(26) 5(7) 11(16)	
Mechanism of injury Blunt trauma Penetrating Combined	 53(76) 4(6) 13(19)	
APACHE II **	13 (5, 34)	
Injury severity score (ISS) **	16(4,50)	
Early Death (≤72 h)	26 (37)	

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

Table 2. Serum cytokine analysis in Trauma hemorrhagic shock on day admission (day 0) compared with healthy control group

S. no	Variable	Trauma hemorrhagic shock group (T/HS) pg/ml (n=70)	Healthy Control group pg/ml (n=30)	*p- value
1	IL-1 beta	**45.4**(2.2,696.5)	**27.5**(10, 52.9)	0.259
2	IL-6	**943.3**(63,26999)	**426.1**(55,1159)	**<0.001**
3	IL8	**431**(91.7, 10573.3)	**116**(186.8, 2381.7)	**0.003**
4	IL10	**41.7**(10.4,649.5)	**13.4**(9.4,53.5)	**<0.001**
5	TNF- α	**247.8**(26, 3438)	360.6 (58,1158)	**0.140**

Data are expressed median (min, max),***Mann-Whitney**, Interleukin (IL), Tumor necrosis alpha (TNF-α),

Cytokines concentrations

On admission, IL-6, IL-10, IL-8 were significantly higher (<0.05) in the T/HS patients and as compared to healthy control (Table 2).

Predictive value of cytokines for T/HS 15-day mortality

The serum levels of cytokines concentrations of survivors and nonsurvivors (death by 15 days) are shown in Table 4. Of the five cytokines studied, only IL-8 was significantly higher in nonsurvivors. Among the cytokines, only IL-8 ROC values above 0.6.

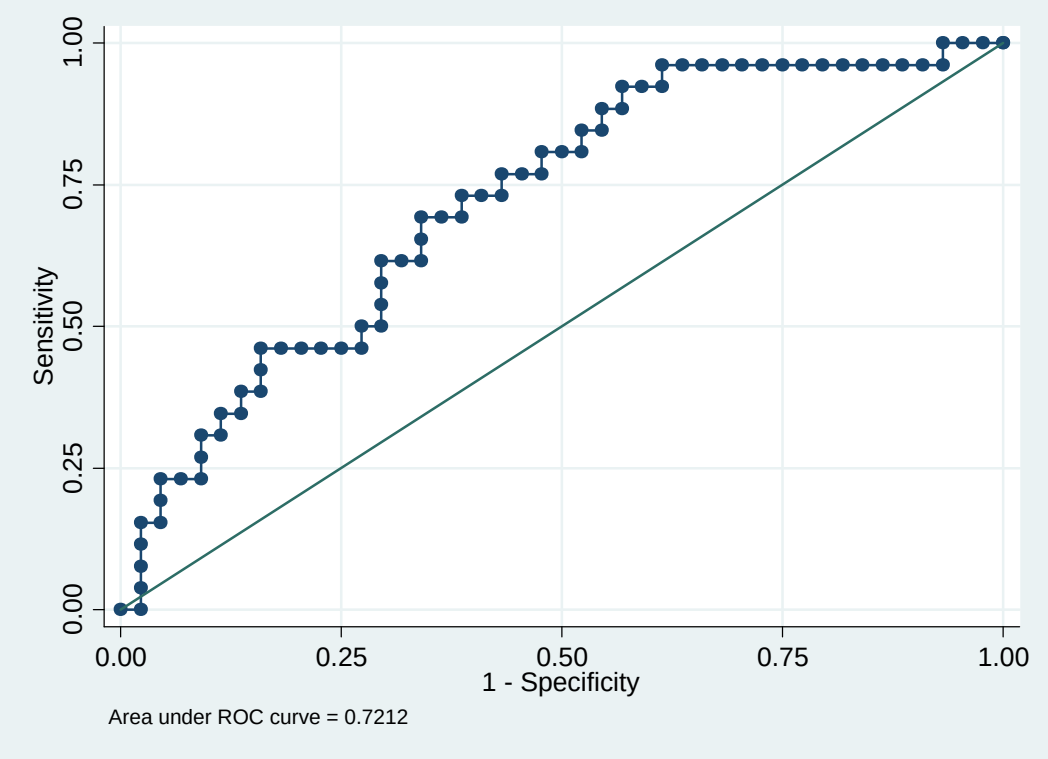
The ROC curve analyses IL-8 for predicting the mortality are shown in fig. 1. The areas under the curve for mortality are, respectively, 0.712 (SE 0.08; 95% confidence interval (CI) 0.52- 0.85 (Fig-1).

Table 3. Serum cytokine concentration: survivors versus nonsurvivors.

S. no	**Variable**	**Survivors (n=44)**	**Non-survivors (≤48 mortality;** n=26)	***p- value**
1	IL-1 β	145.9 (2.2,696.5)	54.6 (3.1,681.6)	0.33
2	IL-6	903.6 (62.9,26999.2)	980 (121.7, 19625)	0.50
3	**IL8**	**232.7 (91.7,10573.3)**	**778.1 (133.8, 8349.9)**	**<0.001**
4	IL10	41.2 (10.4, 201.5)	41.5 (14.7, 649.5)	0.28
5	TNF- α	246.9 (26,3438)	251.7 (45,1372)	0.141

Data are expressed median (min, max),***Mann-Whitney,*** Interleukin (IL), Tumor necrosis alpha (TNF-α)

Figure 1



Receiver operating characteristic curve of IL-8 predicting mortality.

Laboratory Parameter and Outcome

Elevation of greater creatinine (1.2±0.3 vs.1.0±0.40), p < 0.05), PT (24.2±9.5 vs. 20.5±10.3) p < 0.05), APTT (45.3±22.9 vs. 31.9±11.1) was observed in non-survivors patients as compared with survivors, p < 0.05) (Table 4)

Table 4: Laboratory parameter: survivors versus nonsurvivors

S. no	Variable	Survivors	Non-survivors	*p- value
HAEMOGRAM				
1	Hb (gms/dl)	10.1±2.6(n=43)	10.0±3.2(n=26)	0.929
2	HCT (%)	31.4±7.9(n=43)	31.0±9.8(n=26)	0.978
3	TLC ($x10^3$ Cumm)	14791.8±6201.4(n=38)	12765.2±7336.2 (n=23)	0.183
4	Plt ($x10^3$ Cumm)	178.0±103.0(n=42)	155.37±84.9(n=25)	0.492
BIOCHEMISTRY				
5	Sodium (mEq/L) (130-149)	136.3±6.4(n=40)	137.3±6.1(n=23)	0.456
6	Potassium (mEq/L)	4.4±4.1(n=40)	4.1±1.1(n=23)	0.434
7	Urea(mg%)	31.8±21.3(n=40)	33.3±12.9(n=23)	0.206
8	**Creatinine** (mg%)	**1.0±0.40(n=40)**	**1.2±0.3(n=23)**	**0.026**
9	Bil. total(mg%)	1.00±0.52(n=33)	1.1±.81(n=18)	0.879
COAGULATION				
10	**PT** (sec)	**20.5±10.3(n=36)**	**24.2±9.5(n=16)**	**0.012**
11	**APTT** (sec)	**31.9±11.1(n=36)**	**45.3±22.9(n=16)**	**0.007**
12	FFP (unit)	0.0 (0, 6) (n=43)	0.0 (0,6) (n=26)	0.84
13	RBC (unit)	0.0 (0,6) (n=43)	3.0 (0, 6) (n=26)	0.66

Data are expressed mean± SD, median (min, max)*** Mann-Whitney Test,**
Hemoglobin (Hb), platelets (plt), Total leukocyte count (TLC), fresh frozen plasma (FFP), Red blood cells (RBC), Prothrombin time (PT), Activated partial thromboplastin (APTT),

Table 5: Clinical parameter compared with clinical outcome

S. no	Variable	Survivors (n=44)	Non-survivors (n=26)	p-value
1	Age	35.9±13.1	33.4±9.9	0.579
2	Sex Male Female	 36(81.8%) 8(18.2%)	 25(96.1%) 01(3.9%)	0.443
3	RR (12-20)	19.7±3.8	17.3±6.6	0.128
4	SI n=<0.7	1.2±.03	1.1±0.7	0.939
5	**APACHE II	13 (5, 34)	12 (5, 32)	0.93
6	****ISS (0-75)**	**12 (4, 50)**	**17.0 (9, 50)**	**0.05**

Data are expressed mean± SD & **Median (min, max), Respiratory rate (RR), Shock index (SI), Injury severity score (ISS), Acute physiology of chronic health evaluation (APACHE II)

Discussion

Severe trauma and hemorrhagic shock is the leading cause of mortality in a person's between 5 and 44 years[1-2]. Hemorrhagic shock initiate the complex inflammatory response, in which pro and anti-inflammatory cytokines (TNF-α, IL-6, IL-10, IL-8) and MCP-1 are thought to be important role in immune dysfunction resulting multi-organ failure (MOF) and death. Many studies showed laboratory and clinical parameter have poor accuracy for the treatment of patients with trauma hemorrhagic shock. Therefore, the aim of this study to assess the serum cytokine, and to identify early predictor of mortality following T/HS patients.

We have investigated the serum level of proinflammatory cytokines IL-6, IL-8, IL-10 were significantly increased with trauma hemorrhagic patients as compared to healthy control. In s subgroup, serum levels of IL-8 significantly higher in nonsurvivors as compared to survivors group. Recently, reported, elevated levels of pro-inflammatory cytokines in a severely injured patients [12-15]. The increase of pro-inflammatory cytokine levels following injuries indicates their role in the body reaction to trauma [16-17]. The pro-inflammatory cytokines are produced during early immune response and upregulate the expression of endothelial cells adhesion molecules and the activation of neutrophils. Despite, previous studies demonstrated that IL-6 predicts outcome in a patients with multiple injuries [18]. Remick et al suggests that IL-6 predict outcome in the early phase of sepsis in murine model [19].

Excessive release and activation of inflammatory can lead to be MOF [20-22]. Heckbert et al. and some other studies showed, liver is one of the organs most frequently affected by T/HS, and its central role in metabolism and homeostasis makes this organ a critical one for survival of the host after severe injury [23, 24].

Our study demonstrated, In subgroup, serum levels of IL-8 significantly higher in nonsurvivors as compared to survivors group. Interleukin 8 (IL-8) is a neutrophil chemotactic by macrophages, and other cell types such as epithelial cells, airway smooth muscle cells[25]IL-8. It induces adhesion molecules and chemokines, recruiting leukocytes to infected tissues. IL-8 also induces phagocytosis once they have arrived. IL-8 is also known to be a potent promoter of angiogenesis. Interleukin-8 is often associated with inflammation. As an example, it has been cited as a proinflammatory mediator in gingivitis[26] and psoriasis [27]. Interleukin-8 secretion is increased by oxidant stress, which thereby cause the recruitment of inflammatory cells and induces a further increase in oxidant stress mediators, making it a key parameter in localized inflammation.[28] IL-8 was shown to be associated with obesity [29].

Boyle Jr et. al. suggested that after prolonged hypothermic cardiac ischemia neutrophil chemoattractant/ activator IL-8 may contribute to myocyte injury. In a much as neutrophil activation is critical initial step in ischemia reperfusion injury. It has become evident that administration of anti-IL8 antibodies in rabbit prevents cardiopulmonary injury [30]. The finding is an agreement with Endo et. al who considered IL-8 is one of the inflammatory cytokine that reflect the severity of septic shock [31].

Other studies demonstrated that significantly decreased the levels of IL-6 and increased the level of IL-10 in a subsample of four injured patients followed six months after initial injury (T2). These findings are supported by previous findings reporting differences during the first week after the initial trauma [32-38]. However, we consider our findings as only pilot studies of patients with trauma hemorrhagic shock and its correlation with outcome in term of survival or death within 48 h of injury. And further studies to be needed evaluate the long term studies with trauma hemorrhagic shock and outcome.

In a severely injured patients, the significant increase the levels of pro-inflammatory cytokines IL-6 and IL-8, with the highest levels in the severe injured group, is very important as fracture management in patients with multiple injuries continues to be of crucial importance. This may be lead to acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF) with a relatively high morbidity and mortality [32,34, 35,39].

Mukeinda demonstrated that IL-8 developed ARDS and pathophysiological derangements seen after ischaemia/ reperfusion [40]. Previous studies suggest a casual relation between excessive releases of pro-inflammatory cytokine & both morbidity and mortality associated with critical care patients [41]. Many studies

shown IL-8 is a chemokine, can be produced from the locally from heart which may be further enhance leukocyte activation and accumulation in the injured myocardium [42]. Oz et al. hypothesized those human endothelial cells deprived oxygen would secrete IL-8, which might translate in to elevated IL-8 production after cardiac ischemia [43]. The author suggested that neutrophil chemoattractant/ activator IL-8 may contribute to myocyte injury after prolonged hypothermic cardiac ischemia. In a much as neutrophil activation is critical initial step in ischemia reperfusion injury. Previous studies demonstrated, IL-8 blockade that reduced the tissue damage after experimental ischemia & reperfusion [30].

Some previous studies demonstrated that severe trauma induced hyperinflammation which associated with psychological reactions to trauma. Previous studies as well as others studies showed that high serum levels of proinflammatory cytokines IL-6 and IL-8, and low levels of the regulatory cytokine TGF-β and anti-inflammatory IL-4 and IL-10 predicted higher levels of acute stress symptoms soon after injury [44-46]. Moreover, when controlling for age and severity of injury, higher serum levels of IL-8 and lower serum TGF-β predicted higher posttraumatic stress symptoms one month after injury [46]. The author suggests that certain pro-inflammatory cytokines, such as IL-6, may cause or intensify psychological symptoms [50], these results suggest elevated inflammatory cytokines due to injury may be a risk factor for post trauma psychological symptomatology [46-47].

Our study has some limitations that should be acknowledged. 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 [48-50]. In this study cytokines assessed only one time-point; therefore the markers correlation to clinical outcome was not feasible. The sample consists of patients with moderate or severe fractures of the limbs, without involvement of head, chest, abdominal or pelvic injuries. Therefore the abbreviated injury scale (AIS) was used to assess the severity of injuries [51, 52.] The injury severity score (ISS), which is the sum of the AIS squared values of the three most severe injured regions, was not evaluated since this series of patients with fractured limbs did not have any additional injuries of any other different body regions. Further studies to be needed long term assessments of cytokines as biomarkers of systemic inflammatory response and poor outcome or death in T/HS.

Based on the findings of this study of such significant increases in the serum levels of pro and anti-inflammatory cytokines IL-6, IL-8, and IL-10 with the highest levels in the T/HS patients, it is suggested that serum high levels of these cytokines can be used as potential reliable biomarkers for predicting the development of systemic inflammatory response syndrome (SIRS). In a subgroup, we found elevated serum levels of IL-8 can be used as an early predictor of mortality among T/HS. More research is needed to study the expression and regulation of signalling pathway along the different time points during recovery.

Conflict of interests

The authors declare that they have no competing interests.

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