Biochemical Markers of Oxidative Stress in Predialytic Chronic Renal Failure Patients

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Background: The present study was designed to test the hypothesis that elevated reactive oxygen species activity and decreased antioxidant activity may contribute to the pathogenesis of chronic renal failure (CRF) which may be aggravated by immunosuppression.

Methods: Thirty healthy controls of either sex in the age group of 25–70 years and 30 age- and sex-matched CRF patients with serum creatinine levels > 3.0 mg/dL were included in the study. Serum malondialdehyde (MDA), adenosine deaminase (ADA), ferric reducing antioxidant power (FRAP), blood urea nitrogen, and creatinine were estimated.

Results: There were statistically significant decreases in predialysis serum ADA and FRAP, and a statistically significant increase in MDA levels in CRF patients compared to controls. MDA showed significant positive correlation with serum creatinine ($r = 0.79, p < 0.01$). FRAP showed nonsignificant negative correlation with serum creatinine ($r = -0.02, p > 0.05$). Significant negative correlation was observed between MDA and FRAP ($r = -0.55, p < 0.01$) in CRF patients. Nonsignificant negative correlation was observed between ADA and MDA ($r = -0.0087, p > 0.05$), and nonsignificant positive correlation was observed between ADA and FRAP ($r = 0.315, p > 0.05$) in CRF patients.

Conclusion: These results are suggestive of oxidative stress leading to progressive renal injury along with immunosuppression. FRAP can be a useful indicator to monitor and optimize antioxidant therapy, which may potentially become an important adjunct in the management of CRF patients. [Hong Kong J Nephrol 2008;10(2):69–73]

Key words: adenosine deaminase, chronic renal failure, lipid peroxidation, malondialdehyde, total antioxidant capacity

背景：慢性腎衰竭（CRF）的形成涉及多方面的因素，例如免疫抑制會促使病情的加重。本研究旨在探讨，活性氧上升及抗氧化活动下降是否與CRF的形成有關。

方法：研究對象為30位CRF患者，年齡25–70歲，血清肌酸酐水平>3.0 mg/dL；對照組為30位年齡及性別與CRF組相符的健康人士。偵測項目包括血清丙二醛（MDA）、腺苷脫氨酶（ADA）、鐵離子還原抗氧化力（FRAP）、血尿素氮及肌酸酐。

結果：相較於對照組，CRF患者之透析前血清ADA及FRAP顯著較低，MDA水平則顯著較高。MDA與血清肌酸酐呈現明顯的正相關性（$r = 0.79, p < 0.01$）；FRAP則與血清肌酸酐呈現負相關性的傾向（$r = -0.02, p > 0.05$）。在CRF患者之間，MDA與FRAP呈現顯著的負相關性（$r = -0.55, p < 0.01$）；ADA與MDA呈現負相關性的傾向（$r = -0.0087, p > 0.05$）；ADA與FRAP則呈現正相關性的傾向（$r = 0.315, p > 0.05$）。

結論：本研究的結果顯示，氧化應激及免疫抑制可能與腎臟損傷的惡化有關；此外，抗氧化療法可能對CRF患者有所裨益，其效用可採用FRAP作為監測指標。
INTRODUCTION

Adenosine deaminase (ADA), found in large amounts particularly in mononuclear cells, is a marker of T-cell activation and is related to the production of reactive oxygen species (ROS) by neutrophils. A normal ADA activity level prevents adenosine accumulation and thus ensures normal lymphocyte development and function. Chronic renal failure (CRF) may lead to a defect in the cellular arm of the immune response and depress ADA activity. Cell death caused by the accumulation of extracellular adenosine is believed to contribute to the profound loss of T lymphocytes in patients with ADA deficiency. Adenosine-induced apoptosis is associated with the generation of ROS and a reduction in mitochondrial transmembrane potential. Decreased ADA and increased malondialdehyde (MDA) production due to increased neutrophil-derived ROS production might suggest the presence of an interrelationship between T cells and neutrophils in such patients [1,2]. Free radical-induced lipid peroxidative tissue damage has been implicated in the pathogenesis of CRF. To circumvent the damage caused by free radicals, a variety of enzymatic and nonenzymatic antioxidants are present in human serum. Oxidative stress results from the imbalance between oxidative and antioxidative mechanisms with increased levels of pro-oxidants and depletion of antioxidants leading to tissue damage [3].

No single component of serum antioxidant complex could fully reflect the protective efficiency of blood, probably because of interactions that occur in vivo among different antioxidant compounds. Total antioxidant capacity considers the cumulative effect of all antioxidants present in blood and body fluids [4]. There are conflicting and inconsistent data reported on ADA values and oxidative stress in CRF patients [5,6].

The present study was designed to test the hypothesis that elevated ROS activity and decreased antioxidant activity may contribute to the pathogenesis of CRF which may be aggravated by immunosuppression. Serum creatinine, blood urea nitrogen (BUN) and ADA were measured in healthy controls and cases and correlated with serum MDA and ferric reducing antioxidant power (FRAP, which is a measure of total antioxidant capacity). MDA is an indicator of lipid peroxidation while ADA is an indicator of immunity. Total antioxidant capacity may be useful as an indicator of antioxidant defence and in monitoring antioxidant therapy.

METHODS

This study was conducted at M.S. Ramaiah Medical Teaching Hospital in Bangalore and included 30 healthy controls of either sex aged 25–70 years and 30 age- and sex-matched patients who were clinically diagnosed with CRF (on conservative management before dialysis) due to chronic glomerulonephritis or other glomerular diseases, chronic pyelonephritis or obstructive uropathy with serum creatinine > 3.0 mg/dL. Patients with CRF due to diabetes mellitus, liver disease, coronary artery disease, vasculitis, lupus glomerulonephritis or other autoimmune disorders were excluded. This study was approved by the institution’s ethics committee, and informed consent was obtained from every subject.

Under aseptic conditions, 5-mL samples of fasting blood were collected in plain vacutainers. Clotted blood was centrifuged. The clear serum was separated and used for the measurement of creatinine, BUN, ADA, MDA and FRAP levels. All the chemicals used were of the highest analytical grade available in India.

Serum creatinine and BUN were estimated by standard clinical chemistry methods [7]. Total antioxidant capacity was measured by FRAP assay according to the method of Benzie and Strain [8]. At low pH, when ferric tripyridyltriazine (Fe III-TPTZ) complex is reduced to the ferrous (Fe II) form, an intense blue color with an absorption maximum at 593 nm develops.

Lipid peroxidation was measured by serum MDA estimation according to the colorimetric method of Satoh [9]. Lipoproteins are precipitated from the specimen by adding trichloroacetic acid. Then, 0.05 M sulfuric acid and 0.67% thiobarbituric acid (TBA) in 2 M sodium sulfate 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 minutes. The resulting chromogen is extracted in n-butanol, which is measured colorimetrically at 530 nm.

ADA activity was determined by the colorimetric method [10,11] based on the hydrolytic activity of ADA on adenosine, the ammonia formed being measured by the Berthelot reaction. Adenosine 0.675 mM was used as a substrate in 0.2 M phosphate buffer at a pH of 7.05. After incubation at 37°C for 1 hour, the ammonia liberated was estimated by measuring the blue color produced with the phenol–hypochlorite reagent at 640 nm.

Statistical analysis was performed with Student’s t test and Pearson’s correlation coefficient using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). A value of p < 0.05 was taken to indicate statistical significance.

RESULTS

The control group consisted of 16 males and 14 females, and the CRF group also had 16 males and 14 females. Distribution by age were: 8 controls and 8 cases aged 25–35 years; 6 controls and 6 cases aged 35–45 years; 10 controls and 10 cases aged 45–55 years; and 6 controls and 6 cases aged ≥55 years.
Significantly decreased FRAP and ADA levels, and significantly increased MDA level were found in the CRF group compared to the control group (all $p < 0.01$; Table 1). There was a significant positive correlation between MDA and FRAP in the control subjects ($r = 0.52$, $p < 0.01$; Figure 1). In CRF patients, there was a significant positive correlation between creatinine and MDA ($r = 0.79$, $p < 0.01$), and significant negative correlations between BUN and FRAP ($r = -0.39$, $p < 0.01$) and between MDA and FRAP ($r = -0.55$, $p < 0.01$) (Table 2; Figure 2). There were nonsignificant negative correlations between creatinine and FRAP ($r = -0.02$, $p > 0.05$), between ADA and creatinine ($r = -0.11$, $p > 0.05$), and between ADA and MDA ($r = -0.0087$, $p > 0.05$) (Table 2). There were nonsignificant positive correlations between BUN and MDA ($r = 0.00059$, $p > 0.05$) and between ADA and FRAP ($r = 0.315$, $p > 0.05$) in CRF patients (Table 2).

### Discussion

ADA, an enzyme of purine catabolism, catalyzes the conversion of adenosine to inosine and deoxyadenosine to deoxyinosine and helps in the maturation and function of T lymphocytes via noncovalent binding to the T-cell antigen CD26. CRF may lead to a defect in the cellular arm of the immune response. Some studies have demonstrated low predialytic ADA levels in CRF patients while some suggest normal ADA activity levels. Uremic toxins depress ADA activity which results from the deactivation of lymphocytes. Low ADA activity will lead to increased adenosine (a local hormone, produced by mesangial cells) levels, decreasing glomerular filtration rate, exerting immunosuppressive, antiproliferative and anti-inflammatory properties [1]. Depressed ADA activity with increased MDA production suggests increased neutrophil-derived ROS production. ROS cause tissue damage by denaturing or modifying lipids, proteins, carbohydrates, DNA and other molecules. Under normal conditions, ROS produced in the course of metabolism are contained by the body’s natural antioxidant system that consists of a series of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase as well as numerous endogenous and dietary antioxidant compounds that are capable of reacting with and inactivating ROS, thereby protecting functional and structural molecules against ROS-mediated tissue damage [2–4]. Evidence of renal damage mediated by oxidative stress causing glomerular, tubulointerstitial and endothelial alterations have been reported, suggesting that CRF is a pro-oxidant state and that the degree of intracellular and extracellular oxidative stress

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<th>Table 1. Comparison of measured parameters in healthy controls and chronic renal failure (CRF) patients</th>
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<td>MDA (nmol/mL)</td>
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MDA = malondialdehyde; FRAP = ferric reducing antioxidant power; ADA = adenosine deaminase; Cr = creatinine; BUN = blood urea nitrogen.

<table>
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<th>Table 2. Correlation between measured parameters in chronic renal failure patients</th>
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Cr = creatinine; MDA = malondialdehyde; FRAP = ferric reducing antioxidant power; ADA = adenosine deaminase; BUN = blood urea nitrogen.
is related to the severity of renal failure [5,6]. MDA is a three carbon, low molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids of biological membranes. The determination of MDA is used for monitoring lipid peroxidation in biological samples [12,13]. FRAP assay is presented as a novel method of assessing total antioxidant capacity and is considered to be a useful indicator of the system’s ability to regulate damage due to ROS [14]. Studies of total antioxidant capacity in CRF patients have shown varying results [15]. Several studies suggest that there is enhancement of lipid peroxidation and decrease of antioxidant defense in the course of CRF progression, which could promote oxidative damage in the kidneys even before the initiation of dialysis and renal replacement therapy [16–18]. The precise mechanism(s) of CRF-induced oxidative stress has not been elucidated. Upregulation of NADPH oxidase abundance or activity and imbalances in the intracellular redox systems impairing detoxification mechanisms can lead to oxidative stress [19].

In the present study, the significantly decreased predialysis serum ADA concentration in CRF patients compared to controls suggests depressed cellular immunity in CRF. The significantly elevated predialysis serum MDA concentration in CRF patients compared to controls reflects the increased formation of ROS and lipid peroxidation with progressive renal failure. The significantly lower serum FRAP concentration in CRF patients compared to controls reflects a lower total antioxidant capacity. The positive correlation between ADA and FRAP, and the negative correlations between ADA and creatinine and between ADA and MDA suggest a degree of immunosuppression which may also play a role in oxidative stress in CRF.

From these results, increased oxidative stress in CRF patients along with immunosuppression can most likely be concluded. These markers can be useful to monitor and optimize antioxidant therapy which may be an important adjunct in the management of patients with CRF.

REFERENCES

Biochemical markers of oxidative stress in predialytic CRF