Research Article

To evaluate the hemoglobin concentration, lipid peroxidation and antioxidant status in patients with chronic kidney disease

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ABSTRACT

Background: Oxidative stress plays an important role in the progression of CKD as well as in many of the complications associated with the disease. ROS promotes inflammation, accelerated ageing, fibrosis and apoptosis leading to progression of CKD. Therefore, the present study was carried out to evaluate the serum markers for early diagnosis of patients at different stages of chronic kidney disease.

Methods: The study group consisted of 50 patients with chronic kidney disease who were on conservative treatment with the age group of 20 to 60 years. Based on the creatinine clearance values the patients were assigned in to 3 groups; Stage 3, Stage- 4 and Stage- 5 as per NKF DOQI guidelines. Control group consisted of 50 age and sex matched, non-diabetic, nonsmoker healthy volunteers. About 5 ml of blood was collected and serum was used for the estimation of superoxide dismutase, ceruloplasmin and malondialdehyde and haemoglobin level using standard methods. The data was analyzed by applying student’s t test. The p value of ≤0.05 was taken as the level of significance.

Results: The haemoglobin concentration in all the 3 CKD stages was found to be significantly decreased (p<0.000) whereas, the serum malondialdehyde (MDA) was significantly increased (p=0.000) in patients with CKD. But serum SOD and ceruloplasmin levels of normal and patients with CKD showed significant decline (p<0.000) only in stage 5 whereas, in stage-2 and stage-3 patients it did not show significant variation.

Conclusions: The results of the study reinforce the possibility that antioxidant supplementation may be helpful in correcting anaemia in chronic kidney disease. Treatment of renal anaemia is an effective intervention to ensure better quality of life, to prevent adverse cardiovascular outcomes and to retard the progression of chronic kidney disease so as to reduce the burden of end stage renal disease in the long run.

Keywords: Superoxide dismutase, Ceruloplasmin, Malondialdehyde, Haemoglobin, Chronic kidney disease

INTRODUCTION

Chronic kidney disease is a rapidly growing public health problem worldwide, frequently leading to end stage renal disease (ESRD). It is estimated that approximately 1, 00,000 new cases of ESRD develop annually in India.1 The consequences of delayed referral to the nephrologists are far reaching from both clinical and economic perspectives and include metabolic abnormalities, prolonged hospitalizations, increased cost of renal replacement therapy and decreased rehabilitative potential.2

Under normal conditions, ROS are generated as by-products of aerobic metabolism due to partial reduction of oxygen by a variety of enzymes including oxidases (e.g. NADPH oxidase, myeloperoxidase), cyclooxygenase and lipoxygenase. Most important ROS generated are
superoxide anion, hydroxyl radical and hydrogen peroxide. ROS play an important role in numerous biological functions like cell growth, apoptosis, and hormone receptor interaction. They act as signal molecules by activating ion channels (Ca^{2+} and K') and bringing about changes in intracellular pH. Moreover, generation of ROS by activated leucocytes and macrophages play a critical part in the host defence against invading pathogens.

Normally exogenous and endogenous antioxidant systems perform the function of neutralization of the ROS thus limiting their activity. The major endogenous antioxidants can be enzymatic antioxidants which include superoxide dismutase, catalase, glutathione peroxidase and ceruloplasmin or non-enzymatic antioxidants like glutathione, vitamin E and vitamin C.

A delicate balance normally exists between the pro-oxidants that generate ROS and the antioxidant defence mechanisms. The excess production of ROS or impaired antioxidant activity leads to oxidative stress. Here the ROS activity overwhelms the free radical scavenging activity of antioxidants leading to oxidation of macromolecules, and hence tissue damage and dysfunction.

Because of their extreme instability it is difficult to detect ROS directly in vivo. Instead the oxidative stress in humans and animals is assessed by measuring the stable byproducts formed as a result of interaction of ROS with bio molecules such as lipids, carbohydrates, proteins, nucleic acids and nitric oxide.

Oxidative injury to lipids leads to peroxidation of polyunsaturated fatty acids (PUFA), which are the major constituents of cellular and subcellular membranes. Free radical attack of PUFA results in continuous production of hydroperoxides along with consumption of equimolar quantities of PUFA. Peroxidation of membrane lipids alters membrane fluidity, its permeability to ions and solute transport.

Lipid peroxidation is a chain reaction as it leads to generation of more hydroperoxides and other free radicals which initiate further peroxidation of other PUFA. Subsequent degradation of oxidized lipid molecules leads to formation of several specific metabolites such as malondialdehyde (MDA). MDA is one of the most frequently used biomarkers that provide an indication of the overall lipid peroxidation level and oxidative stress.

Several studies suggest that antioxidant activity is depressed in CKD. Superoxide dismutase is an antioxidant enzyme that scavenges superoxide anion, one of the most potent ROS. Serum levels of superoxide dismutase were found to be decreased in studies by Nitin et al and Sasikala et al with progression of renal disease. Vaziri et al have shown a downregulation of SOD and upregulation of NADPH oxidase (a major source of ROS) in kidney and liver of CRF rats.

Oxidative stress plays an important role in the progression of CKD as well as in many of the complications associated with the disease. ROS causes hypertension in CKD by oxidation of nitric oxide and arachidonic acid and resultant vasoconstriction. The risk for cardiovascular events like myocardial infarction and stroke are increased in CRF due to oxidation of lipoproteins and atherogenesis.

Neurological diseases like encephalopathy and peripheral neuropathy develop in CKD due to nitration of brain proteins and oxidation of myelin. Moreover ROS promotes inflammation, accelerated ageing, fibrosis and apoptosis leading to progression of CKD. Therefore, the present study was carried out to evaluate the serum markers for early diagnosis of patients at different stages of chronic kidney disease.

**METHODS**

The present study was conducted among chronic kidney disease patients who attended the Nephrology outpatient department and those admitted in the Nephrology wards of a tertiary care hospital at Calicut. The study was conducted the institutional ethical clearance and informed consent from all the participants.

The study group consisted of 50 patients with chronic kidney disease who were on conservative treatment with the age group of 20 to 60 years. Based on the creatinine clearance values the patients were assigned in to 3 groups; Stage 3 (Creatinine clearance=30-59 mL/min), Stage 4 (Creatinine clearance=15-29 mL/min) and Stage-5 (Creatinine clearance ≤15 mL/min) as per the National Kidney Foundation Diseases Outcome Quality Initiative (NKF DOQI) guidelines.

The creatinine clearance values were calculated from the serum creatinine levels using the Cockroft and Gault equation. Patients with history of smoking, diabetes, acute infections and malignancy were excluded from the study. Control group consisted of 50 age and sex matched, non-diabetic, non-smoker healthy volunteers.

About 5 ml of blood was collected by venous puncture using disposable syringes and needles under aseptic precautions and transferred into clean dry bottles. 4mL blood was allowed to clot and the serum separated by centrifugation at 3,000 rpm for 15 minutes.

Superoxide dismutase, Ceruloplasmin and Malondialdehyde levels were assayed in serum using UV–Vis Spectrophotometer 118 (Systronics), photoelectric colorimeter (Systronics 114) and Semiautomatic analyzer (Erba). Haemoglobin was estimated using cyanmethaemoglobin method of Drabkin (25).
Statistical analysis

The data obtained was represented as mean and the standard deviation. Determination of significance of difference between the two groups that are compared is done by applying student’s t test. The p value of ≤0.05 was taken as the level of significance.

RESULTS

The haemoglobin concentration in control group, stage 3, stage 4 and stage 5 of Chronic Kidney Disease was shown in Figure 1.

The haemoglobin concentration in all the 3 CKD stages was found to be significantly decreased (p<0.000). The Serum Malondialdehyde (MDA) in control group and patients of different stages of chronic kidney disease was shown in Figure 2. When compared for statistical analysis, it showed significantly very high (p=0.000) malondialdehyde level in patients with CKD.

Table 1: Comparison of serum MDA values in stage 3, stage 4 and stage 5 CKD with control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/dL)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Normal</td>
<td>81.07</td>
<td>12.34</td>
</tr>
<tr>
<td>Stage 3</td>
<td>82.61</td>
<td>6.71</td>
</tr>
<tr>
<td>Stage 4</td>
<td>91.94</td>
<td>27.50</td>
</tr>
<tr>
<td>Stage 5</td>
<td>118.21</td>
<td>33.80</td>
</tr>
</tbody>
</table>

The serum SOD levels of normal and patients with CKD showed significant decline (p<0.000) in stage 5 (Figure 3). On comparison of stage 2 (p=1.00) and stage 3 (p=0.458) with normal subjects showed a slight decline but was not significant.

The serum ceruloplasmin level was found to be significantly declined (p<0.000) in patients with stage 5 of chronic kidney disease when compared that of normal subjects (Figure 4). Whereas, in stage-2 and stage 3 patients it did not show significant variation (p=1.00 respectively).

DISCUSSION

In the present study, the haemoglobin values in chronic kidney disease patients were significantly lower than the control group indicating that the severity of anaemia
increased with the progression of the disease. Similar fall in haemoglobin levels were obtained in patients with progressive renal disease in studies by Bhagwat et al, Clemens et al and Mircesu et al.

Anaemia in chronic kidney disease is caused primarily by depressed erythropoiesis and shortened erythrocyte lifespan. Depressed erythropoiesis in CKD is due to erythropoietin deficiency, iron and folic acid deficiency and oestitis fibrosa associated with hyperparathyroidism. Shortened erythrocyte life span occurs due to RBC membrane damage caused by oxidative stress.

The creatinine clearance values recorded were significantly lower in all the three stages of chronic kidney disease when compared with that of the control group. There was a progressive decrease in creatinine clearance value from stage 3 to stage 5 CKD.

Similar results were obtained by Radteke et al and Williams et al in their study on predialysis patients with varying degrees of renal disease. Glomerular filtration rate as represented by the creatinine clearance value is an index of overall kidney function. The creatinine clearance values decline in chronic kidney disease due to reduction in the filtering area of glomerular capillary bed as a result of destruction of glomeruli and also due to decreased rate of renal blood flow.

A highly significant elevation in serum MDA levels were found in all the 3 stages of chronic kidney disease when compared with that of the control group. There was a progressive increase in malondialdehyde levels from stage 3 to stage 5. Our findings were consistent with the results obtained by Annu. M et al, Nitin et al, Talia et al and Martin et al.

It is said that, in chronic kidney disease, renal cells as well as infiltrating cells like macrophages and neutrophils upon activation produce free radicals like superoxide radical and hydroxyl radical which causes peroxidation of lipids. Lipid peroxidation is a chain reaction which leads to further production of more and more free radicals resulting in cellular damage and dysfunction. Serum malondialdehyde is a product of this major chain reaction leading to definite oxidation of polyunsaturated fatty acids such as linoleic and linolenic acids and thus it serve as a reliable marker of oxidative stress.

The serum levels of superoxide dismutase (SOD) were found to be lower in patients in all the 3 stages of chronic kidney disease. Nitin et al and Sasikala et al have reported similar reduction in SOD levels in CKD patients. In the present study the fall in SOD level was highly significant only in stage 5, where as in the study by Nitin et al, the reduction in SOD level was found to be highly significant in patients in all the 3 stages of CKD.

Superoxide dismutase functions as an antioxidant by scavenging superoxide anion which is formed from molecular oxygen by single electron transfer. SOD converts the highly reactive superoxide radical into less toxic hydrogen peroxide and decreases cell damage.

The lower serum SOD levels point towards the deficient antioxidant mechanisms in chronic kidney disease. The catalytic activity of SOD depends on a prosthetic group containing copper. Zinc stabilizes the apoenzyme in the native configuration. Decreased levels of copper and zinc have been reported in CKD patients who may contribute to deficient activity of SOD enzyme.

The serum ceruloplasmin levels progressively decreased from stage 3 to stage 5 suggesting a lowering of antioxidant activity with the progression of chronic kidney disease. Studies by Bhagwat et al also showed similar results. In the present study, the fall in serum ceruloplasmin level was significant only in patients with stage 5 chronic kidney disease.

Free iron and copper are powerful catalysts of free radical damage. By binding copper, ceruloplasmin prevents free copper from catalyzing oxidative damage. The ferroxidase activity of ceruloplasmin facilitates iron loading into transferrin and prevents free ferrous ions from participating in harmful free radical generating reactions.

CONCLUSION

In the present study, the evaluation of the oxidant and antioxidant status in plasma of patients with renal anaemia indicated the existence of increased free radical activity and decrease in function of antioxidant enzyme systems. This resulted in oxidative stress which caused increased lipid peroxidation and destruction of red cell membrane.

The results of the study reinforces the possibility that antioxidant supplementation may be helpful in correcting anaemia in chronic kidney disease. Treatment of renal anaemia is an effective intervention to en

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