Clinical Significance of Ischemia Modified Albumin in Critically Ill Patients with Sepsis

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Abstract The study was conducted on 38 patients admitted into the intensive care unit with a provisional diagnosis of sepsis and 25 apparently healthy volunteers as controls. Serum procalcitonin (PCT) was assayed by an electrochemiluminescence method. Serum ischemia modified albumin (IMA), expressed as absorbance units was assayed by the albumin cobalt binding test. Patients with sepsis had significantly higher IMA levels (1.087 ± 0.786) as compared with those without sepsis (0.085 ± 0.234) with a p value <0.0001 . The receiver operator characteristic (ROC) plot showed a sensitivity of 100 % and a specificity of 86.2 %. The area under the curve of the ROC plot was 0.917 with a p value of <0.0001 . The higher levels of IMA serve to highlight the occurrence of ischemic damage which could be a prelude to poorer prognosis. The performance characteristics of IMA warrants its inclusion along with PCT as a parameter in the diagnosis of sepsis.

Keywords Ischemia modified albumin · Pro-calcitonin · Oxidative stress · Sepsis

Introduction

Sepsis is a condition characterized by systemic invasion of micro-organisms breaching the normal host barriers. Sepsis occurs in 1–2 % of all hospitalizations and accounts for as much as 25 % of intensive care unit (ICU) bed utilization. Sepsis ranks among the top 10 causes of death in the world [1]. Sepsis is a fatal condition and patients with the disease show an impaired quality of life [2]. A study conducted internationally showed that mortality from sepsis in hospitals ranged from around 14.7–29.9 % [3].

Procalcitonin (PCT), polypeptide containing 116 amino acid residues, is formed by the C cells of the thyroid gland as the precursor of calcitonin [4]. Almost all of the PCT is cleaved to form the hormone calcitonin within the thyroid gland itself. In healthy subjects, the plasma levels of PCT are therefore generally low. The levels of PCT have been found to increase in patients with sepsis and it also has been shown to correlate with the severity of the condition of the patient [5]. PCT is now being routinely used as a gold standard diagnostic marker for sepsis in patients admitted to the ICU [6]. On comparison with other markers PCT has a sensitivity of 85 % and specificity of 91 % in diagnosing sepsis [7].

Ischemia modified albumin (IMA) is an emerging marker which has been found to be elevated in pathological conditions associated with ischemia and oxidative stress such as myocardial infarction, pulmonary embolism, COPD and cerebrovascular accidents [8–10]. It has been hypothesized that oxidative stress causes a conformational change at the N-terminal end of the albumin molecule. Metallic ions, such as copper and cobalt that normally bind to the N-terminus of albumin, fail to do so, due to the conformational change and this modified form of albumin is generally referred to as 'IMA'. The exact mechanism

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that leads to the formation of IMA is still not known. Studies have shown that IMA can be used as a ubiquitous indicator for oxidative stress and ischemic damage.

To the best of our knowledge there very few reports which link IMA and sepsis in critically ill patients. This study sought to highlight the fact that sepsis is associated with oxidative stress, by estimating the levels of IMA in critically ill patients with a proven diagnosis of sepsis. The second objective of the study was to compare the sensitivity and specificity of the new parameter IMA with that of the established parameter PCT as a biomarker of sepsis in critically ill patients.

Materials and Methods

Ethical clearance for carrying out the study was obtained from the Institutional Human Ethics Committee of PSG Institute of Medical Sciences and Research, Coimbatore. The samples used for this study were sent to the lab along with request forms duly signed by the treating clinician. Serum PCT was one of the tests requested. General consent was obtained from all patients.

Subjects

The study population consisted of 32 critically ill patients admitted to ICU of PSG Hospitals, Coimbatore. Patients suspected of having sepsis with a request from the clinician for the estimation of serum PCT formed the subjects of the study. The control subjects consisted of 25 healthy human adult volunteers consisting of 19 males and 6 females and ages ranging from 44 to 75 years. Thirty-eight critically ill patients were considered initially. Due to insufficient sample volume for performing the tests, samples from six patients were excluded. Hence, there were a total of 32 subjects in the critically ill group of which 19 were males and 13 were females with ages ranging from 22 to 82 years. Patients with other conditions such as chronic liver disease and diabetes mellitus that could possibly alter IMA values were excluded from the study.

5 mL of a random sample of venous blood was collected using plain Beckton-Dickinson 'vacutainers' from each patient and transported without delay to the clinical biochemistry laboratory, serum was used for the estimation of PCT within 3 h after collection. The remaining serum was used for the estimation of IMA within 8 h.

Serum PCT was estimated using Roche e-411 electrochemiluminescence analyser using Roche kits. The PCT in the sample reacts with a biotinylated monoclonal specific antibody and also with a monoclonal PCT specific labelled antibody with a ruthenium complex to form a sandwich complex. A second reagent, streptavidin-coated micro

particles, interacts with the complex, becomes bound to the solid phase via interaction between biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with a Pro-Cell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined by a calibration curve which is instrument- specifically generated by a two point calibration. A PCT value ≥ 0.5 ng/mL was considered to be diagnostic of sepsis.

The assay of IMA is based on the premise that ischemia causes changes in human serum albumin (HSA) that are demonstrated by reduced exogenous cobalt (II) binding. Reagents for this assay were prepared in the laboratory. Preparations for the Co (II) albumin binding protocol involved the addition of 200 μ L of the patient's serum to 50 μ L solution of 1 g/L cobalt chloride, followed by vigorous mixing and 10-min of incubation at 37 °C. Dithiothreitol (50 μ L of a 1.5 g/L solution) was then added and mixed. After 2 min incubation, 1.0 mL of a 9.0 g/L solution of NaCl was added. The absorbance of the assay mixture was read at 470 nm using a spectrophotometer. The blank was prepared similarly with the exclusion of DTT. The final IMA results were expressed as absorbance units (ABSU) [11].

Statistical Analysis

The students't' test was used for comparing the IMA levels in the two groups of patients (those with sepsis and those without sepsis) and a *p* value <0.05 was taken as significant. The ROC plot was constructed in order to determine the sensitivity and specificity of IMA, using PCT as the classification variable. The receiver operator characteristic plot (ROC) is a statistical tool that is used to compare the performance characteristics of an 'emerging diagnostic parameter' with an 'established parameter' which is considered to be the gold standard. The ROC plot was done using the Medcalc software.

Receiver Operator Characteristic Curve

In the ROC curve the true positive rate (sensitivity) is plotted as a function of the false positive rate (100-specificity) for different cut-off points. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC curve that passes through the upper left corner (100 % sensitivity, 100 % specificity). Therefore the closer the ROC curve to the upper left corner, the higher is the overall accuracy of the test [12].

Results

Patients with sepsis had significantly higher IMA levels ($1.087 \text{ ABSU} \pm 0.786$) as compared to those without sepsis ($0.085 \text{ ABSU} \pm 0.234$) with a p value <0.0001 which is very highly significant. Patients with sepsis had IMA values ranging from 0.085 to 2.422 ABSU. The range of IMA values in the control group was 0–0.033 ABSU. The dot plot graph (Fig. 1) shows the spread of values in the two groups. This graph also shows that IMA was elevated in four subjects in the control group. These patients had PCT levels which were $<0.05 \text{ ng/mL}$ and therefore did not have sepsis. The reason for the high IMA in these patients is not known with certainty. There could be some other cause for oxidative stress in these patients.

Despite the discrepant findings in these four subjects the ROC plot has shown that IMA is a very good discriminatory parameter for the diagnosis of sepsis using PCT as a classification variable (Fig. 2). The area under the curve was found to be 0.917 with a highly significant p value of <0.0001 . The criterion value was found to be 0.033 with a sensitivity of 100 % and specificity of 86 %.

Thus, it is seen that IMA can be used as a highly sensitive diagnostic marker for sepsis. The concern however is with regard to its specificity due to the ubiquitous response of IMA to oxidative stress.

Discussion

An important observation in this study is that, sepsis is associated with the alteration in the structural

Fig. 1 Comparison of IMA values in patients with sepsis and patients without sepsis

Fig. 2 ROC plot showing the sensitivity and specificity of IMA sepsis defined using a PCT values $\geq 0.5 \text{ ng/mL}$

characteristics of the albumin circulating in the plasma, leading to the generation of significant quantities of IMA. Raised levels of IMA have been linked to episodes of acute myocardial infarction, cerebro-vascular occlusion, pulmonary ischemia, gastro-intestinal ischemia and skeletal muscle ischemia [7]. That a similar molecular event occurs in sepsis serves as a pointer to the fact that oxidative stress and free-radical mediated damage is a key event in patients developing sepsis.

Sepsis, often initiated by an infection, is a state of disrupted inflammatory homeostasis. Neutrophils show a burst of oxygen consumption most of which is used to generate reactive oxygen species such as superoxide anion, hydroxyl ions as well as reactive nitrogen species such as peroxynitrite [13]. This microenvironment leads to the oxidative modification of several biomolecules of which albumin represents a measurable component. Measurement of IMA therefore serves to quantify oxidative stress in sepsis. This in turn denotes that damage at the level of biomolecules is an inevitable event and may be a prelude to the development of organ dysfunction.

It has been known for more than a century that the biological function of a protein is intricately linked to its molecular architecture and that even slight alterations in structure could lead to a compromise in its function. Plasma albumin performs several crucial functions which includes its role in acid–base homeostasis, as well of transport of several endogenous and exogenous ligands [14]. To name a few, free fatty acids, hormones, bilirubin and calcium ions are some of the endogenous substances. Albumin also possesses binding sites for drugs such as salicylates and diazepam [15]. It is conceivable that these properties could be affected when albumin gets converted from its native state into IMA. In the context of patients

who are critically ill and administered several drugs, alteration in pharmacokinetics might have a direct effect on treatment outcomes.

The second aspect to be considered is the possible contribution of IMA as a parameter in the diagnosis of sepsis. Here again the performance characteristics of IMA warrants its inclusion in patients with sepsis. The ROC plot that was constructed has shown a high degree of sensitivity and specificity. Since the methodology is simple and inexpensive it could be assayed along with PCT in patients with sepsis. While PCT helps to rule in or rule out sepsis, IMA helps in quantifying oxidative stress.

To conclude, this study has clearly demonstrated that IMA rises in critically ill patients with sepsis. Its estimation helps to identify and quantify oxidative stress and could serve as a surrogate marker of ischemic damage to tissues and organs.

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