Haematological & Inflammatory Markers in Septicaemia and Its Prognostic Implications in Okhla Industrial Area

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ABSTRACT

BACKGROUND

Sepsis remains an important cause of hospitalisation and mortality worldwide among patients admitted in intensive care units (ICU). Due to increasing mortality and morbidity of sepsis early diagnosis and treatment are essential. This can be achieved with the help of biomarkers–haematological and inflammatory i.e., C-reactive protein (CRP) and pro calcitonin (PCT). They are found to be highly sensitive and useful markers for early detection and management of sepsis.

METHODS

This is a cross-sectional study conducted from May 2019 to March 2020 in the Department of Biochemistry, ESI Hospital, Okhla. A total of 104 subjects were recruited for the study. Of them, 52 subjects were healthy controls, and 52 cases were ICU patients. Samples were taken to evaluate differential leucocyte count (DLC), red cell distribution width standard deviation (RDW SD), platelet count (PLT), absolute neutrophil count (ANC) and platelet crit as haematological markers whereas C-reactive protein, fibrinogen and pro calcitonin indices were chosen as inflammatory markers.

RESULTS

There was significant increase in the TLC (21583.51 ± 2240.36), total neutrophil count (7889.48 ± 1482.83), immature neutrophil (2.97 ± 1.67), fibrinogen levels (518.07 ± 66.74), CRP (0.048 ± 0.01) and pro calcitonin levels (0.062 ± 0.22) of the cases as compared to the healthy controls. The platelet count was observed to be higher in healthy subjects as compared to the patients in ICU.

CONCLUSIONS

The study results showed that clinical examination along with laboratory testing which include hematologic and inflammatory biomarkers is the most effective combination of parameters for rapidly and accurately predicting or diagnosing sepsis in a critically ill patient.

KEY WORDS

Sepsis, Haematological, Inflammatory, CRP, PCT, Fibrinogen

BACKGROUND

Sepsis remains an important cause of hospitalisation and mortality worldwide among patients admitted in intensive care units.[1] In a 5 year prospective study conducted by Sharmila Chatterjee et al. it was found that ICU mortality was 56 % in patients admitted with sepsis.[2] Sepsis can be defined as a life threatening organ malfunction caused by the dys regulated host response to an infection.[3] It is based on three cardinal symptoms-altered mental status, fast respiratory rate (> 22 breaths / minute) and low blood pressure (≤ 100 mm Hg systolic). There are three levels of severity within sepsis which are identified as sepsis, severe sepsis, and septic shock which depends upon increasing organ system involvement and coinciding mortality rates.[4] Early diagnosis and treatment is very important as mentioned in a study by Kumar et al. stating that mortality increases by 8 percent each hour if treatment is delayed.[5] Organ dysfunction or failure was assessed with the help of SOFA (Sepsis related Organ Failure Assessment) score which was developed by Vincent et al.[6] SOFA was designed to describe the sequence of complications in a critically ill patient and thus, helped to evaluate morbidity in sepsis. This score included six physiological systems (respiratory, coagulation, hepatic, cardiovascular, renal and neurological) and assigned points based on the degree of organ system dysfunction. A SOFA score of ≥ 2 and a documented or suspected infection is considered as sepsis. Though newer guidelines have changed however, this SOFA score is still used in cases of proven or suspected infection. Due to increasing mortality and morbidity of sepsis early diagnosis and treatment is essential for the clinician. Positive blood or body fluid culture requires 24 to 48 hours to develop and many at times it can be falsely negative. Hence, there is an urgent need for biomarkers-inflammatory and haematological for early diagnosis of sepsis.

Following any infection there is a phase of hyper-inflammatory response, which is called systemic inflammatory response syndrome (SIRS) which either may resolve or if further complicated can progress to severe sepsis. Whenever body is affected by harmful stimulus like infection and trauma, we can see release of pro inflammatory cytokines like interleukin 6, interleukin 1, TNFα and gamma interferon.[7] These cytokines trigger the liver to release acute phase proteins such as CRP and fibrinogen. Fibrinogen is elevated in initial stages of sepsis. ESR determines the rate at which RBC by virtue of its rouleaux formation settles down when placed in a vertical tube for an hour and indirectly measures the amount of fibrinogen, hence, increased levels of fibrinogen can increase ESR, which generally increases within 24 to 48 hours. Acute inflammation due to infectious diseases, tissue trauma, ischemia or tumour can lead to high ESR.[8] CRP is a highly sensitive marker of inflammation. It was first discovered by Tillet and Francis in 1930 when they showed it reacted to the C-polysaccharide of *Streptococcus pneumoniae* in patients with pneumococcal pneumonia. Normal range is 2 to 10 mg / l and its level starts to rise after 4 to 6 hours and peaks by 36 to 50 hours and can increase 100 to 1000 fold during acute inflammation.[9] PCT was first described in 1993 as a useful biomarker of sepsis and infection.[10] Normal value of PCT is less than 0.1 ng / ml. Higher values of PCT over 0.5 ng / ml is highly suggestive of bacterial infection and antibiotics are recommended. The pro inflammatory cytokines like IL6, IL1 and TNF-α like CRP and fibrinogen stimulates the release of

PCT but gamma interferon which is produced in viral infection does not stimulate PCT release, thereby proving PCT to be a marker highly suggestive of bacterial infections only.[11] PCT is detectable within 3 to 4 hours of an infection and peaks within 6 to 24 hours making it a marker superior than CRP in sepsis.

Endothelial dysfunction plays a key role in the pathogenesis of sepsis and is responsible for haematological changes which occur during sepsis. The endothelial injury generally occurs after entry of bacterial endotoxins or due to the effect of pro inflammatory cytokines and thus, can further lead to micro vascular coagulopathy and acute organ damage.[12] In sepsis we can find alterations in the property of red cell membrane which can lead to decrease in oxygen carrying capacity and change in mechanical properties of red cell disrupting the microcirculation and decreasing the oxygen delivery to the tissue. In early sepsis there is decrease in the red cell deformability which can decrease blood flow and increasing time for cell to transit the microcirculation.[13]

The most common laboratory finding includes anaemia with fall in haematocrit (HCT) values, low RBC count or low haemoglobin concentration, thereby decreasing oxygen carrying capacity. Neutrophilic leucocytosis is also commonly observed. Neutropenia is more prevalent in paediatric age group with severe sepsis.[14] Peripheral smear include neutrophilia with toxic granulation and Dohle bodies, increased proportions of non-segmented or band neutrophils and circulating immature granulocytes (metamyelocytes and myelocytes).[15] Thrombocytopenia is seen in cases of severe sepsis which is a marker of hematologic organ dysfunction.[16] In 20 to 40 % cases of ICU patients platelet count was found to be less than 100×10^9 / L and sepsis was found to be the major reason for thrombocytopenia.[17] There can be signs of disseminated intravascular coagulation (DIC) which can include combination of thrombocytopenia along with prolongation of prothrombin time (PT) with international normalised ratio (INR) and activated partial thromboplastin time (APTT) in severe sepsis. Factor VIII is decreased in severe sepsis and elevated in liver disease.[18]

Thus, the present study was conducted to investigate the significance of the various haematological and inflammatory markers to ascertain their role in early diagnosis and management of the patients admitted in ICU.

METHODS

The present study is a cross-sectional study conducted from May 2019 to March 2020 in the Department of Biochemistry, ESI Hospital, Okhla. A total of 104 subjects were recruited for the study. Informed written consent was obtained from them. Of them, 52 subjects were healthy controls, and 52 cases were ICU patients.

Sampling Method

Inclusion criteria for ICU admission were threatened airway, respiratory arrests, pulse rate < 40 or > 140 beats / min, sudden fall in level of consciousness, repeated or prolonged seizures, deranged arterial blood gas (ABG), respiratory rate ≥ 40 or ≤ 8 breathes / min. Exclusion criteria included: age below 23 years; known pregnancy patients; primary cases of trauma, burns, active seizure, acute cerebral vascular accident,

acute coronary syndrome, status asthmaticus, cardiac arrhythmias, active gastrointestinal bleeding, drug overdose; requirement for immediate surgery; active chemotherapy; and haematological malignancy.

The various assumptions used in sample size calculation are

1. Power - 85 %
2. Effect size - 0.5
3. Tails - Two
4. 3 allocation ratio - 1:1:1

A detailed clinical history including age, sex, occupation, socio-economic status, duration of diabetes and any associated risk factor contributing for the illness was elicited from the subjects. Under all aseptic precautions, blood samples (5 ml) were drawn by venipuncture and collected in EDTA and plain tubes respectively to analyse for haematological parameters like differential leucocyte count, red cell distribution width standard deviation, platelet count, absolute neutrophil count and platelet crit whereas C-reactive protein, fibrinogen and pro calcitonin indices were chosen as inflammatory markers.

Statistical Analysis
The sample size calculations were done on G power software version 3.1.9.2. For difference between two independent means. Student t-test was applied to calculate mean standard deviation and P-value.

RESULTS

Variables	Mean ± SD
TLC cumm	21583.51 ± 2240.36
TNC cumm	7889.48 ± 1482.83
Band cells %	2.97 ± 1.67
PLT count lac / cumm	163.77 ± 29.035
NUC.RBC count	0.06 ± 0.01
Fibrinogen (MG / DL)	518.07 ± 66.74
CRP (MG / DL)	0.048 ± 0.01
PCT (NG / ML)	0.062 ± 0.22
Table 1. Haematological & Inflammatory Markers in ICU Patients	

Variables	Mean ± SD
TLC cumm	9154.74 ± 1934.90
TNC cumm	3876.629 ± 1073.24
Band cells %	0.11 ± 0.02
PLT Count lac / cumm	21471.74 ± 22860.44
NUC.RBC Count	1.44 ± 1.23
Fibrinogin (MG / DL)	187.96 ± 47.49
CRP (MG / DL)	1.22 ± 042
PCT (NG / ML)	0.010 ± 0.03
Table 2. Haematological & Inflammatory Markers in Normal Individuals	

Table 1 & Table 2 show the haematological and inflammatory markers in both ICU admitted patients and healthy controls respectively. There was significant increase in the total leucocyte count (21583.51 ± 2240.36), total neutrophil count (77889.48 ± 1482.83), immature neutrophil (2.97 ± 1.67), fibrinogen levels (518.07 ± 66.74), CRP (1.22 ± 042) and pro calcitonin levels (0.062 ± 0.22) in the cases as compared to the healthy controls. The level of platelet count was observed to be higher in the healthy subjects (21471.74 ±

22860.44) as compared to the admitted patients in ICU (163.77 ± 29.035). The results are presented in mean ± SD. All the biochemical and haematological parameters were compared by using unpaired t-test between cases and controls. The Pearson's correlation coefficient was calculated among the study parameters. The P-value < 0.05 was considered significant. (Table 3)

Variables	Septic (52) Mean ± SD	Non-Septic (52) Mean ± SD	t Value	P Value	Conclusion
TLC cumm	21583.51 ± 2240.36	9154.74 ± 1934.90	21.81	0.001	Significant
TNC cumm	7889.48 ± 1482.83	3876.629 ± 1073.24	11.39	0.001	Significant
Band cells %	0.11 ± 0.02	2.97 ± 1.67	19.04	0.001	Significant
PLT count lac / cumm	163.77 ± 29.035	21471.74 ± 22860.44	4.84	0.001	Significant
NUC.RBC count	0.06 ± 0.01	1.44 ± 1.23	17.02	0.001	Significant
Fibrinogen (MG / DL)	518.07 ± 66.74	187.96 ± 47.49	20.94	0.001	Significant
CRP (MG / DL)	1.22 ± 042	0.048 ± 0.01	16.85	0.001	Significant
PCT (NG / ML)	0.062 ± 0.22	0.010 ± 0.03	1.17	0.001	Significant
Table 3. Correlation of Haematological & Inflammatory Markers between Normal Individuals and ICU Patients					

DISCUSSION

CRP is the most widely accepted marker of infection and inflammation and has been more used in paediatrics[19] to detect any infectious or inflammatory disease. Nowadays it is more commonly used in cardiovascular or atherosclerotic conditions.[20] Numerous comparative studies of CRP and PCT were conducted in diagnosing sepsis where initially PCT was found to be more sensitive and specific than CRP for identifying bacterial infection.[21] A large meta-analysis done by Uzzan et al.[22] collected 33 studies between April, 1996, and October, 2004, where it included 3,943 patients of sepsis and it was observed that the receiver operating characteristic (ROC) curve for PCT was much higher than CRP (0.78 versus 0.71). A recent meta-analysis done by Tang et al.[23] that collected 30 studies showed that PCT has a low accuracy in distinguishing sepsis from SIRS. Muller et al.[24] in 2007 conducted a study in patients with community acquired pneumonia, where he projected that PCT concentration helped to distinguish bacterial from viral pneumonia. In 2007 Kofoed et al.[25] concluded that combined use of three or six pro inflammatory markers was more successful in accurately identifying patients with bacterial infection. Similar multi marker approach was used by Shapiro et al.[26] in 2009 in diagnosing severe sepsis. A study done by Young et al.[27] investigated the potentiality of PCT as a marker to diagnose septic shock in patients with acute pyelonephritis occurring secondary to ureteral calculi. It concluded that high PCT and low platelet count are high risk factors of septic shock in such cases. In a study done by Heffner AC et al. in division of critical care medicine, it was observed that more than 50 % of the patients with severe sepsis have negative culture results. Hence, culture and sensitivity offer a limited prospectus in management of severe sepsis.[28] In a study done by Canan BalcI[29] in 2002, it was observed that PCT has the maximum specificity and sensitivity in differentiating patients with SIRS

from sepsis. However, the best approach in early identification of sepsis would be selection of combination of markers like haematological, pro inflammatory and anti-inflammatory markers.

CONCLUSIONS

Sepsis is the leading cause of death in hospitalised patients in ICU settings. Patients with sepsis often present with nonspecific symptoms of inflammation which rapidly progress to a more severe condition if not treated. In uncontrolled cases of sepsis, acute organ dysfunction and shock may develop. Because of this rapid progression, it is of utmost importance that patients should be diagnosed and treated in a requisite time frame.

The current literature and changing guidelines demonstrate that the bedside physical examination along with laboratory testing (haematologic and inflammatory biomarkers) are the most effective combination of parameters that clinicians can rely upon to accurately predict or diagnose sepsis in a critically ill patient. We included all the readily available cost effective parameters.

Limitations of the Study

We considered blood and fluid culture reports of the patients. Since the cost of interleukin assessment was high, we couldn't incorporate the data of the same. There should be data collection of the entire tertiary health care centres in the district so as to effectively analyse it on a large scale. The future implication of the study lies in the development of better cost-effective biomarkers for early diagnosis of sepsis patients.

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