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Original Article

Internal Medicine Section

Markers of Oxidative Stress and Clinical Outcome in Critically ill Septic Patients: A Preliminary Study from North India

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Introduction: Sepsis is the leading cause of mortality in non-coronary Intensive Care Units (ICUs). Oxidative stress is one of the important features in pathogenesis of sepsis.

Aim: This study was undertaken to evaluate levels of oxidants and antioxidants in patients with sepsis admitted to ICU.

Study Design: This was a non-interventional clinical case-control study undertaken at a tertiary level teaching hospital in New Delhi, India.

Materials and Methods: Forty-six consecutive non-pediatric patients admitted to ICU with sepsis were included and subjected to detailed history, physical examination and investigations. Blood samples were drawn to evaluate oxidant Malondialdehyde (MDA) and antioxidant (alpha-tocopherol) levels. Acute Physiology and Chronic Health Evaluation II (APACHE II) and Organ Dysfunction and/or Infection (ODIN) scores were calculated and patients followed up for outcomes. Twenty age and sex matched healthy subjects served as controls.

Results: Mean levels of malondialdehyde were higher in patients than controls (17.2±3.8nm/ml versus 4.6±1.6nm/ml, p<0.001) while levels of alpha-tocopherol were lower (3.2±1.3µg/ml versus 9.9±2.0µg/ml, p<0.001). The mean APACHE II and ODIN scores were 18.1±9.3 and 1.7±1.3 respectively in patients. These scores were two to three fold higher in non survivor patients (n=22) in comparison with survivors (n=18) (p<0.001). There was no significant difference between the two groups in oxidants and antioxidants levels (p>0.05). However, a significant and positive correlation was observed between oxidant -antioxidant levels and APACHE II, ODIN and International Normalized Ratio (INR) scores in septic patients overall.

Conclusion: The oxidants in septic patients were significantly higher while antioxidants were significantly lower than healthy controls. There was also a significant correlation with APACHE II and ODIN scores. A large patient population based study may draw more specific conclusions.

Keywords: Antioxidants, APACHE II, ICU, Oxidants, Sepsis

INTRODUCTION

The incidence of sepsis has increased considerably since the last few decades. Malnutrition, poverty, lack of access to vaccines and timely treatment, all contribute to death and in the developing world the mortality rate have been reported to be ranging from 60-80% due to sepsis [1]. Sepsis is defined as systemic response to infection [2]. It has been reported to be the commonest, resource consuming and causative factor for frequently fatal conditions, associated with many deaths in non-coronary Intensive Care Units (ICU) [3]. It is also said to reduce the long term quality of life of the survivors [4,5]. Patients with sepsis are exposed to severe oxidative stress, resulting from an oxidant antioxidant imbalance. Oxidative damage results due to an imbalance between oxidants and antioxidants and includes oxidative modification of cellular macromolecules, induction of cell death as well as structural tissue damage [6]. The use of antioxidant therapy has long been advocated for blunting the oxidative stress and thereby reducing the inflammatory response and its negative impact on the body [7,8]. The oxidant and antioxidant state in septic patients has been scarcely evaluated and only few studies have been done on septic patients correlating the severity of illness and patient outcome with oxidant and antioxidant levels [9-12]. It was thought that a well-structured study of patients of sepsis and their levels of oxidants and antioxidants would be of immense help in understanding the role of oxidative stress in sepsis. Many studies have reported a higher antioxidant state, determined by different compounds in non-surviving than in surviving septic patients [10-12]. MDA is a low molecular weight aldehyde that results from free radical attacks on polyunsaturated fatty acids. Hence, MDA measurement can be considered as a valuable screening tool of indicator oxidative damage [13]. Recently, Lorente and colleagues have demonstrated increased levels of MDA in the serum of adults with severe sepsis. They reported that the non–survivors exhibited higher MDA levels than survivors suggesting that oxidative stress mediated by lipid peroxidation, contributes to sepsis pathophysiology [14]. Therefore, measurement of MDA levels is appropriate for the early estimation and quantification of oxidative damage. In healthy individuals, oxidant levels are stabilized by anti-oxidants. Alpha tocopherol – an endogenous lipid soluble chain breaking and the most active form of Vitamin E- is a powerful anti-oxidant and protects polyunsaturated fatty acids found in cell membrane phospholipids from the effects of free radicals by donating its hydrogen atom [15]. Vitamin E is shown to attenuate endotoxin- induced activation of alveolar macrophages [16] and is involved in septic shock in humans [17]. The present preliminary study was undertaken with the objective of measuring MDA and alpha tocopherol levels in patients with sepsis and to find out any correlation of these to disease severity and final outcome.

MATERIALS AND METHODS

This was a non-interventional clinical case control study undertaken in the Departments of Internal Medicine and Biochemistry at Maulana Azad Medical College and associated Lok Nayak Hospital, a tertiary level teaching hospital in New Delhi, India. The study recruited patients from medical and surgical ICUs over a 3 month period. The criteria used for labeling a patient as having sepsis were those set by Bones et al., [18]. Sepsis = the systemic response to infection, manifested by two or more of the following conditions as a result of infection: (1) Temperature >38°C or <36°C; (2) Heart rate >90 beats per minute; (3) Respiratory rate >20 breaths per minute or $PaCO_2$, <32mm Hg; and white blood cell count >12,000/cumm, <4,000/cumm,

or >10% immature (band) forms. Exclusion criteria were: age <12 years, pregnancy, lactation, Human Immunodeficiency Virus (HIV), and white blood cell count <1,000 cells/µl, solid or haematological tumour, or immunosuppressive, steroid or radiation therapy.

Forty six consecutive non-pediatric patients (age more than 12 years) diagnosed with sepsis were included in the study. Ethical approval was obtained from the Institutional Ethics Committee of the hospital. The patients were subjected to a detailed medical history that included demographic data, presenting complaints, history of present illness and any past history of prolonged illness or hospitalization. A thorough physical examination was done and following clinical variables were recorded for each patient. Sex, age, cultures and routine investigations – haematological, biochemical and radiological diabetes mellitus, chronic renal failure, Chronic Obstructive Pulmonary Disease (COPD), site of infection, leukocytes, platelets, International Normalized Ratio (INR), Severity of illness assessed by Acute Physiology and Chronic Health Evaluation II (APACHE-II) score [19], and Organ Dysfunction and/or Infection (ODIN) [20] scores were calculated for each patient.

Twenty healthy age and sex matched controls with no evidence of sepsis or underlying infectious etiology were selected for the purpose of comparison from the outpatients coming to Department of Medicine. Blood samples were collected from patients within 24 hours of making a diagnosis of sepsis for estimating serum MDA and alpha tocopherol levels.

MDA Serum and Plasma Alpha-Tocopherol Level Analysis: MDA levels were measured by Thiobarbituric Acid Reactive Substance (TBARS) reaction using microplate spectrophotometer reader and following the method described by Kikugawa et al., [21]. The serum concentration of the MDA level was expressed in nmol/ml. Plasma alpha tocopherol was estimated by Hansen and Warwick's method by plotting relative fluorometer units versus concentration of the tocopherol standards on a standard curve. The plasma concentration of alpha tocopherol was expressed as µg/ml [22]. To avoid the possible dispersion of serum MDA and alpha tocopherol level results, all the samples were processed at the same time.

STATISTICAL ANALYSIS

Continuous variables are reported as medians and interquartile ranges. Categorical variables are reported as frequencies and percentages. Comparisons between groups for categorical variables were carried out with chi-square test and Spearman's Rank Correlation Coefficient was used to determine correlation between MDA and alpha tocopherol levels and correlation of these with APACHE II and ODIN scores. Comparisons between pairs of groups separately for survivors and non-survivors in Oxidant and Antioxidant levels were carried out with Student's t-test for repeated measures. The difference of means was used to calculate significance of difference of various parameters between survivors and non survivors. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Twenty five of the total patients (n=46) were females and 21 were males. Mean age was 43.2 ± 17.2 years and ranged from 14 to 76 years. More than half the patients (n=30) fulfilled three of the four criteria of sepsis and the rest conformed to four (n=11) and two (n=5) criteria respectively. Tachycardia was seen in 44 patients, temperatures of more than 38°C in 37 and hypothermia in 2, tachypnea in 22 patients, 19 (41.3%) patients were on ventilators and neutrophilic leucocytosis observed in 18 with one patient demonstrating leucopenia. The most common site of infection precipitating sepsis was the respiratory tract (41.3%) followed by abdominal (30.4%) urinary (19.5%) and skin (8.6%). Fever (n=43) remained the most frequently encountered presenting symptom in these patients, although a wide spectrum of symptomatology was observed. Chronic Obstructive Pulmonary Disease (COPD) (n=9; 19.5%) Hypertension (n=9; 19.5%), diabetes (n=8; 17.3%) and chronic renal failure (n=5; 10.8%) were common associated chronic conditions. Sixteen (34.7%) patients' demonstrated gram positive cultures, Gram negative organisms were isolated in 13 (28.2%) patients.

In terms of biochemical results the levels of MDA in patients (17.2± 3.8 nM/ml) were about four folds than in controls (4.6 ± 1.6 nM/ml) (p<0.001) while the alpha tocopherol levels were less than one third (3.2 ± 1.3 versus 9.9 ± 2.0 µg/ml, p<0.001) [Table/Fig-1,2]. APACHE II and ODIN scores were 18.1 ± 9.3 and 1.7 ± 1.3 respectively. The APACHE II and ODIN scores of patients who did not survive were much higher as compared to those who survived (p<0.001) [Table/Fig-3]. These two groups (of survivors and non-survivors) however, failed to demonstrate any significant differences in terms of oxidant and antioxidant levels [Table/Fig-3]. For the patient group as a whole, we observed a significant and positive correlation between oxidant-antioxidant levels with APACHE II, ODIN scores and INR [Table/Fig-4].

Characteristics	Value
Gender	
Male Female	21 (45.6%) 25 (54.3%)
Age (Yrs.)	43.2 ± 17.2 (14-76)
COPD	09 (19.5 %)
Diabetes Mellitus	08 (17.3%)
Chronic Renal Failure	05 (10.8%)
Hypertension	09 (19.5%)
Ventilator Support	19 (41.3%)
Site of Infection	
Respiratory Abdominal Urinary Skin Neurological	19 (41.3%) 14 (30.4%) 09 (19.5%) 04 (8.6%) 01 (2.1%)
Responsible Microorganisms	
Gram Positive n (%) Gram Negative Unknown	16 (34.7%) 13 (28.2%) 17 (36.9 %)
Creatinine (mg/dl) Bilirubin (mg/dl)	1.52 (0.7 0-3.24) 1.42 (0.62-3.14)
INR	1.34 (0.99-2.4)
APACHE II Score	18.3±9.3
ODIN Score	1.7±1.3
Oxidant (Malondialdehyde- MDA (Median nmol/ml)	17.2±3.8nmol/ml
Antioxidant (Alpha Tocopherol (µg/ml)	3.2±1.3 µg/ml

[Table/Fig-1]: Clinical and demographic characteristics of the septic patients (n=46).
COPD: Chronic Obstructive Pulmonary Disease; INR: International Normalized Ratio; APACHE II = Acute Physiology and Chronic Health Evaluation –II; ODIN Score: Organ Dysfunction and/or Infection Data are presented as number (Percentage) or interquartile range (Median).

Investigations	Cases (n=46)	Controls (n=20)	p-value
Oxidant Malondialdehyde (nmol/ml)	17.2±3.8	4.6±1.6	<0.001
Antioxidant Alpha Tocopherol (µg/ml)	3.2±1.3	9.9±2.0	<0.001

[Table/Fig-2]: Malondialdehyde and alpha tocopherol (oxidants/antioxidants) serum levels in septic patients and controls.
The table describes the biochemical values obtained in cases and controls, and calculated p-values for statistical significance of the difference in means.

Value	Non survivors (n = 22)	Survivors (n = 24)	Total (n = 46)	p-Value
APACHE II*	24.1 ± 5.9	12.0 ± 8.0	18.1 ± 9.3	< 0.001
ODIN**	2.6 ± 0.9	0.9 ± 1.0	1.7 ± 1.3	< 0.001
Oxidant (Malondialdehyde) (nM/ml)	17.0 ± 4.2	17.5 ± 4.0	17.2 ± 3.8	> 0.05
Antioxidant (Alpha tocopherol) (µg/ml)	2.9 ± 0.7	3.0 ± 1.5	3.2 ± 1.3	> 0.05

[Table/Fig-3]: Comparison of outcome with studied parameters.
The table describes the comparisons between different parameters for Survivors vs. Non-Survivors. The p value described refers to the statistical significance of difference of means between these two groups.
* APACHE II - Acute Physiology and Chronic Health Evaluation II
** ODIN - Organ Dysfunction and/or Infection

Parameters	MDA Serum Levels (Oxidant)	Alpha Tocopherol Levels (Antioxidant)
Age	r=0.16; p= 0.08	r=0.29; p=0.21
*APACHE II	r=0.32; p=0.004	r=0.42; p<0.001
**ODIN Score	r=0.38; p=0.002	r=0.34; p<001
INR	r=0.28; p<0.001	r=0.32; p<0.001

[Table/Fig-4]: Correlation of oxidant -antioxidant levels with age, APACHE score II, ODIN score and INR.
* APACHE II - Acute Physiology and Chronic Health Evaluation II
** ODIN - Organ Dysfunction and/or Infection
INR- International Normalized Ratio
r=Spearman's Rank Correlation Coefficient

DISCUSSION

There is no published study about oxidative stress and oxidant/anti-oxidant system evaluation from septic patients over 12 years. of age in the existing literature in India. To our knowledge, our study is the first hospital based pilot study providing data on oxidative and anti oxidative state in septic patients. The relevant findings can be summarized as: First, oxidant-antioxidant imbalance was evident in patients with sepsis. Second, the MDA serum levels (oxidant) were significantly higher in septic patients than controls and alpha tocopherol levels (antioxidants) were significantly lower in septic patients than controls. Third, a significant correlation was observed between oxidant and antioxidant serum levels and several indicators of sepsis. Taken together the results from our study indicate alteration of the oxidative and anti-oxidative state may represent a potential therapeutic target in the pathophysiology of sepsis.

We found that septic patients had higher mean serum levels of MDA than healthy controls (17.2 ± 3.8 nM/ml vs. 4.6 ± 1.6 nm/ml) respectively reflecting a state of lipid hyperoxidation. Antioxidant levels were significantly less in patients than controls (3.2±1.3 vs. 9.9±2.0). Recently, Lorente and colleagues have demonstrated increased levels of MDA in the serum of adults with severe sepsis than healthy controls [14]. A study by Scott L and Clifford reported an increase in serum levels of the biomarker of oxidative stress-induced lipid peroxidation, in adults with severe sepsis, particularly in non survivors [23]. Their finding further reinforced the role of oxidative stress as a mechanism of organ dysfunction, and thus a potential therapeutic target - in sepsis. In an another study by Ameen et al., it was assumed that lipid peroxidation resulted in production of Reactive Oxygen Species (ROS) which played an important role in pathogenesis of multiple organ dysfunction and septic shock associated with neonatal sepsis. The MDA levels were extremely higher in both full term and preterm neonates with sepsis than that in their corresponding controls [24]. Our findings too are in agreement with the results of above mentioned studies [14,24] and other one small study done on septic patients [9].

Goode HF and colleagues [17] reported both an increased level of oxyradical stress, as monitored by Thiobarbituric Acid Reactive Species (TBARS) and urine nitrite excretion, as well as a significant decrease in the level of several key antioxidant systems (alpha tocopherol, retinol, beta-carotene and lycopene). Higher levels of lipid peroxidation products [9,17] and lower plasma selenium [17] and ascorbic acid levels [25] were associated with higher incidence of Ministry of Finance (MOF) and worse prognosis.

In terms of culture positivity we found that positive cultures were seen in 62.9% patients, similar to other workers. Gram negative organisms accounted for 16 (34.7%) and gram positive organisms were obtained in 13 (28.2%) of the isolates. This is similar to the results of cultures performed by Lorente and colleagues [14] and Sands et al., [26] where 24.1% and 40% isolates were found to be gram negative respectively. The reason for relatively high percentage of gram negative organism isolates in our study could be attributed to the difference in prevalent bacterial flora in our ICU.

We found a significant correlation between serum MDA levels, Alpha tocopherol and several indicators of severity in sepsis, including INR, APACHE II score and ODIN score in these septic patients. Previous studies have reported a positive correlation between MDA and severity in septic patients [9,12,14]. In the study by Goode HF et al., patients with three or more failing organs showed higher MDA levels than patients with fewer failing organs [17]. Andresen et al., reported a positive correlation between peak MDA and peak lactate levels. However, no correlation was observed at day 1 [27].

In our study we found, non-surviving septic patients had significantly higher APACHE II and ODIN scores than surviving septic patients (p<0.001). However, we could not find a significant difference in oxidant –antioxidant levels between survivors and non survivors (p=0.05). This may due to the fact that our sample size was too small to demonstrate that oxidant and antioxidant levels were independently associated with survival in our patients with sepsis. Based upon this it seems evident that in our study oxidative stress failed to predict the poor prognosis and mortality, contrary to some previous studies. Alonso de Vega et al., observed in their study that the non survivors had a more severe oxidative stress, higher APACHE III scores, and higher plasma concentrations of markers of leukocyte activation, in comparison with survivors [28]. Here, we will like to emphasize that, none of the clinical studies done on septic patient has been able to establish a causal Relationship between oxidative stress and mortality. In a study by Cowley et al., oxidative stress was present in survivors as well as non-survivors at time of diagnosis [12]; but antioxidant levels increased to normal or supernormal level in patients who eventually recovered. This indicates that oxidative stress is universal in all patients with sepsis and it is recovery from this stress which is affecting patient outcome. Our study also endorses same view that it is not static value of oxidative stress, but dynamics of balance between oxidant and antioxidant forces that affects the disease progression and prognosis. Nature of these factors which leads to recovery in some patients still remains to be elucidated.

STRENGTHS AND LIMITATIONS

The strength of our study lies in the fact that it was a case control study conducted in the ICU setting and patients were enrolled consecutively which removes selection bias. Certain limitations should be recognized such as the fact that the sample size of the study was not pre -determined and was relatively small. Secondly, we studied a narrow selection of oxidant –antioxidant compounds and included a portion of the full cohort. We did not consider other potentially broad relevant oxidants- antioxidants which may act as a biomarker. An ideal study would encompass measuring of a broader range of other compounds of oxidants and antioxidants involving all subjects in the cohort. However, our preliminary study lays the foundation for a more comprehensive examination of a broader range of oxidants-antioxidants modulators in septic patients.

CONCLUSION

With the information gathered from our study, it can be foreseen that oxidant/anti-oxidant level may play an important role in pathogenesis of sepsis. Our study shows significant difference of oxidant and antioxidant parameters in septic patients in comparison to the healthy controls. Therefore, the study points at the importance of oxidative stress as a prognostic factor for sepsis and probably alpha tocopherol and MDA as promising molecules that deserves the attention of future research. The values of APACHE II and ODIN scores, which were found to be two to three folds higher in non survivors than survivors, may act as better indicators for prognostication and prediction of outcome. Will this translate into therapy for sepsis rather than only prognostic indicator? Further studies should also try to establish the possible benefits of combination of oxidants-antioxidants supplementation in septic patients. A study involving a large number of patients could perhaps lead to a better insight into the role of oxidative stress in sepsis.

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