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ASSESSMENT OF OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN PATIENTS OF ACUTE RENAL FAILURE WITH SEPSIS

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ABSTRACT

Role of reactive oxygen species in the pathogenesis of a variety of human diseases including toxic or ischemic acute renal failure (ARF) and sepsis has been reported. Therefore, in the present study, to assess the oxidative stress and antioxidant status, the plasma peroxidation products, ascorbic acid, vitamin E, catalase, total thiol groups and the erythrocyte reduced glutathione levels were measured in 65 patients of ARF with sepsis and 30 age and sex-matched normal healthy volunteers. A significant increase in plasma MDA (lipid peroxidation products; $p<0.001$) and catalase activity ($p<0.01$) with a concomitant significant decrease in plasma ascorbic acid ($p<0.001$), vitamin E ($p<0.01$), total thiols ($p<0.001$) and erythrocyte reduced glutathione ($p<0.001$) was observed in ARF patients with sepsis compared to normal healthy persons. These observations indicate that imbalance between pro-oxidant and antioxidant levels may play an important role in the pathophysiology of ARF.

KEYWORDS: Oxidative stress, Antioxidants, Acute Renal Failure, Sepsis

INTRODUCTION

Reactive oxygen species (ROS), an important biochemical intermediates have been implicated in a very large number of human diseases. These are produced in much larger amounts than normal under a variety of pathological conditions.[1, 2] These oxygen species can attack any biochemical component of the cell. Lipid component of the cells or organelle

membranes are the most frequently damaged resulting in lipid peroxidation. Oxidation of biomolecules by ROS can lead to tissue injury and cell death.[3] The role of ROS in the pathogenesis of a variety of renal diseases and in particular of toxic or ischemic acute renal failure (ARF) has also been documented.[4, 5]

Septic shock is the commonest cause of mortality in intensive care unit (ICU). Increased amounts of ROS are generated in sepsis by phagocytes and reperfusion. In response to infection, more free radicals are produced by activated phagocytes.[6] Additionally due to transitory circulatory insufficiency to organ like heart, lungs, kidneys & other organs accompanying sepsis and during reperfusion/reoxygenation, free radicals are generated. This occurs due to the fact that a high concentration of oxygen is required to achieve adequate arterial oxygenation.[7] Sepsis, especially septic shock is one of the main causes of Acute Renal Failure (ARF). The prevalence of ARF in sepsis ranges from 9% to 40%.[8] However, another French multicenter study by Brivet *et al* has shown the rate of ARF in sepsis to be as high as 48%.[9] A Madrid ARF study indicated that sepsis caused acute tubular necrosis in 35% patients in ICU and 27% of non-ICU patients.[10]

Therefore, the present study was carried out to examine the oxidative stress and antioxidant status in patients of ARF with sepsis by estimating the plasma levels of lipid peroxidation products, ascorbic acid, vitamin E, total thiol groups, catalase and erythrocyte reduced glutathione. The results were compared with age and sex-matched healthy volunteers.

MATERIALS AND METHODS

The study group consisted of sixty-five patients with clinically defined ARF with sepsis admitted at Medical wards of Govt. Medical College and Hospital, Chandigarh and Medical College and SSG Hospital, Baroda. ARF in these patients was defined as plasma creatinine >3mg/dl and 24h urinary output less than 400ml. Diagnosis of sepsis was made if two or more of the following criteria were met: a) hyperthermia >38^{o}C; b) heart rate >100 beats/min; c) laboratory evidence of infection (gram stain culture); d) Leucocyte count >12×10^{6} L. Patients with pregnancy, uncontrolled haemorrhage acquired immunodeficiency syndrome, diabetes mellitus and also on non-steroidal anti inflammatory or immuno-suppressive drugs were excluded. For the present study, all the chemicals used were of analytical grade.

Five milliliters of heparinized blood were collected in a clean glass tube and centrifuged at 1000g for 10 min in a cold centrifuge. Plasma was separated. Packed erythrocytes were

washed with cold normal saline thrice and centrifuged each time. Hemolysate was prepared from washed and packed erythrocytes and centrifuged at 13000g for 1h at 4^{O}c.

The plasma was used for sodium, potassium, urea and creatinine estimation for monitoring renal function and lipid peroxidation, ascorbic acid, vitamin E, total thiol groups and catalase to assess oxidative stress. The hemolystate was used to assay reduced glutathione (GSH) content.

The electrolytes, sodium and potassium were measured using AVL 9120 ion-selective electrode analyzer. Urea and creatinine were measured by standard kits from M/s Accurex Biomedical Pvt. Ltd. The lipid peroxides (as malondialdehyde, MDA, thiobarbituric acid reactive substances, TBARS) was measured by the method of Beuge and Aust.[11] The concentration in the sample was calculated using an extinction coefficient of 1.56×10^{5} M^{-1} cm-1. The plasma ascorbate was estimated by the method of Roe and Kuether[12] using dinitrophenylhydrazine. The vitamin E levels were estimated as described by Martinek[13] using 2, 4, 6 tripyridyl–S–triazine. The plasma total thiol groups were measured according to the method of Koster *et al*[14] using DTNB [5, 5'–dithiobis (2-nitrobenzoic acid)]. Catalase was estimated according to method of Luck[15], using hydrogen peroxide. The reduced glutathione in the hemolysate was estimated by the method of Beutler *et al*[16] using DTNB. Hemoglobin (Hb) in the hemolysate was measured by Cyanmethemoglobin method.

Statistical Analysis

Results have been expressed as mean ± SD. Statistical significance was determined by student's 't' test. The 'p' value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Table 1 shows the comparison of renal function parameters between patients of ARF with sepsis and healthy volunteers. No significant difference was observed in serum electrolytes i.e. sodium and potassium between the two groups. However, plasma urea and creatinine levels were significantly higher ($p<0.001$) in patient group when compared to control group. Plasma lipid peroxidation (measured as TBARS) and catalase were significantly high ($p<0.001$ and $p<0.01$ respectively). The increase in TBARS levels was almost two fold. The patients had significantly low levels of antioxidant vitamins i.e. plasma ascorbic acid ($p<0.001$), vitamin E ($p< 0.01$), total thiol groups ($p< 0.001$) and erythrocyte reduced GSH ($p<0.001$) as compared to healthy controls group (**Table 2**). As evident an imbalance between

pro and antioxidants was observed that increased oxidative stress and depression of antioxidant status in patients of ARF with sepsis.

The results of the present study i.e. increased urea and creatinine levels in patients of ARF with sepsis as compared to the healthy control group are consistent with those reported in the literature.[8-10] These results demonstrate the impairment of renal functions in this group of patients.

Increased amounts of ROS generation have been reported in a variety of renal diseases including toxic or ischemic ARF.[4, 5] and sepsis.[6, 17] Large amounts of ROS produced under pathological conditions can challenge the defense of the body and lead to lipid peroxidation resulting in the formation of malondialdehyde (MDA). Measurement of MDA due to its easy estimation has been reported to be a good indirect method to measure the oxidative degradation of polyunsaturated fatty acids.[18] A significant increase in the plasma lipid peroxidation levels ($p<0.001$) measured as TBARS was observed in patients indicating overwhelmed production of ROS leading to tissue injury. Lipid peroxidation has been shown to increase with the severity of the disease, thereby reflecting the extent of tissue injury.[19,20]

Ascorbate is the water-soluble antioxidant vitamin, reacts rapidly with superoxide, peroxyl and hydroxyl radicals to give semidehydroascorbate.[21] Vitamin E, a lipid soluble antioxidant, is capable of donating electron to free radicals and thereby quenching it. It is capable of breaking free radical chain reaction by scavenging free radicals.[22] A significant decrease in plasma ascorbic acid ($p<0.001$) and vitamin E ($p<0.01$) was observed in ARF patients. The synergistic role of ascorbic acid and vitamin E has been reported in the past that helps in protection against peroxidation.[23] Studies have demonstrated that ascorbate can restore the antioxidant properties of oxidized vitamin E by recycling of vitamin E radical.[24,25] Thus the decrease in plasma ascorbate may be attributed to increased utilization to protect against lipid peroxidation which in turn may have led to decreased vitamin E regeneration.

Glutathione (GSH), a tripeptide, is the most prevalent intracellular thiol occurring in high concentration in virtually all mammalian cells. It is under tight homeostatic control both intracellularly and extracellularly. A dynamic balance is maintained between GSH synthesis, its recycling from GSSG / oxidized glutathione, and its utilization.[26] The importance of GSH in protecting cells against ROS has been demonstrated in several studies.[27] In the present

study, ARF patients showed depletion of erythrocyte reduced GSH levels which may be due to its increased utilization in the detoxification of ROS. Ascorbate is also known to play an important role in the restoration of GSH.[28] Further, studies have also shown that the protective effects of ascorbate and GSH against lipid peroxidation are mediated through vitamin E.[29] Thus, decreased levels of ascorbate and vitamin E as observed in the present study together with reduced GSH levels adds on to the oxidative stress in ARF patients. One of the first events that take place in human plasma upon peroxyl radical generation is the oxidation of protein sulphydryl groups.[30] Thus thiols also play an important role in chain-breaking activity. A significant decrease in plasma total thiol groups was observed. Reduced thiols have long been reported to be essential for recycling of antioxidants ascorbic acid and vitamin E.[31] The decrease in reduced GSH content owing to oxidative stress leads to a concomitant decrease in plasma total thiol groups, thereby suggesting, their role in maintenance of sulfhydral groups of protein in the reduced form.

Catalase, an enzyme present in most cells decomposes H_2O_2 to water and oxygen and it acts faster than glutathione peroxidase.[32] The activity of plasma catalase was found to be increased despite reduced GSH levels and increased oxidative stress in patients as compared to the control group may indicate the body's adaptive response to the oxidative stress. Although the source of increased plasma catalase is unclear, it may be explained on the basis of i) increased synthesis of catalase ii) release of catalase from the damaged cells iii) decreased clearance of catalase from the blood stream by the liver. Our findings are also in agreement with those reported earlier in patients of sepsis.[33, 34]

TABLE 1: Sodium, Potassium, Urea and Creatinine levels of normal healthy persons and patients of ARF with sepsis.

	Normal healthy persons (n=30) (control group)	Patients of ARF with sepsis (n=65)
Sodium (mEq/L)	139.62 ± 1.97	135.14 ± 5.7
Potassium (mEq/L	3.98 ± 0.27	4.33 ± 0.61
Urea (mg/dl)	27.55 ± 1.2	139.75 ± 10.6***
Creatinine (mg/dl)	0.86 ± 0.09	4.82 ± 0.94***

Values are Mean ± SD of number of observations (n).

***indicates $p<0.001$ when compared with normal healthy controls.

TABLE 2: Plasma thiobarbituric acid reactive substances (TBARS), ascorbic acid, vitamin E, total thiol groups, catalase and erythrocyte reduced glutathione (GSH) content of normal healthy persons and patients of ARF with sepsis.

	Normal healthy persons (n=30) (Control group)	Patients of ARF with Sepsis (n=65)
MDA (µmol TBARS/L	3.10 ± 0.60	5.94 ± 0.93***
Plasma Ascorbic acid (mg/dl)	0.90 ± 0.34	0.51 ± 0.12***
Plasma Vitamin E (mg/dl)	0.89 ± 0.11	0.74 ± 0.09**
Plasma total thiol groups (µmol/L)	342.21 ± 47	266.14 ± 28***
Plasma catalase (µmol/ml)	74.16 ± 7.67	89.05 ± 10.29**
Erythrocytes reduced GSH (µmol/g Hb)	12.58 ± 0.12	9.40 ± 0.72***

Values are Mean ± SD of number of observations (n).

**indicates p<0.01; ***indicates p<0.001 when compared with normal healthy controls.

CONCLUSION

Results of our study suggest that increased oxidative stress and depression in antioxidant status may lead to profound alterations of renal function. ARF once established may also itself contribute to accumulation of ROS and further depletion of the antioxidant defense system. Antioxidant therapy may have an important role to play in delaying the onset and/or progression of ARF.

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