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Research article

LIPID PEROXIDATION AND DNA DAMAGE IN DIFFERENT STAGES OF ACUTE RENAL FAILURE

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ABSTRACT: Introduction: Oxidative stress is an imbalance between the formation of Reactive oxygen species and protective antioxidant defense. It is known that oxidative stress increases the Acute renal failure. Measurement of oxidative stress parameters may be a simple tool for monitoring the progression of acute renal failure. **Aim:** The aim of this study was to evaluate oxidative stress in acute renal failure patients by Lipid peroxidation product Malondialdehyde (MDA), Total antioxidant capacity (TAC) and oxidative DNA damage product and compare its level among the patients of varying severity as per RIFLE classification. **Materials and methods**. We conducted a cross sectional study to compare oxidative stress parameters. Blood samples were collected from 62 patients with ARF, admitted to the Vinayaka Mission's Medical College & Hospital, Salem from March 2009 to July 2010. We further subdivided the patients according to RIFLE classification. Results: The levels of MDA, Index of oxidative status MDA/TAC and DNA damage product 8 OH deoxy guanosine were significantly higher among failure group when compared to risk and injury. Total antioxidant capacity and super oxide dismutase (SOD) were found to be decreased.

Keywords: Acute renal failure; RIFLE; oxidative DNA damage

INTRODUCTION

Acute renal failure (ARF) also known as acute kidney injury is the deterioration of renal function over a period of hours to days resulting in retention of nitrogenous and non- nitrogenous waste products that are normally excreted by the kidney (Schrier and Wang 2004). Acute renal failure is a common clinical problem with serious consequences, unsatisfactory therapeutic options, and an enormous financial burden to society. Acute renal failure is an independent risk factor for death. Interestingly, it has recently been demonstrated that even mild elevations in serum creatinine are associated with increased mortality (Chertow et al 2005). As it is often preventable, identification of patients at risk and initiation of appropriate preventive measures are crucial (Rachel Hilton 2006). The Acute Dialysis Quality Initiative (ADQI) devised the RIFLE classification which defines three grades of increasing severity of (Risk, Injury and Failure) and two outcome variables (loss and End stage renal disease) (Bellomo et al 2004). A clear feature of the RIFLE classification is the three grades of severity of renal dysfunction which are based on an individual change in serum creatinine reflecting changes in Glomerular Filtration Rate (GFR) or duration and severity of decline in urine output from the baseline.

Cells can protect themselves against oxidative damage with antioxidants, both enzymatic and non enzymatic (Baliga R *et al.,* 1999) .This balance between the formation of Reactive Oxygen Species (ROS) and protective mechanisms can be destabilized in situations of excessive production of free radicals and/or of deficient antioxidant defense, resulting in oxidative stress.

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Any imbalance in this can lead to hyperproduction of reactive oxygen species (ROS) damaging proteins, lipids, carbohydrates and nucleic acids. Oxidative stress constitutes the mechanism of production and progression of acute renal failure indirectly by promoting hypertension and atherosclerosis or directly by inducing glomerular damage and renal ischemia. Oxidative reactions that fuel various biochemical functions are coupled with the continuous generation of highly reactive and potentially cytotoxic reactive oxygen species (ROS). Under a variety of abnormal conditions, the rate of ROS production may exceed the natural antioxidant capacity leading to oxidative stress in which uncontrolled ROS can attack the functional or structural molecules, and thereby produce tissue injury and dysfunction. Acute renal failure (ARF) is associated with oxidative stress that leads to Oxidative DNA damage.

MATERIALS AND METHODS

This study includes 62 patients of ARF, admitted to the Vinayaka mission's medical college, Salem from January 2009 to July 2010. The control group consists of 50 subjects. The ARF patients were further subdivided into three groups as per RIFLE depending on serum creatinine and GFR criteria. Informed consent was obtained from all patients prior to the study. This clinical research received approval from the institutional ethical committee. We excluded cases with Diabetes mellitus, chronic renal failure, kidney transplanted patients, patients on antioxidant.

About 5ml of venous blood was drawn under aseptic precautions in a sterile tube from selected subjects and serum was separated by centrifugation and stored at -20C. Serum urea and creatinine were measured by enzymatic method in auto analyzer and eGFR was calculated by Modification of Diet in Renal Disease (MDRD) formula. Total antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) method (Benzie and strain 1996), lipid peroxidation was estimated by Satoh method. The MDA / TAC ratio was calculated as an index of oxidative status (Kocyigit et al.,2004). Oxidative DNA damage was measured by estimation of 8 OH deoxy guanosine by competitive enzyme immune assay. Super oxide dismutase was measured by Cayman's superoxide assay kit. Values were expressed as mean ± SD. Statistical significance between more than two groups were tested using one way ANOVA followed by the Bonferroni multiple comparison test.

RESULTS

Table 1: Renal parameters among the RIFLE groups of Acute renal failure

Parameters	Control	Risk(22)	Injury (26)	Failure (14)
Urea mg/dl	31.6± 6.14	84.7± 29.6	86.1± 19.6	121.5±7.42a,b **
Creatinine mg/dl	0.92 ± 0.12	1.7 ± 0.18	3.0± 0.28[a]	4.4± 0.64a,b **
GFR	95.94 ± 18.3	53.3 ± 7.0	34 ± 8.5 [a]	17.35±7.65[a,b] **
Urea/Creatinine	35.56±10.92	35.1± 13.1	27± 7.02	26.4 ± 9.03
Urine output	1377± 15.18	774.5± 200.6	761.4 ± 366.2	604.1 ± 305.4

Values are expressed as mean ± SD. **P<0.001 between the study group. a P <0.05 as compared with Risk b:p<0.05 as compared with Injury.

The changes in renal parameters in RIFLE groups were shown in Table 1. Urea and creatinine values were found to be significantly elevated with the significant reduction in GFR across the study group.

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Reduction in GFR leads to accumulation of urea and creatinine and this increase was found to be higher in failure group when compared to risk and injury ($p<0.05$ & $p<0.001$). The increase in creatinine in injury group was found to be significant when compared to Risk. A significant increase in serum creatinine and a decrease in the GFR was observed in failure group compared to risk and injury. In this study, GFR was significantly decreased when compared to control. As kidney function deteriorates GFR was found to be decreased.

Table 2: Oxidative stress parameters and DNA damage among the RIFLE groups of Acute renal failure

Parameter	CONTROL	RISK	INJURY	FAILURE
MDA(nmol/ml	1.5 ± 0.51	3.6± 0.90	4.07± 0.97	4.5 ± 1.5a*
TAC (umol/L)	822.1 ± 57.90	655.1± 161.3	582.7± 95.8	560 ± 111.4
(MDA/TAC)	0.001 ± 0.0006	0.005 ± 0.002	0.0071±0.001	0.008 ± 0.003a*
SOD (units/ml)	3.1 ± 0.6	3.09 ± 1.0	2.7 ± 0.64	2.3 ±1.0 a*
DNA DAMAGE ng/ml)	0.92 ± 0.15	2.35 ± 0.77	3.95± 0.75 a	4.23± 0.66 a*

Values are expressed as mean ± SD

MDA level was found to be increased among the RIFLE group and the level of MDA was found to be highest in failure group when compared to the risk and injury. No significant reduction in the level of Total antioxidant capacity was observed among the RIFLE groups of ARF patients. The level of oxidative stress index was found to be increased as ARF progresses from risk to failure group. Among the group OSI was found to be higher in failure group as compared with risk and injury. The level of Super oxide dismutase in Risk group was found to be near normal in comparison with control. But a significant decrease in SOD level was found in the failure group when compared to the Risk. The DNA damage in the Injury and failure group patients were significantly higher, when compared to the Risk group patients. The increase in blood level of 8 OH dG revealed that increased oxidative stress leads to increased DNA damage

Table 3: Correlation between Index of Oxidative Status and DNA damage

Parameters	r Value	P value
Index of Oxidative Status and GFR	-0.265	<0.05
Index of Oxidative Status and Creatinine	0.401	<0.05
8 OH dG and Creatinine	0.745	<0.05
8 OH dG and SOD	-0.271	<0.05

*p<0.05, between the study group. a p <0.05 as compared with Risk : b p<0.05 as compared with Injury.

Oxidative stress increases as ARF progresses from risk to failure and correlates significantly and inversely with the level of Glomerular filtration rate ($r=-0.265$, $p< 0.03$). Oxidative stress was significantly and positively correlated with creatinine ($r=0.401$, $p=<0.001$). A significant positive correlation was found between 8 OH dG and Creatinine ($r=0.745$; $p<0.05$). Additionally a negative correlation was observed between SOD ($r=-0.271$; $p<0.05$).

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DISCUSSION

Lipid peroxidation, as a free radical generating system, is closely related to tissue damage. The increase in lipid peroxidation observed in the present study is in agreement with Paller et al 1984. MDA was found to be higher in RIFLE group and being high in failure as compared with risk and injury. This suggests that lipid peroxidation was increased along with the progression of acute renal failure. Paolo Lentini et al 2010 previously demonstrated that AKI patients in failure class had markedly elevated level of Advanced Oxidation Protein Products (AOPP). Our study also showed the increase in lipid peroxidation as a consequence of the overproduction of ROS and of a rapid depletion of the endogenous stores of antioxidants. Lipid peroxidation disrupts the structural integrity of the lipid bilayer and leads to increased membrane permeability and subsequent impaired ion transport, electron transport for oxidative phosphorylation in mitochondria, and increased lysosomal permeability.

The increased lysosomal permeability leads to release of hydrolytic enzymes, further enhancing cell injury (Greene 1991). Total antioxidant capacity was found to be decreased significantly in the ARF group when compared to the control. The results of the present study demonstrated that though there was a reduction in Total antioxidant capacity among RIFLE group, the reduction was not found to be statistically significant among them. Some authors found that serum levels of antioxidants were much the same and remained stable in various stages of non-metabolic kidney disease despite a progressive increase in oxidative stress (Karamouzis et al 2008). These findings imply that it is not the amount of antioxidants, but rather the ratio of oxidative stress to antioxidant levels and the resulting antioxidant capacity that seem to be important . Index of Oxidative Status was found to be significantly higher in acute renal failure as compared with controls and found to be elevated in the subgroups of acute renal failure and found to be statistically significant when compared to the control. The cutoff value for Oxidative stress index in the control subjects in our study was considered as 0.001. All the ARF patients had >0.001 value for OSI. Though Lipid peroxidation, Total antioxidant capacity and Superoxide dismutase were measured separately, MDA/TAC ratio is a better predictor of renal damage due to oxidative stress. The increase in oxidative stress in ARF is likely to come from a combination of increased ROS production and reduced clearance as well as an ineffective antioxidant defense mechanism.

In the present study blood 8 OH deoxy Guanosine (8OHdG) in ARF patients was significantly increased compared to control. Free radicals induced oxidative DNA damage was found to be elevated even in the early stage (risk) of acute renal failure and positively associated with the progression of kidney disease, being higher in injury and failure group. The increase in blood level of 8 OH dG reveals that increased oxidative stress leads to increased DNA damage. Previous study shows a 2.5-fold higher level of 8-OHdG in the hemodialysis patients compared with the control subjects (Mastalerz Migasi et al). In the present study, the level of 8 OH dG was 3.6,4.2and 4.6 fold higher in risk, injury and failure groups respectively as compared with that in healthy subject , which may be taken as evidence of intensive oxidative stress in these patients. In the present study Increase in 8 OH dG was seen along with an increase in creatinine and MDA and decrease in TAC. This shows the casual link between the degree of renal failure, oxidative stress and oxidative DNA damage.

CONCLUSION

The results suggest that oxidative stress and oxidative DNA damage takes place even during the early phase of ARF and remains at a high level while the disease progresses. Hence assessment of the same may help in early detection of patients at risk. Further therapeutic administration of antioxidants to decrease the index of oxidative status may help to prevent the progression into injury and failure stages of ARF.

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