

Effect of Haemodialysis on Oxidative Stress in Chronic Kidney Disease Patients

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ABSTRACT

Introduction: The progressive damage to the kidney by oxidative stress and depletion of nitric oxide have been pointed out in recent years. Oxidative stress has been implicated to various mechanisms leading to accumulation of superoxide anions.

Aim: We have estimated serum levels of Superoxide Dismutase (SOD), Ceruloplasmin, Total Antioxidant (TAO) and Malondialdehyde (MDA) to study the oxidative stress in chronic kidney disease as well as compare it with its post haemodialysis status.

Materials and Methods: The study group consisted of 96 individuals with chronic kidney disease (cases) and 60 age/sex matched healthy individuals (control). Blood sample was collected from cases before and after haemodialysis as well as from the control group. Blood sample was processed and estimated for

MDA, SOD, TAO and ceruloplasmin. Student's t-test was used to find the significance and Pearson correlation was used to find the degree of relationship between the study parameters.

Results: A statistically significant ($p < 0.001$) increase in serum MDA was observed in cases compared to controls. Serum SOD, ceruloplasmin and TAO levels were decreased in cases in chronic kidney disease compared to control group. The decrease in serum ceruloplasmin and TAO activity was statistically significant ($p < 0.001$). After haemodialysis, statistically significant increase in serum MDA level ($p = 0.004$) as well as ceruloplasmin ($p < 0.001$) level were observed. However, serum SOD and TAO activity were decreased significantly ($p < 0.001$).

Conclusion: In chronic kidney disease oxidative stress is higher, which is further increased after haemodialysis as indicated by increased MDA levels and decreased SOD and TAO activity.

Keywords: Antioxidant, Ceruloplasmin, Malondialdehyde, Superoxide dismutase

INTRODUCTION

Chronic kidney disease has become a public health concern in India as about 100,000 end stage renal disease cases are reported annually [1]. Increased cardiovascular risk is associated with chronic kidney disease [2]. Reactive Oxygen Species (ROS) are produced by mitochondria, phagocytes, vessel walls, non-phagocytic cells like glomerular cells, tubular cells mainly in the cortex of the kidney [3,4]. It can be produced in different areas of the kidney but mainly in the cortex [5,6]. Oxidative stress develops when there is an imbalance in the steady-state of pro-oxidants to antioxidants where, the bioactive oxidation products greatly overwhelm the total capacity of endogenous antioxidants to neutralise it [7].

Oxidative stress is a major contributor for several conditions like diabetes mellitus, atherosclerosis which can lead to cardiovascular disease and chronic kidney disease [8]. Haemodialysis is a modality of treatment in end stage chronic kidney disease. There is a growing body of evidence which points towards oxidative stress following haemodialysis. It has been demonstrated by some researchers that MDA level is increased after the haemodialysis. Antioxidant vitamin level also found to be decreased after haemodialysis. These can contribute to increased oxidative stress due to accumulation of free radicals [9-16]. The free radicals damage the membrane lipids leading to lipid peroxidation which is assessed by the byproduct MDA. It was shown to have positive correlation with cardiovascular disease [17].

SOD is an enzymatic front line defender against the damage causing effect of free radicals. It neutralises superoxide anions formed due to single electron gain by molecular oxygen. Superoxide anion is converted to less toxic hydrogen peroxide by SOD and helps to prevent oxidative damage to cell. When the serum level of SOD decreases, it indicates the decrease in the antioxidant defence mechanism [18].

Oxidative stress is due to reduced antioxidant defense or increased production of ROS. It can be assessed by measuring the MDA, SOD and TAO level representing the sum of all the antioxidant activity machineries available [19].

This study was conducted to evaluate the oxidative stress in chronic kidney disease patients undergoing haemodialysis by evaluating the serum levels of MDA, SOD, ceruloplasmin and TAO.

MATERIALS AND METHODS

The present study was a prospective case control study. The study group consisted of 96 individuals (cases) with chronic kidney disease who are non-smokers and non-alcoholics attending dialysis centre at A.J. Institute of Medical Sciences and Research Center, Mangalore, Karnataka, India, between September 2015 to June 2016 for haemodialysis. Age and sex-matched 60 individuals visiting the hospital for regular health checkup who are healthy and all parameters in the normal range were recruited as controls.

Exclusion criteria: Chronic kidney disease patients unwilling to participate, with fever indicating infection, with multiorgan involvement and terminally ill patients were excluded from the study.

Sampling technique: Purposive sampling technique was used for collection of samples. Institutional ethical committee clearance (AJEC/Rev/103/A/2013-14) was obtained. After taking informed consent from the individuals, 5 mL of early morning blood sample in fasting state was obtained in a clean and dry test tube from control group as well as from individuals with chronic kidney disease. Another blood sample was collected from the cases immediately after haemodialysis.

Blood samples were stored between 4-8°C and processed on the same day to obtain serum by centrifugation at 3000 rpm for 30 minutes. Reagents were prepared manually using the reagent grade chemicals and methods were standardised for the estimation

of study parameters. Serum MDA was estimated by thiobarbitric acid method described by Ohkawa H et al., [20]. Serum SOD was estimated by pyrogallol method described by Marklund S et al., and Marklund SL et al., [21,22]. Serum TAO capacity was estimated by FRAP (Ferric Reducing Ability of Plasma) assay described by Benzie FF et al., and serum ceruloplasmin was estimated by measurement of its p-phenylenediamine oxidase activity as described by Sunderman FW et al., [23,24]. Measurements were done by using UV-VIS spectrophotometer 117.

STATISTICAL ANALYSIS

Statistical analysis included descriptive and inferential statistics. Results are presented as Mean±SD. Significance is assessed at 5% level of significance. Student's t-test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Student's t-test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group. Pearson correlation between study variables was performed to find the degree of relationship.

Statistical software: Analysis of the data was done using Statistical Package for the Social Sciences SPSS 18.0, and R environment version 3.2.2. Microsoft Excel has been used to create graphs.

RESULTS

Serum MDA level in cases was increased significantly ($p<0.001$) in comparison to control group. There is a decrease in serum SOD in cases when compared to controls which was statistically not significant. Serum ceruloplasmin was decreased significantly (<0.001) in cases when compared to controls group. TAO level was decreased significantly (<0.001) in cases in comparison to controls [Table/Fig-1].

Variables	Cases	Controls	p-value
Serum malondialdehyde (nmol/dL)	1363.61±261.11	454.54±118.40	<0.001**
Serum superoxide dismutase (U/mL)	3.74±1.37	4.08±0.69	0.180
Serum total antioxidant activity (mmol/L)	0.81±0.34	1.84±0.42	<0.001**
Serum ceruloplasmin (mg/L)	17.54±5.24	37.03±7.54	<0.001**

[Table/Fig-1]: Comparison of clinical variables in case-controls studied. Significance (p-value: 0.05<p<0.10) * Moderately significant (0.01<p≤0.05) ** Strongly significant (p≤0.01), Student's t-test (two tailed, independent).

Oxidative stress parameters and serum ceruloplasmin levels were compared in chronic renal failure patients before and after haemodialysis. A statistically significant ($p<0.004$) increase in serum MDA after haemodialysis when compared to before haemodialysis was observed; whereas serum SOD levels and TAO levels after haemodialysis were significantly (<0.001) decreased compared to before haemodialysis [Table/Fig-2]. Serum ceruloplasmin level was increased in post-haemodialysis serum compared to pre-haemodialysis samples.

Variables	Before Haemodialysis	After Haemodialysis	p-value
Serum malondialdehyde (nmol/100mL)	1363.61±261.11	1499.05±259.67	0.004**
Serum superoxide dismutase (U/mL)	3.74±1.37	2.50±0.74	<0.001**
Serum total antioxidant activity (mmol/L)	0.81±0.34	0.48±0.20	<0.001**
Serum ceruloplasmin (mg/L)	17.54±5.24	24.07±6.99	<0.001**

[Table/Fig-2]: Assessment of clinical variables before and after haemodialysis. Student's t-test (two tailed, dependent). Significance (0.05<p<0.10) * Moderately significant (0.01<p≤0.05) ** Strongly significant (p≤0.01).

A negative correlation between MDA and SOD before and after haemodialysis was observed. This inverse relation between the MDA and SOD was statistically highly significant before haemodialysis ($p<0.001$). An inverse relation was also observed between MDA and TAO before and after haemodialysis, which was statistically insignificant [Table/Fig-3,4]. Serum SOD was positively correlated with TAO and ceruloplasmin before and after haemodialysis however, it was statistically not significant [Table/Fig-3,4]. A statistically significant inverse association between serum ceruloplasmin and TAO was observed after haemodialysis ($p<0.023$) [Table/Fig-4].

Before haemodialysis	Serum Malondialdehyde		Serum Superoxide dismutase		Serum Total antioxidant activity	
	r-value	p-value	r-value	p-value	r-value	p-value
Serum Malondialdehyde (nmol/100mL)			-0.553	<0.001**		
Serum Total antioxidant activity (mmol/L)	-0.013	0.923	0.113	0.394		
Serum Ceruloplasmin (mg/L)	-0.311	0.017*	0.059	0.658	-0.017	0.897

[Table/Fig-3]: Correlation between serum MDA, serum SOD, serum TAO and serum ceruloplasmin before haemodialysis. Significance (0.05<p<0.10) * Moderately significant (0.01<p≤0.05) ** Strongly significant (p≤0.01), Student's t-test (two tailed, dependent), Pearson correlation.

After haemodialysis	Serum Malondialdehyde		Serum Superoxide dismutase		Serum Total antioxidant activity	
	r-value	p-value	r-value	p-value	r-value	p-value
Serum Malondialdehyde			-0.120	0.365		
Serum Total antioxidant activity	-0.159	0.230	0.027	0.842		
Serum Ceruloplasmin	0.008	0.954	-0.244	0.062	-0.295	0.023*

[Table/Fig-4]: Correlation between serum MDA serum SOD, serum TAO and serum ceruloplasmin after haemodialysis. Significance (0.05<p<0.10) * Moderately significant (0.01<p≤0.05) ** Strongly significant (p≤0.01), Student's t-test (two tailed, independent), Pearson correlation.

DISCUSSION

Oxidative stress due to the imbalance in the oxidants and antioxidants play a major role in chronic kidney disease [4]. The source for the ROS in kidneys are macrophages, various glomerular and vascular cells. The imbalance in the production of the ROS and the defence by the antioxidants like SOD will lead to oxidative stress [25]. In the present study, we observed an increase in the levels of MDA and decrease in the level of SOD, TAO in patients with chronic kidney disease when compared to controls. An inverse relationship between MDA, SOD as well as MDA and TAO levels before haemodialysis was observed. These findings indicate increased oxidative stress. Sheeba V et al., and Nagane NS et al., also observed increased serum levels of MDA and decreased SOD levels in chronic kidney disease when compared to controls [18,25]. Sasikala M et al., also reported decreased SOD level in chronic kidney disease [7].

Chen MF et al., observed an increase in the plasma level of superoxide in patients on haemodialysis when compared to normal individuals [9]. Griendling KK et al., had shown that significant reduction in the cellular SOD in chronic renal failure was associated with the elevation of NADPH oxidase which was a major source of superoxide in animal tissues [26]. Superoxide anion accumulated leads to inactivation leading to deficiency of nitric oxide which is an important antioxidant protecting renal tissues by improving the blood flow [8,27-29]. This is a major contributor for oxidative stress in chronic renal failure and after haemodialysis [29]. Oxidants contribute to progressive damage to the kidney by causing ischemia, cell death and ultimately

causing inflammatory process [8,30]. Several studies have emphasised that chronic kidney disease is a result of deficiency of nitric oxide. Decrease in the functional mass of nephrons leads to increased activity of the remaining healthy nephrons. It leads to increased utilisation of oxygen by the healthy nephrons resulting in formation of excess amount of ROS. It might lead to further tubular damage, inflammation and fibrosis of interstitial tissues [31].

We observed statistically significant increase in serum MDA level after haemodialysis and an inverse relationship between MDA, SOD as well as MDA and TAO after haemodialysis. Meerashivashekar et al., also found an increase in the serum malondialdehyde in chronic kidney disease after haemodialysis [32]. In the present study, statistically significant ($p < 0.001$) decrease in the serum level of SOD as well as TAO was observed in chronic kidney disease patients after haemodialysis. From this we can infer that the oxidative stress is further increased in chronic kidney disease after the haemodialysis. Meerashivashekar et al., reported a decrease in serum SOD after dialysis, but it was not statistically significant [32].

Various studies have tried to explain the mechanism for increase oxidative stress following haemodialysis. Exposure of blood to dialyzer membrane and dialysate during haemodialysis which may activate the complement factors, thrombocytes and leucocytes leading to increase in oxidative stress within minutes [10-14]. Sela S et al., had shown that activation of polymorphonuclear leucocytes during dialysis as the significant contributor of oxidative stress. [15]. Maher ER et al., had shown increase in the MDA with in few minutes of haemodialysis and hypothesised that it may be due to increase in free fatty acids due to heparin or activation of complement factors [11]. There are evidences that antioxidant vitamins level are decreased in patients undergoing haemodialysis which further increases oxidative stress following haemodialysis [29]. These explain further increase in oxidative stress following haemodialysis observed in our study.

In chronic kidney disease, we observed decreased serum ceruloplasmin level when compared to controls and an inverse relationship between the TAO and ceruloplasmin. Sheeba V et al., had also reported decrease in the serum ceruloplasmin level in chronic kidney disease [18]. A significant increase in serum ceruloplasmin level and statistically significant inverse relation between serum ceruloplasmin and TAO was observed after haemodialysis. Our finding were consistent with the observations of Ashok KJ et al., they have also reported increase in serum ceruloplasmin in chronic renal failure after haemodialysis [33]. Mehdi WA et al., also observed decrease in serum ceruloplasmin level in patients with chronic kidney disease and it increased after haemodialysis [34]. Ceruloplasmin has ferroxidase activity responsible for its antioxidant activity. In chronic kidney disease ferroxidase activity of the ceruloplasmin is reduced and contributes to oxidative stress [2]. Ceruloplasmin might lead to vasculopathic effect and ROS by disrupting normal copper binding to ceruloplasmin. It may contribute to increased oxidative stress [35].

LIMITATION

Limitations of this study include the sample size of the study group. The duration, cause and stage of the disease were not taken into account.

CONCLUSION

In our study on chronic kidney disease patients we found oxidative stress is increased significantly. After haemodialysis decreased SOD, TAO and increased MDA, indicates the further increase in oxidative

stress. The increase in oxidative stress may be due to the increased production of lipid peroxidation product (MDA) due to activation of polymorphonuclear leucocytes or complement factor. In addition decreased antioxidant vitamins also may be a contributing factor. This increased oxidative stresses in renal failure patients poses a risk for cardiovascular mortality.

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