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Research Article

Evaluation of Serum Granulocyte Colony Stimulating Factor in Patients admitted with Trauma Haemorrhagic shock

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Abstract

Background: Hematopoietic progenitor cells (HPC) mobilized from bone marrow to peripheral blood has been observed in severe trauma and hemorrhagic shock patients. Granulocyte colony stimulating factor (G-CSF) is a potent stimulator that mobilized HPC from bone marrow to peripheral blood. **Objective:** Our aim of the study was to investigate the serum G-CSF levels in patient with trauma hemorrhagic shock (T/HS). **Methods:** Peripheral blood sample from 37 T/HS was collected on arrival for determination of G-CSF and compared with healthy control (n=15). Determination of serum levels of G-CSF by sandwich ELISA. We found optimal cutoff of serum G-CSF level for T/HS patients with the help of trade-off between the sensitivity and (1- specificity) across a series of cutoff points using ROC curve. **Results**: Significantly increased the serum level of G-CSF in T/HS group when compared with control group (264.8vs.79.1 pg/ml). n**Conclusions:** Our studies suggest serum level of G-CSF elevated in T/HS patients. The elevated G-CSF was also associated with mobilization of HPC from BM to peripheral blood HPC. Increased the levels of G-CSF in T/HS may play a significant role in alternation of hematopoietic compartment.

Keywords: Granulocyte Colony Stimulating Factor (G-CSF), Trauma hemorrhagic shock (T/HS) and mortality.

Introduction

Severe trauma and hemorrhagic shock is the leading cause of mortality in individuals between 5 and 44 years[1-2]. Anemia is a multifactorial process in a trauma hemorrhagic shock (T/HS) not only the excessive pro-inflammatry subsequently increases catecholamine which changes the behavior of HPC in bone marrow (BM) . BM dysfunction is a one component of this response, which lead to persistent anemia that increased the susceptibility to infection and sepsis mainly as a result of the BM essential role in erythropoiesis and myelopoesis [3]. Animal and human studies has been shown bone marrow failure associated with impaired growth of HPCs and stromal cells in T/HS.[3-4]

The anemia leads to repeated transfusion requirement despite no ongoing blood loss. Transfusion in trauma patients is an independent risk factor for death,

infection, organ failure and ICU admission [5-6] and understanding the mechanism behind post traumatic BM dysfunction is important in order to design successful therapeutic strategies. Severe trauma and T/HS induced suppression of hematopoietic progenitor cell growth and mobilization of HPCs into the peripheral blood and leads to persistent anemia. Shah S. et. al. demonstrated that in a severe trauma injury, the mobilization of hematopoietic progenitor cells from BM to peripheral blood in to injured or inflammatory tissue, is a beneficial for wound healing and maintaining immune response [7].When excess, the peripheral blood HPCs results BM dysfunction [8]. Recent studies has been proved, Granulocyte colony stimulating factor (G-CSF) is one potent stimulator of hematopoietic mobilization in severe trauma and neutropenic patients, but still remain about its release with T/HS patients [9-10]. Therefore the purpose of this study was to evaluate the serum levels of G-CSF in T/HS patients.

Materials and Methods

Patients

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 sample were collected on admission for determination of G-CSF level and laboratory parameter. The period of study was October 2011 to November 2014.

Inclusion criteria

Trauma victims with hemorrhagic-shock group
Age group >18, <60 years
Systolic Blood pressure of ≤90 mmHg.
Patients or patients relatives 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

Sample collection

Peripheral blood sample were collected from T/HS 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.

Measurement of G-CSF levels

Serum levels of G-CSF were determined using a commercially available kit, (Bio-plex, (Bio-Rad): USA) following the manufacturer's instructions.

Clinical and laboratory parameter

The results of clinical examination and Laboratory parameter (Hemogram, Biochemistry, Coagulation profile) were recorded on admission .

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.

Statistics

Data analysis

Categorical data are expressed in frequency (%) and continuous data are in Mean ± SD or Median (Minimum, Maximum). the differences of G-CSF (pg/ml) level between two groups (i.e. control and THS group) was seen by using Mann-Whitney test for

two independent groups. ROC curve analysis was done to determine the optimal cutoff value of G-CSF level for T/HS patients using trade-off between the sensitivity and (1- specificity) across a series of cutoff points using ROC curve. All the p-values less than 0.05 were taken as significant. All statistical analysis was done by using software stata 12.1.

Results

Study subjects

Peripheral blood was collected from 37 patients between 18 years and 60 years of age, with 29 males (78%) and 8 females (22%) (Table1).The results of clinical examination and Laboratory were recorded on admission (Table 1, 2).

Table 1. Patient Characteristics

Characteristics	Trauma hemorrhagic shock (T/HS) Patients (n=37)	Healthy volunteer (n=15)
Demographics		
Age (years) *	34.9±12.0	34.1±13.1
Sex (%) Male Female	 29(78) 8 (22)	 15(100)
Mode of injury RTA FALL RTI Other	 21(57) 10(27) 3(8) 3(8)	NA
Mechanism of injury (%) Blunt trauma Penetrating Combined	 29(78) 2(6) 6(16)	NA
APACHE II **	13 (5, 34)	NA
Injury severity score (ISS) **	19(7,34)	NA
Shock Index (SI)*	1.2±0.4	NA

Road traffic accident (RTA), railway track injury (RTI), *mean ±sd, **median (min, max)

Table 2: Routine Laboratory parameters of T/HS patients

S. no	Variable	T/HS
HAEMOGRAM		
1	Hb(gms/dl)(n=34)	10±2.6
2	HCT(%)(n=34)	31.6±8.4
3	TLC($x10^3$ Cumm)(n=34)	5924.7±13232.3
4	Plt (150-400 ($x10^3$)(n=35)	156.0±95.7
BIOCHEMISTRY		
5	Sodium (mEq/L) (130-149)(n=32)	136.0±7.0
6	Potassium (mEq/L) (3.5-5.0)(n=32)	3.6±0.7
7	Urea**(10-50)(n=32)	28(12,137)
8	Creatinine (mg%) (0.5-1.5)(n=32)	1.0±0.3
9	Bil. Total**	0.9 (0.3,2.9)
COAGULATION		
10	PT** (13-15 sec)	17.3(14.2,62.8)
11	APTT (28-32 sec)	33.9±13.0

Data are expressed in mean± sd /**median (min, max) **;**Hemoglobin (Hb), platelets (plt), Total leukocyte count (TLC), Activated partial thromboplastin (APTT), Hematopoietic progenitor cells (HPCs).

Measurement of Granulocyte colony stimulating factor (G-CSF)

In T/HS patients, the serum levels of G-CSF were significantly elevated when compared to the levels in normal healthy volunteers (p<0.001) (Table 3). Using ROC curve (Fig 1), the cutoff value of 129.9 pg/ml was determined for G-CSF level for T/HS patients**.** A test value below 129.9 was considered to be normal and above 129.9 to be abnormal. The sensitivity and specificity for cut point were 72.97 % and 73.33 % respectively (Table 5). There was significant association was found between the categorization of G-CSF level and THS group (p=0.004). The odds of having THS is 7.4 times higher if the level of ≥129.9 G-CSF level value as compared to the level of<129.9 [7.4(1.9, 28.8)] (Table 4).

Table 3. Control vs. T/HS

S. no	Variable	Control group pg/ml (n=15)	Trauma hemorrhagic shock group (T/HS) pg/ml (n=37)	p- value*
1	G-CSF(pg/ml)	79.1(65,305)	264.88(118,12080)	<0.001

Data are expressed in median (min-max)**, ***Mann-Whitney U Test

Fig1: Roc curve to find the cut-off of G-CSF (pg/ml) for Trauma hemorrhagic shock

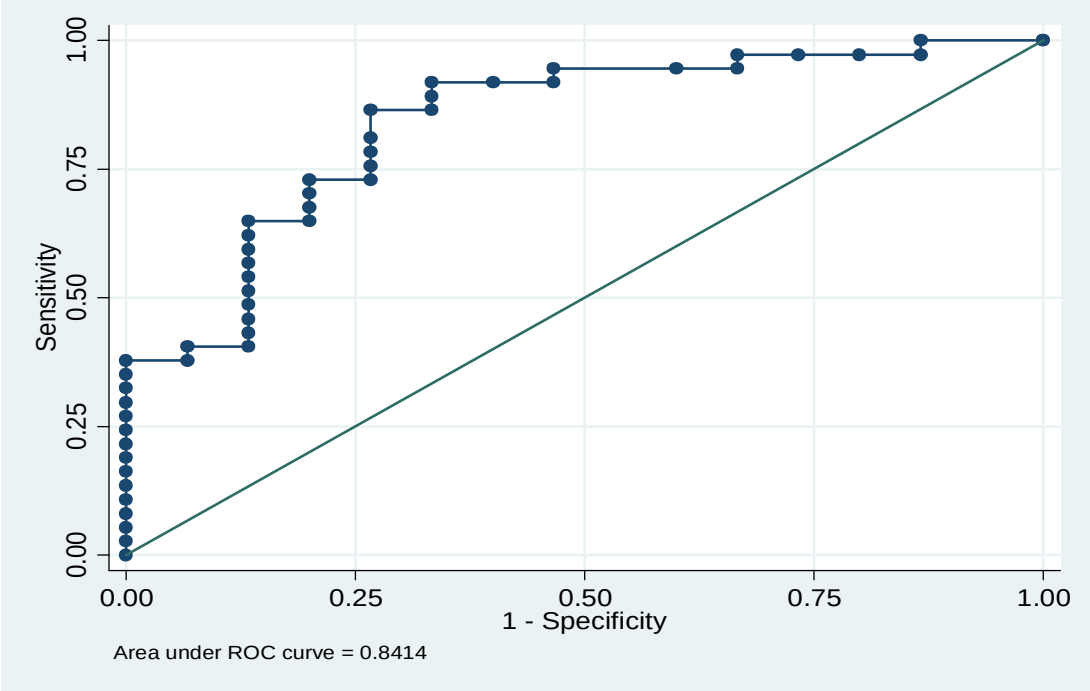

Table 4. Control vs. T/HS

G-CSF (pg/ml)	T/HS n(%)	Control n(%)	p-value*	Odds (95% CI)
<129.9	10 (47.6)	11(52.4)	0.004	1.0
≥129.9	27(87.1)	4(12.9)		7.4(1.9, 28.8)

*Fisher's exact test

Table 5: Sensitivity and specificity cut point of G-CSF

Cut point (G-CSF)	Sensitivity (%)	Specificity (%)
≥126.81	78.38	73.33
≥129.64	75.68	73.33
≥129.9	72.97	73.33
≥131.52	72.97	80.0
≥135.25	70.27	80.0

Discussion

Animal and human studies have been shown bone marrow failure associated with impaired growth of HPCs and stromal cells in trauma hemorrhagic shock [3-4]. Previous studies demonstrated, in a severe trauma injury, the mobilization of hematopoietic progenitor cells from BM to peripheral blood in to injured or inflammatory tissue, is a beneficial for wound healing and maintaining immune response [5].When excess, the peripheral blood HPCs results BM dysfunction [8].

We have investigated the serum level of granulocyte colony stimulating factor (G-CSF) was significantly increased with T/HS patients as compared to healthy control. In a previous animal studies and Baranski et. al showed that increased HPCs mobilization in to peripheral blood, plasma G-CSF levels significantly more than double three hours following both hemorrhagic shock and lung contusion hemorrhagic shock (LC/HS)[11]. G-CSF is one potent stimulator of hematopoietic mobilization, and has been well studied in BM dysfunction after severe trauma and neutropenic patients [10].

G-CSF or GCSF, also known as colony-stimulating factor 3 (CSF 3), is a glycoprotein that stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream. Functionally, it is a cytokine and hormone, a type of colony-stimulating factor, and is produced by a number of different tissues. The G-CSF-receptor is present on precursor cells in the bone marrow, and, in response to stimulation by G-CSF, initiates proliferation and differentiation in to mature granulocytes. G-CSF is also a potent inducer of HSCs mobilization from the bone marrow into the bloodstream, although it has been shown that it does not directly affect the hematopoietic progenitors that are mobilized [12]. Tanaka et.al demonstrated elevation of G-CSF was reported in a study of 19 trauma patients with increases in circulating G-CSF levels also similar results was found in burn and septic patients [13-15]. Endogenous production of G-CSF and release from endothelium, macrophages and other immune cells is incompletely understood, but appears to be mediated by inflammatory markers such as IL-6 and TNFα [10]. Gardner et. al. demonstrated that G-CSF plays a central role in posttraumatic resistance to infection and prioritization of bone marrow responses [16]. The rise in plasma concentrations of chemokines and growth factors likely contribute to the mobilization of monocytes and granulocytes [17]. Previous studies have evaluated that G-CSF treatment increases local cellularity after rotator cuff repair, but this finding did not translate to improve structural healing of the supraspinatus tendon-bone complex [18].

Our findings indicate, patients with T/HS (systolic blood pressure less than 90 mmHg) increased level of G-CSF compared to control. Elevated serum level of G-CSF associated with mobilization of HPCs from BM in to peripheral blood. Tanaka et.al demonstrated a significant correlation between injury severity score and G-CSF. While there is an association between increasing ISS and hemorrhagic shock, the correlation is imperfect and likely explains the disparate findings, especially if the sample size is small [13]. Our previous studies demonstrated that increased the peripheral blood HPCs associated with mortality following T/HS [19].

This study was supported by murine research. Hierholzer et al demonstrated G-CSF locally produced in liver and lung of hemorrhagic shock animals which appears to be mediated through NFkB activation[20]. Previous studies indicated downregulation of the innate immune system when elevated NFkB in polytrauma patients [20-21]. Finnerty et al describe a peak in circulating G-CSF at day two after severe burn, which remains elevated through 3 weeks of injury[15]. Tanaka et al found G-CSF elevated in septic patients

and remain elevated throughout the course of infection with a return to baseline after recovery of illness [12]. Recently studies demonstrated T/HS induced stress condition, increased catecholamine level especially norepinepherine level and G-CSF promote mobilization of HPCs from bone marrow[22]. Excessive release of inflammatory cytokines leads to sustained elevation of catecholamine concentrations[4, 23]. Therefore trauma induced hypercathecolamine that alter the regulation of CXCR4 and SDF1 results suppress bone marrow HPCs and continued mobilization of HPC in to peripheral blood lead to persistent anemia[10]. Similarly, stress condition induced by catecholamine in burn injuries[24]. Kollet et. al.showed, trauma hemorrhage induced stress condition activation of osteoclast resulting mobilized progenitor cells in peripheral blood, when comparing patients with myocardial infarction to those with stable angina[25]. Induction of HPC mobilization by recombinant G-CSF administration results in a peak plasma concentration within 24 hours of dosing and a rise in peripheral HPCs in 4-6 days[26-27]. Similar results were observed in trauma patients, with an elevated and sustained number of circulating progenitors peaking at 5-8 days following trauma.

Cook et al. find high and sustained G-CSF levels are at least partly responsible for the observed bone marrow cell mobilization and subsequent dysfunction. Plasma G-CSF levels may serve as a useful marker in the investigation of post-injury immune suppression and modulation of G-CSF plasma levels, especially later in a patient's post-injury course, may have a salutary effect on the bone marrow function, anemia and infection, Additionally, these patients with high G-CSF required three units of packed red blood cell transfusions compared to one in the lower initial G-CSF group [10]. Miller et al demonstrated recombinant G-CSF is clinically used to mobilize and collect stem cells for bone marrow transplantation. While this medication and technique is safe and approved, there is a documented drop in haemoglobin among healthy patients, approximately 10%, which returns to baseline within a year [26].

Previous studies and Bungart et al. suggest that association between G-CSF and anemia has also been investigated in animal models. In animal, high dose G-CSF maintained erythropoiesis in normal rats, there is resultant anemia in impaired animals[28]. Van Os demonstrated that bone marrow has a limited reserve capacity and G-CSF may function to activate stem cells to differentiate at the expense of self-renewal which may ultimately cause a permanent loss of stem cells[29]. In the rare patient, G-CSF administration has been reported to lead to myelodysplastic syndromes and chronic leukemias.

Our study has some limitations that should be acknowledged. In this study, we did not assessed serum level of G-CSF with trauma patients without hemorrhagic shock, and G-CSF assessed only one time-point, and small sample size ; further studies to be recruit more patients and evaluate the serum level of G-CSF at different time point and design therapeutic strategies for G-CSF. Recent studies demonstrated that granulocyte colony stimulating factor (G-CSF) is one potent stimulator of hematopoietic mobilization, and has been well studied in BM dysfunction after severe trauma [10]. These findings support our study and strongly suggest connection between elevated serum level of G-CSF and bone marrow dysfunction in T/HS patients.

Conclusion

In summary, our study was demonstrated serum level of G-CSF was elevated in T/HS patients. The elevated level of G-CSF was associated with BM dysfunction following T/HS patients. Serum G-CSF levels may be used predictor of mobilization of HPCs following T/HS patients. Further studies to be evaluate the levels of G-CSF at different time points and therapeutic strategies for modulation of G-CSF.

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