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Lipid Peroxidation and Total Antioxidant Capacity in Patients with Chronic Renal Failure

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Abstract: This study was carried out to assess the status and correlation between lipid peroxidation and antioxidant status in CRF patients. Forty predialytic CRF patients, of either sex and age >25 years with serum creatinine levels >3.0 mg dL^{-1} and forty healthy controls were included in the study. Serum MDA levels were estimated by colorimetric method and Total Antioxidant Capacity (TAC) was estimated by Ferric Reducing Antioxidant Power (FRAP) assay. It was observed that statistically significant increase in MDA levels in CRF patients correlating positively with creatinine and BUN and significant decrease in TAC in CRF patients correlating negatively with creatinine and BUN. Significant negative correlation was observed between MDA and TAC in CRF patients which is suggestive of increased lipid peroxidation and depletion of antioxidants causing an imbalance between OFR and TAC leading to progressive renal injury. This study would be beneficial to identify patients with increased risk of CRF and also for monitoring and optimization of antioxidant therapy.

Key words: Chronic renal failure, lipid peroxidation, malondialdehyde (MDA), ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC), oxidative stress

INTRODUCTION

Oxygen free radicals (OFR) play a significant role in the pathogenesis of many chronic diseases such as diabetes mellitus, cancer, chronic renal failure etc. (Psotova *et al*., 2001). Studies in patients with varying degrees of kidney impairment suggests that patients with chronic renal disease are in a state of oxidative stress compared with healthy controls. Chronic Renal Failure (CRF) is a pro-oxidant state and the degree of intracellular and extracellular oxidative stress is related to the severity of renal failure (Massy and Khoa, 2002). Studies have shown that OFR are involved in progressive renal injury. Attack of OFR on polyunsaturated fatty acids initiate lipid peroxidation which leads to alterations in biological membranes and causes progressive renal injury. A team of endogenous and exogenous antioxidants representing the Total Antioxidant Capacity (TAC) of extracellular fluids provides greater protection against attack by OFR (Oka *et al*., 2001). In the literature, there are limited and conflicting data on the relationship between lipid peroxidation and total antioxidant capacity in CRF patients (Mircescu *et al*., 2005). Evaluating oxidative stress in CRF patients by measuring lipid peroxidation and TAC can lead to a better understanding of free radical damage in CRF patients. The comparison of serum MDA and serum FRAP may find its use as an indicator of the progressive follow up in the CRF patients and for antioxidant therapy.

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MATERIALS AND METHODS

The study was carried out at MS Ramaiah Medical Teaching Hospital, Bangalore, in the year 2006, by taking forty patients of either sex and within the age group of 25-70 years as cases. Patients with clinically diagnosed chronic renal failure (on conservative management before dialysis) due to chronic glomerulonephritis and other glomerular diseases, chronic pyelonephritis and obstructive uropathy with serum creatinine levels more than 3.0 mg dL^{-1} were included in this study. Patients with CRF due to diabetes mellitus, essential hypertension, liver diseases, coronary artery disease, vasculitis and other autoimmune disorders were excluded. The clinical history and other necessary details were obtained from their case sheets. The study was conducted after informed consent was obtained from them and approved by the ethical committee of the institution. Control group consisted of forty healthy males and females with age group of 25-70 years.

Under aseptic precautions 5 mL of fasting venous blood samples were collected. Clotted blood was subjected to centrifugation. The clear serum was separated and used for the following biochemical investigations: Malondialdehyde, Ferric Reducing Antioxidant Power (FRAP) assay, Serum creatinine and Blood Urea Nitrogen (BUN). All the chemicals used were of highest analytical grade available in India.

Lipid peroxidation was measured by serum MDA estimation according to the colorimetric method of Satoh (1978). Lipoproteins are precipitated from the specimen by adding TCA. 0.05 M sulphuric acid and 0.67% TBA in 2 M sodium sulphate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling water bath for 30 min. The resulting chromogen is extracted in n-butanol, which is measured colorimetrically at 530 nm.

Total antioxidant capacity was measured by Ferric Reducing Antioxidant Power (FRAP) assay according to the method of Iris Benzie and Strain (1996). At low pH, when a ferric tripyridyltriazine (Fe^{III}-TPTZ) complex is reduced to the ferrous (Fe^{II}) form, an intense blue color with absorption maximum at 593 nm develops. FRAP directly analyzes total low molecular weight antioxidants.

Serum creatinine and BUN were estimated by standard clinical chemistry methods (Newman *et al*., 1999).

RESULTS AND DISCUSSION

Statistically significant increase in MDA levels were found in CRF patients as compared to controls ($p<0.001$) and statistically significant decrease in TAC in CRF patients as compared to controls ($p<0.001$). MDA showed significant positive correlation with serum creatinine ($r = 0.794$) ($p<0.01$) and BUN ($r = 0.492$) ($p<0.01$). TAC (FRAP assay) showed significant negative correlation with serum creatinine, $r = -0.76$, ($p<0.01$) and BUN, $r = -0.398$, ($p<0.05$). Significantly negative correlation was observed between MDA and TAC, $r = -0.752$, ($p<0.01$) in CRF patients (Table 1, 2, Fig. 1).

Table 1: Comparison of the parameters in controls and CRF patients

Parameters	Controls (n = 40) Mean±SD	Study group (n = 40) Mean±SD	Comparison
MDA (nmol mL^{-1})	0.965±0.208	3.254±0.827	p<0.001 highly significant
FRAP (μmol L^{-1})	1069±143.88	626±186.20	p<0.001 highly significant
Serum creatinine (mg dL^{-1})	0.795±0.181	7.1±3.8	p<0.001 highly significant
BUN (mg dL^{-1})	11.075±3.38	69.987±44.77	p<0.001 highly significant
Serum Na^+ (mEq L^{-1})	140±4.624	136.07±10.45	p 0.033 significant
Serum K^+ (mEq L^{-1})	4.065±0.564	5.070±1.095	p<0.001 highly significant

Table 2: Correlation between serum creatinine, serum MDA and serum FRAP in CRF patients

Correlation between parameters	CRF patients		r-value	p-value	Level of significance
Serum MDA and serum creatinine	Serum MDA 3.254±0.827	Serum creatinine 7.1±3.81	0.794	<0.01	Highly significant
Serum FRAP and serum creatinine	Serum FRAP 626±186.20	Serum creatinine 7.1±3.81	-0.760	<0.01	Highly significant
Serum MDA and serum FRAP	Serum MDA 3.254±0.827	Serum FRAP 626±186.2	-0.752	<0.01	Highly significant

Serum MDA-nmol mL^{-1}, Serum creatinine-mg dL^{-1}, Serum FRAP-µmol L^{-1}

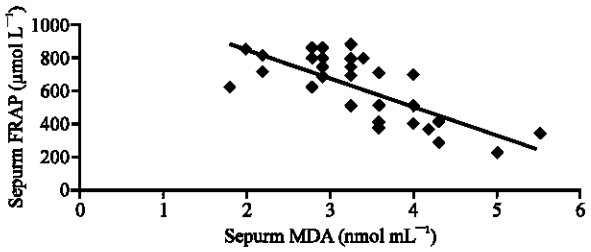

Fig. 1: Correlation between MDA and FRAP values in CRF patients

Oxidative stress is defined as an imbalance between formation of reactive oxygen species and antioxidative defence mechanisms. ROS can damage proteins, lipids, carbohydrates and nucleic acids. Plasma membranes are critical targets of free radical reactions. ROS can easily produce injuries to cell membranes by initiation of polyunsaturated fatty acid peroxidation, inactivation of membrane enzymes and receptors and protein crosslinking and fragmentation (Oka *et al*., 2001; McCall and Frei, 1999). Thus responsible for pathogenesis of a variety of diseases like diabetes mellitus, cancer or chronic renal failure (Massy and Khoa, 2002; Halliwell, 1991).

There are conflicting results regarding the status of antioxidant systems in patients with CRF. Also, there is a wide variation in the published levels of malondialdehyde (MDA) in CRF patients (Annuk *et al*., 2001; Luciak and Trznadel, 1991; Bolton *et al*., 2001). Several studies suggest that concentration of MDA, a byproduct of lipid peroxidation is significantly increased in CRF patients before initiation of dialysis and renal replacement therapy when compared with the control group (Fiorillo *et al*., 1998; Mircescu *et al*., 2005; Leonardo *et al*., 2005). In the present study, there was a significant increase in MDA levels in CRF patients. MDA concentrations significantly increase with the severity of kidney dysfunction. This suggests increased lipid peroxidation in these patients (Leonardo *et al*., 2005; Martin-Mateo *et al*., 1999). Total Antioxidant Capacity (TAC) parameter summarizes the overall activity of antioxidants and antioxidant enzymes (Psotova *et al*., 2001; Haugen and Nath, 1999). It should be noted that co-operation between different antioxidant pathways provides greater protection against attack by Reactive Oxygen Species (ROS) or nitrogen radicals, compared to any single compound. Thus, the overall antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual biomarkers (Malliaraki *et al*., 2003). The FRAP assay is presented as a novel method for assessing antioxidant power/TAC (Iris Benzie and Strain, 1996). This parameter is able to give information regarding the total charge of antioxidants present in the serum. The index thus obtained is considered as a measure of the system's ability to regulate the damage due to the ROS production (Psotova *et al*., 2001; Iris Benzie and Strain, 1996). Studies of TAC in patients with chronic renal failure have shown varying results (McCall and Frei, 1999; Gazdikova *et al*., 2000). Increased levels of TAC indicate absorption of stock organ antioxidants eg. uric acid and the induction or activation of antioxidant enzymes as an

adaptation to the oxidative stress, but at a later phase of oxidative stress, the TAC falls due to depletion of antioxidants (Psotova *et al*., 2001). In addition, high concentration of a number of metabolites, including uric acid can lead to pro-oxidant effects, introducing a further decrease of the antioxidant capacity. This could be the consequence of increased exposure to the oxidative attacks or lipid peroxidation and its over consumption as an antioxidant (Malliaraki *et al*., 2003). A number of enzymes and enzymatic pathways contribute to the detoxification of free radicals and a decreased efficiency of these pathways including NADPH, NADH and GSH can also readily explain for the reduced activity of antioxidant systems. Imbalances in the intracellular redox systems may impair detoxification mechanisms (Pias and Aw, 2002). The results of this study reflect the significant increase in serum MDA levels and depletion of antioxidative defence mechanisms with progressive renal injury as evidenced by significant decrease in FRAP levels in CRF patients. The significant negative correlation between MDA and FRAP levels in CRF patients supports the mechanism of oxidative stress caused by an imbalance between formation of ROS and attenuation of antioxidative defence mechanisms.

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