

## Research Article

# Correlation of hemoglobin with creatinine clearance, antioxidant status, lipid peroxidation and ceruloplasmin in patients with chronic kidney disease

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## ABSTRACT

**Background:** Anaemia in CRF is caused primarily by a combination of depressed erythropoiesis and shortened erythrocyte lifespan caused by oxidative stress. Therefore, the present study was designed to investigate the correlation between Hb concentration and antioxidant and lipid peroxidation levels.

**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, non-smoker 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 correlations between the different groups are performed by applying Pearson's correlation test. The p value of  $\leq 0.05$  was taken as the level of significance.

**Results:** A positive correlation of Hb with creatinine clearance ( $r=0.46$ ,  $p=0.001$ ), SOD level ( $r=0.4$ ,  $p=0.009$ ), serum ceruloplasmin ( $r=0.3$ ,  $p=0.07$ ) was observed. Significant positive correlation was found between creatinine clearance and SOD level ( $r=0.4$ ,  $p=0.008$ ), ceruloplasmin ( $r=0.3$ ,  $p=0.04$ ). A negative correlation was obtained between serum malondialdehyde levels and haemoglobin concentration ( $r=-0.4$ ,  $p=0.007$ ) and between creatinine clearance and MDA levels ( $r=-0.4$ ,  $p=0.01$ ).

**Conclusions:** The study provides a better understanding of the biochemical parameters underlying anaemia in chronic kidney disease. The increased production of ROS and deficiency of antioxidant enzymes altered the oxidant and antioxidant equilibrium in the plasma of CKD patients.

**Keywords:** Ceruloplasmin, Chronic kidney disease, Creatinine clearance, Malondialdehyde, Haemoglobin, Superoxide dismutase

## INTRODUCTION

The diseases of kidney which lead to progressive destruction and scarring of renal tissue are characterized by a common pattern of metabolic and functional change. The diseases that bring about the alterations are many and include diabetes mellitus, hypertension, non-diabetic

glomerular disease, cystic kidney disease and tubule-interstitial disease. No matter what the nature of underlying disease process may be, the functional disorganization ultimately encountered differs little from one disease process to another. This uniformity of pathophysiologic disintegration was recognized by Bright as early as in 1836 and these disease processes which

follow the uniform course have since been collectively referred to as Chronic Bright's disease.<sup>1,2</sup>

In CRF, compensatory and adaptive mechanisms maintain acceptable health until GFR is about 10-15mL/min and life sustaining renal excretory and homeostatic functions continue until GFR is <5mL/min.<sup>3</sup> As the GFR of the whole kidney falls, the still functioning nephrons produce an increased volume of filtrate (hyperfiltration) and their tubules respond appropriately by excreting fluids and solutes in amounts that maintain homeostatic balance.

In cases of chronic reduction in nephron number oxygen consumption by the remaining nephrons increases which lead to increased production of reactive oxygen species. This oxidative stress probably enhances not only tubular damage but also interstitial inflammation and fibrosis via release of proinflammatory and profibrotic molecules. Increased apoptosis of tubular cells has been reported in CKD and reactive oxygen species (ROS) is thought to play a role.<sup>4</sup>

Oxidative stress is involved in the pathogenesis of hypertension, endothelial dysfunction, neurological disorders, shortened erythrocyte lifespan, atherosclerosis and inflammation in CRF. Oxidative stress can cause hypertension in CKD by promoting ROS mediated inactivation of nitric oxide (NO), generation of vasoconstrictive isoprostanes (by oxidation of arachidonic acid) and resultant vasoconstriction. The combination of nitric oxide inactivation by ROS and depressed NO biosynthesis are largely responsible for endothelial dysfunction in CRF.

In addition, peroxynitrite, the reaction product of superoxide radical and NO, constitutes a strong oxidant molecule which is able to oxidize proteins, lipids and nucleic acids causing vascular cell damage.<sup>5</sup> Finally superoxide anion facilitates oxidative modification of low density lipoprotein (LDL) that plays a key role in the formation of atherosclerotic lesions. ROS can inflict neurotoxicity via peroxidation of cell membrane phospholipids and cause excitotoxicity by facilitating glutamate release.<sup>6</sup>

Anaemia in CRF is caused primarily by a combination of depressed erythropoiesis and shortened erythrocyte lifespan. The latter is caused by oxidative stress via oxidation of cell membrane phospholipids, glutathione depletion and altered intracellular redox state. These processes deepen anaemia in CRF and make difficulties in its treatment.

In chronic kidney disease patients, the severity of anaemia increased with the progression of the disease. The majority of patients with chronic kidney disease had normocytic normochromic anaemia. The serum malondialdehyde (MDA) levels increased with the advancement of chronic kidney disease. This favoured

the fact that there was an increased oxidative stress in these patients.<sup>7,8</sup> The levels of the antioxidant enzymes - superoxide dismutase (SOD) and ceruloplasmin decreased with the progress of chronic kidney disease. This may be due to increased consumption of antioxidant enzyme systems as a result of the increased oxidative stress. Therefore, the present study was carried out to evaluate the correlation haemoglobin with serum markers of antioxidants and lipid peroxidation 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 after obtaining 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 Cockcroft and Gault equation.<sup>9,10</sup> 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 (Systronics-118), photoelectric colorimeter (Systronics-114) and Semiautomatic analyzer (Erba). Haemoglobin was estimated using cyanmethaemoglobin method of Drabkin.<sup>11</sup>

## Statistical analysis

The data obtained was represented as mean and the standard deviation. Correlations between the different groups are performed by applying Pearson's correlation test. The p value of ≤0.05 was taken as the level of significance.

## RESULTS

A highly significant positive correlation ( $r=0.46$ ,  $p=0.001$ ) was found between creatinine clearance and Hb

concentration in patients with different stages of chronic kidney disease (Table 1). A negative correlation ( $r=-0.4$ ,  $p=0.007$ ) was obtained between serum malondialdehyde

levels and haemoglobin concentration in patients with chronic kidney disease (Table 2) and it was found to be highly significant.

**Table 1: Correlation between creatinine clearance and haemoglobin concentration in normal and patients with different stages of chronic kidney disorders. Values are mean±SD.**

Group	Creatinine clearance (mL/ min)	Hb (g/dL)	r- value	p- value
Normal	93.80±19.71	13.79±1.90	r= 0.46	p=0.001
Stage 3	41.08±7.12	9.42±2.22		
Stage 4	19.31±3.40	9.25±1.43		
Stage 5	8.72±3.09	7.09±1.72		

**Table 2: Correlation between malondialdehyde and haemoglobin concentration in normal and patients with different stages of chronic kidney disorders. Values are mean±SD.**

Group	MDA (nmol/dL)	Hb (g/dL)	r- value	p- value
Normal	81.07±12.34	13.79±1.90	r= -0.4	p=0.007
Stage 3	82.61±6.71	9.42±2.22		
Stage 4	91.94±27.50	9.25±1.43		
Stage 5	118.21±33.80	7.09±1.72		

**Table 3: Correlation between superoxide dismutase and haemoglobin concentration in normal and patients with different stages of chronic kidney disorders. Values are mean±SD.**

Group	SOD U/mL	Hb ( g/dL)	r- value	p- value
Normal	3.39±1.63	13.79±1.90	r= 0.4	p=0.009
Stage3	2.67±1.95	9.42±2.22		
Stage4	2.60±1.37	9.25±1.43		
Stage 5	1.64±1.32	7.09±1.72		

**Table 4: Correlation between superoxide dismutase and haemoglobin concentration in normal and patients with different stages of chronic kidney disorders. Values are mean±SD.**

Group	Ceruloplasmin mg%	Hb ( g/dL)	r- value	p- value
Normal	31.50±7.66	13.79±1.90	r= 0.3	p=0.07 (NS)
Stage3	29.12±5.63	9.42±2.22		
Stage4	28.85±10.24	9.25±1.43		
Stage 5	22.45±6.79	7.09±1.72		

**Table 5: Correlation between creatinine clearance and malondialdehyde concentration in normal and patients with different stages of chronic kidney disorders. Values are mean±SD.**

Group	Creatinine clearance (mL/ min)	MDA (nmol/dL)	r- value	p- value
Normal	93.80±19.71	81.07±12.34	r= -0.4	p=0.01
Stage3	41.08±7.12	82.61±6.71		
Stage4	19.31±3.40	91.94±27.50		
Stage 5	8.72±3.09	118.21±33.80		

The serum levels of superoxide dismutase (SOD) were found to be lower in patients in all the 3 stages of chronic kidney disease. A highly significant positive correlation ( $r=0.4$ ,  $p=0.009$ ) was found between SOD level and Hb concentration (Table 3). 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. A positive correlation was also found between serum ceruloplasmin and haemoglobin values ( $r=0.3$ ,  $p=0.07$ ) but it was not

found to be significant (Table 4). A negative correlation was found between creatinine clearance and MDA levels ( $r=-0.4$ ,  $p=0.01$ ) and it was found to be significant (Table 5). A highly significant positive correlation ( $r=0.4$ ,  $p=0.008$ ) was found between creatinine clearance and

SOD level (Table 6). A significant positive correlation was found between creatinine clearance and ceruloplasmin values in patients with different stages of chronic kidney disease. ( $r=0.3$ ,  $p=0.04$ ) and it was found to be significant (Table 7).

**Table 6: Correlation between creatinine clearance and superoxide dismutase concentration in normal and patients with different stages of chronic kidney disorders. Values are mean±SD.**

Group	Creatinine clearance (mL/ min)	SOD U/mL	r- value	p- value
Normal	93.80±19.71	3.39±1.63	r= 0.4	p=0.008
Stage3	41.08±7.12	2.67±1.95		
Stage4	19.31±3.40	2.60±1.37		
Stage 5	8.72±3.09	1.64±1.32		

**Table 7: Correlation between creatinine clearance ceruloplasmin level in normal and patients with different stages of chronic kidney disorders. Values are mean±SD.**

Group	Creatinine clearance (mL/ min)	Ceruloplasmin mg%	r- value	p- value
Normal	93.80±19.71	31.50±7.66	r= -0.4	p=0.01
Stage3	41.08±7.12	29.12±5.63		
Stage4	19.31±3.40	28.85±10.24		
Stage 5	8.72±3.09	22.45±6.79		

## DISCUSSION

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. A highly significant negative correlation was found between creatinine clearance and serum malondialdehyde levels. Martin et al and Nitin et al have obtained similar results in their studies on chronic kidney disease patients.<sup>12,13</sup> The increase in malondialdehyde levels with fall in creatinine clearance indicates that lipid peroxidation and oxidative damage increases with progression of chronic kidney disease.

A negative correlation was obtained between serum malondialdehyde levels and haemoglobin concentration in patients with chronic kidney disease and it was found to be highly significant. Siems et al and Sommerberg et al have also got similar findings.

In uraemia, peroxidation of polyunsaturated fatty acids on the RBC membrane causes increased permeability to sodium ions, rapid influx of calcium ions and entry of water by osmosis into the cell leading to cell damage.<sup>14-16</sup> Increased RBC destruction as a result of lipid peroxidation leads to decreased haemoglobin levels in chronic kidney disease patients.

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.<sup>13,17</sup> In the present study the fall in SOD level was highly significant only in stage 5, whereas 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.<sup>13</sup>

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.<sup>18</sup>

A highly significant positive correlation was found between creatinine clearance values and superoxide dismutase enzyme levels. Similar results were obtained by Nitin et al and Sasikala et al.<sup>13,17</sup> The antioxidant enzyme deficiency increased with the progression of chronic kidney disease subjecting the end stage renal disease patient to severe oxidative stress and its consequences. The decrease in antioxidant defence was more marked in the final stage of CKD as in the initial stages the transcriptional upregulation of antioxidant

enzyme levels in response to increased ROS activity keep the SOD levels from falling greatly.<sup>19</sup>

A highly significant positive correlation was found between serum superoxide dismutase levels and hemoglobin concentration. In CKD, the defective antioxidant environment exposed the RBCs to severe oxidative damage by superoxide radical, decreasing their life span. A significant negative correlation was found between serum malondialdehyde and superoxide dismutase enzyme levels. The increased oxidative damage caused excess consumption of antioxidants which led to the imbalance between oxidants and antioxidants.

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.<sup>20</sup> In the present study, the fall in serum ceruloplasmin level was significant only in patients with stage 5 chronic kidney disease.

A positive correlation was found between creatinine clearance and serum ceruloplasmin levels and it was found to be significant. The result obtained in the present study was consistent with the finding of Vikram Kolagal.<sup>21</sup> A positive correlation was also found between serum ceruloplasmin and haemoglobin values but it was not found to be significant. Decreased levels of ceruloplasmin in CRF led to increased availability of copper and iron which can generate free radicals to cause further damage of bio molecules in these patients.

## CONCLUSION

The study provides a better understanding of the biochemical parameters underlying anaemia in chronic kidney disease. The increased production of ROS and deficiency of antioxidant enzymes altered the oxidant and antioxidant equilibrium in the plasma of CKD patients. The deficiency of antioxidant enzymes - superoxide dismutase and ceruloplasmin resulted in increased susceptibility of RBC membrane to oxidative damage induced by ROS mediated lipid peroxidation.

The accelerated peroxidation reactions probably resulted in shortening of erythrocyte survival and contributed to the development of anaemia in chronic kidney disease. It is therefore suggested that antioxidant supplementation may help the chronic kidney disease patients to cope with the oxidative stress and prevent destruction of RBC.

The study paves the way for further research work which is needed to design effective antioxidant treatment protocols for the management of oxidative stress and anaemia and to delay the progression of chronic kidney disease. Large scale clinical intervention studies are required to realize this goal and this will help to reduce

the burden of end stage renal disease in the general population.

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