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ORIGINAL ARTICLE

Oxidative Stress And Antioxidants In CRF Patients Before And After Dialysis

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ABSTRACT

Chronic Renal Failure (CRF) is a debilitating condition which is responsible for high morbidity and mortality. Tissue injury due to free radicals is commonly seen in a variety of disease processes. The aim of the present study was to investigate the possible free radical mediated tissue damage in CRF before and after dialysis, by measuring Melondialdehyde (MDA) which is marker of oxidative stress. Antioxidants like Vitamin A, Vitamin E and Vitamin C, which prevent oxidative damage, were also measured. The study included 30 healthy persons as controls and 30 patients of CRF (before dialysis and after dialysis) as the study cases. All parameters were assessed by spectrophotometric methods. **Results:** MDA levels were found to be significantly increased in pre dialysis CRF patients as compared to the controls (P< 0.001) and the levels further increased in post dialysis CRF patients as compared to that in pre dialysis CRF patients (P <0.001). The levels of antioxidants like Vitamin A, Vitamin E and Vitamin C were significantly decreased in pre dialysis patients as compared to the controls (P=<0.001) and the values further significantly decreased in post dialysis patients as compared to the pre dialysis CRF patients(P<0.001). **Conclusion:** This study indicates that there is considerable oxidative stress in patients with CRF, which is further exacerbated by hemodialysis, as evidenced by increased lipid peroxidation and low antioxidant levels.

Key Words: Antioxidants, Chronic renal failure, Dialysis, Melondialdehyde,

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Introduction

Chronic renal failure is a debilitating condition which is responsible for high morbidity and mortality and it is a financial burden on both the government and the society. True data on the incidence and the prevalence of End Stage Renal Disease

(ESRD) in India is lacking, because no National Registries exist here. A high incidence of ESRD has also been noted in Asians of Indian origin in the United Kingdom. If the incidence of ESRD is indeed 100 patients/ million population/ year, this would mean ≈ 100,000 new patients every year for a population of 1 billion in India [1].

The present study is focused on the role of free radical mediated tissue damage in chronic renal failure (CRF) and dialysis. The sources of free radicals (FR) are activated macrophages, vascular cells and various glomerular cells, including fibroblasts and renal interstitial cells. Different cellular enzymes including mitochondrial oxidases, lipooxygenases, myeloperoxidase, NADPH oxidase, xanthine oxidase and nitric oxide synthase have been identified as the cellular sources for the formation of reactive oxygen species (ROS) [2].

Generation of oxidative free radicals (OFR) is increased in many pathological conditions. The cellular constituents and biomolecules are subject to free radical attack. Polyunsaturated fatty acids (PUFAs) present in cell membranes are readily attacked by oxygen free radicals. The oxidative destruction of polyunsaturated fatty acids is known as lipid peroxidation. It is a destructive chain-reaction that can damage other cellular components by the production of reactive aldehydes [3]. Lipid peroxidation has been implicated in a wide range of tissue injuries and diseases [4]. Proteins are subject to free radical mediated destruction which may lead to structural loss of the enzymes or enzymatic deactivation. Nucleic acids are prone to base hydroxylation, cross linking or stand scission, which may result in mutation or even cell death [5]. Extracellular tissue components including hyaluronic acid and collagen are vulnerable to injury by toxic free radicals (FRs), thus resulting in damage to the architectural integrity of the tissues [6].

The present study was planned to investigate the possible FR mediated injury in CRF patients who were undergoing haemodialysis by measuring the oxidative stress. Oxidative stress is assessed by measuring malondialdehyde (MDA), which is a marker and a product of lipid peroxidation. The study also included the assessment of the non-enzymatic antioxidants Vitamin E, Vitamin A and Vitamin C in CRF patients before and after dialysis, which prevent oxidative damage and scavange or neutralize free radicals which are produced in disease processes.

Materials

The sample size included 60 subjects, that is, 30 normal healthy controls and 30 CRF patients who were attending the dialysis unit over a period of one year. These 30 CRF patients had a urine output of less than 600ml per day. The average serum creatinine level was 8.2±3.1 mg/dl and the average blood urea level was 159±40 mg/dl. The causes for CRF in these patients were Hypertensive Nephropathy, Obstructive Uropathy, Acute Gastroenteritis, Acute Pyelonephritis and Diabetic Nephropathy. Most of these patients had Microcytic Hypochromic Anaemia. Ultrasonography findings showed grade II-III nephropathic changes.

Patients with reversible renal involvement, advanced form of major extra renal complications like cerebro vascular accidents, coronary artery disease, neoplasia and active infection elsewhere in the body were excluded from the study. The study was approved by the Medical College ethical and research committee

Methods

6 ml of blood was drawn by venipuncture from the controls and the CRF patients who were selected for dialysis (before and immediately after dialysis) and was

collected in a heparinized tube (5 units/ml of blood) by using aseptic precautionary measures.

1ml of blood was processed for the estimation of Malondialdehyde by the Thiobarbituric acid method [7] and the rest of the sample was centrifuged and plasma was separated for
the analysis of antioxidants like Vitamin E (α-tocopherol) by the Quaife et al. method [8],Vitamin A (Retinol) by the Bessay et al method (9) and Vitamin C (Ascorbic acid) by the Evelyn and Malloy method [10].

Estimation of Malondialdehyde (MDA) [7]

This reaction depends on the formation of a pink coloured complex between malondialdehyde and thiobarbituric acid (TBA), having a maximum absorption at 532 nm.

Estimations of Plasmaα-Tocopherol[8]

This method is based on the Emmerie Engel reaction. The Xylene extract of plasma containing α-tocopherol reacts with ferric chloride, thus reducing the ferric ions to ferrous ions. The ferrous ions then react withα, α' – dipyridyl to give a red coloured complex which is measured at 520 nm. Carotenoids, which were also extracted into xylene, were estimated by their absorbance at 460 nm and a correction was applied at 520 nm. The carotenoid absorption at 520 nm was 29% of the absorption at 460 nm.

Retinol [9]

Proteins get precipitated on the addition of ethanol and the concentration of Retinol can be determined by reading the extinction value of the heptane extract of retinol at 327nm.

Ascorbic Acid [10]

When ascorbic acid reacts with 2, 6-dichlorophenol indophenol, reduced 2, 6-dichlorophenol indophenol is formed which is colourless. The decrease in colour is proportional to the concentration of ascorbic acid which is present in the solution. Decrease in the optical density was measured at 520nm and the concentration was calculated from standards which were treated similarly.

Statistical analysis of all the obtained parameters in patients with chronic renal failure (before and after dialysis) and the control groups were done using paired and unpaired student's 't' test. Also, Logistic regression analysis was performed for all the parameters.

Results

[Table/Fig 1] shows the mean and standard deviation of oxidant and anti-oxidant levels in controls and in CRF patients, before and after dialysis. The levels of MDA were significantly increased in pre-dialysis CRF patients as compared to the controls ($p<0.001$) and the levels were further significantly increased in post-dialysis patients as compared to the pre-dialysis patients ($p<0.001$). The levels of Vitamin E, Vitamin A and Vitamin C showed significant decrease in pre-dialysis CRF patients as compared to healthy controls ($p<0.001$) and further significant decrease was seen in post-dialysis CRF patients as compared to the pre-dialysis patients ($p<0.001$).

(Table/Fig 1) MDA & Antioxidant levels in controls and CRF Patients before and after dialysis

	MDA(nm/ml)	Vitamin E (mg%)	Vitamin A (µg%)	Vitamin C (mg%)	p-value
Control (n=30)	5.94 ±1.01	0.91 ± 0.08	32.23 ± 4.86	0.87±0.12	–
Pre-dialysis (n=30)	10.30±0.99	0.68 ± 0.10	22.43 ± 2.08	0.57 ± 0.09	P<0.001
Post-dialysis (n=30)	13.04±1.08	0.47 ± 0.07	18.96 ± 2.23	0.36 ± 0.09	P<0.001

The following variables (MDA, Vitamin E, Vitamin A and Vitamin C) were also considered for multiple regression analysis as shown in [Table/Fig 2] , [Table/Fig 3]. The model used for regression analysis showed that the R square was 0.75. By this, we can explain that 75% of the changes in the dependent variable (MDA) can be attributed to the variables which were considered for the regression (Vitamin E, Vitamin A and Vitamin C).

(Table/Fig 2) Logistic regression analysis for controls and CRF (Pre-dialysis).

Parameters	B	SE	Df (Degree of freedom)	Significance
MDA	9.67	4115.2	1	0.998
Vitamin E	-57.79	56938.89	1	0.99
Vitamin A	-1.86	1494.09	1	0.99
Vitamin C	-27.50	42749.7	1	1.00

(Table/Fig 3) Logistic regression analysis for Pre-dialysis and Post-dialysis CRF patients.

Parameters	B	SE	Df (Degree of freedom)	Significance
MDA	-31.86	3043.25	1	0.992
Vitamin E	-251.87	33985.90	1	0.994
Vitamin A	-10.79	1164.10	1	0.993
Vitamin C	-1.59	698.42	1	0.998

Discussion

CRF leads to imbalance between the production of oxygen free radicals and cellular antioxidants system. The Hexose Mono Phosphate (HMP) shunt pathway which is required for NADPH synthesis in RBC is impaired in CRF, thus resulting in the accumulation of free radicals. This enhances the process of oxidative stress and plays a role in the development of the disease, thus leading to an increase in MDA levels [11], [12],[13]. Further increase in the levels of MDA in post-dialysis CRF patients may be due to the exacerbation of the lipid peroxidation process by haemodialysis. Haemodialysis leads to the activation of complement system, which in turn stimulates free radical generation, mainly superoxides through the NADPH system. Heparin which is used in haemodialysis causes lipolysis, thus increasing free fatty acid in plasma, which leads to the exacerbation of lipid peroxidation. The red cell MDA increases the rigidity of the RBCs and makes them more susceptible to lysis during haemodialysis [14], [15].

Antioxidants

In the present study, plasma concentrations of Vitamin E, Vitamin A and Vitamin C were significantly decreased. The levels were further significantly decreased in post-dialysis CRF patients as compared to pre-dialysis CRF patients.

Plasma α-tocopherol (Vitamin E)

Decreased levels of α-tocopherol in predialysis CRF patients as compared to healthy controls may be due to enhanced lipid peroxidation. There may also be impaired absorption of dietary α-tocopherol due to altered lipid metabolism. After dialysis, further decreased levels may be due to increased consumption in an attempt to reduce the effect of the oxygen free radicals [16], [17], [18].

Plasma Vitamin A

Decreased levels of Vitamin A in predialysis CRF patients as compared to healthy controls, was due to enhanced lipid peroxidation, thus leading to the exhaustion of hepatic storage. So, there was reduced conversion of β-carotene to vitamin A and decreased secretion of vitamin A from the liver into the circulation. In post-dialysis CRF patients, the enhanced production of oxygen free radical leads to the increased utilization of vitamin A and its microsomal degradation, thus resulting in its reduced levels [14].

Plasma Vitamin C

Decreased levels of Vitamin C in pre-dialysis CRF patients as compared to healthy controls, may be due to the utilization of ascorbic acid to generate α-tocopherol from the α-tocopheroxyl radical at the water lipid interface. The ascorbate may be reduced because it is an efficient quencher of superoxide peroxyl and hydroxyl radicals. In addition to the above, there may be associated nutritional deficiency of ascorbate to limit potassium intake in CRF patients. During haemodialysis, there may be additional loss of ascorbic acid from plasma, as it is a water soluble vitamin [14],[16],[17].

Conclusion

The result of the present study indicates that there is considerable oxidative stress in patients with CRF, which is further exacerbated by haemodialysis, as evidenced by increased lipid peroxidation and low antioxidant levels. Increased levels of Malondialdehyde (MDA) which is a reliable marker and a product of lipid peroxidation in CRF and dialysis patients, indicates the existence of oxidative stress.

The decreased levels of non-enzymatic antioxidants like Vitamin E, Vitamin A and Vitamin C indicate the increase in oxidative stress. Inflammatory processes during dialysis appear to be the main factors which are involved in oxidative stress. Exogenous supplementation of non-enzymatic antioxidants may decrease the damage to renal tissue by quenching and preventing the free radical action which are responsible for the disease process.

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