

## **Study of lipid profile and oxidative stress in chronic renal failure**

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### **Abstract**

**Chronic renal failure is a debilitating disease which leads to many complications, most common being cardiovascular. Hence this study was undertaken to know the risk of cardiovascular morbidity in CRF patients. The levels of serum TG, TC, LDL-C, VLDL-C, TC/HDL-C and LDL-C/HDL-C ratio were significantly increased and HDL-C was significantly decreased in cases when compared to controls. Serum TG, TC, VLDL-C and HDL-C are significantly increased in conservatively managed patients than hemodialysis patients. Serum MDA was significantly increased and SOD was significantly decreased in cases when compared to controls. These changes were more pronounced in hemodialysis patients when compared to conservatively managed patients. We also did the correlation between lipid and oxidative stress parameters, in which serum MDA was positively correlated with all the lipid parameters except HDL-C and serum SOD was negatively correlated with all lipid parameters except for HDL-C. This study suggests that increased lipoproteins along with increased oxidative stress leads to accelerated atherosclerosis in CRF patients.**

**Key words:** Chronic renal failure; Dyslipidemia; Oxidative stress; Malondialdehyde; Superoxide dismutase.

*Accepted May 27 2010*

### **Introduction**

Chronic Renal Failure (CRF) is the state which results from a permanent and usually progressive reduction in renal function, in a sufficient degree to have adverse consequences on other systems.[1] In the developing countries, the awareness and burden of CRF on society has been highlighted during last decade. In India, incidence of CRF is not well documented because of lack of national registry and data regarding its incidence. It has been estimated that the prevalence of CRF in India may be up to 785 people per million populations.[2]

Chronic Renal Failure leads to many complications over a period of time. The most common include cardiovascular, cerebrovascular and peripheral vascular diseases. Deaths due to cardiovascular complications are 4-20 fold higher in CRF patients than any other cause in general population.[3] These complications are due to many metabolic and endocrinal disturbances among which dyslipidemia is one of the constant feature of CRF. Lipid abnormalities can be detected as early as renal function begins to decline (Glomerular Filtration Rate (GFR) < 50ml/min) but the type and severity vary among different patients.[3,4].

CRF patients are also subjected to enhanced oxidative stress due to reduced antioxidant systems and increased prooxidant activity.[5] During this process polyunsaturated fatty acids, present in cell membrane are oxidized in vivo to form aldehydes of variable chain length like malondialdehyde (MDA). This lipid peroxidation product can structurally alter DNA, RNA, body protein and other biomolecules.[6]

Lipid abnormalities and enhanced oxidative stress in CRF patients, accelerates the process of atherosclerosis resulting in cardiovascular complications. Hence this study was undertaken to assess the alterations in the serum lipid profile and determination of oxidative stress to know the risk of development of cardiovascular complications in CRF patients.

### **Materials and Methods**

A case control study of serum lipid profile and oxidative stress in chronic renal failure patients were carried out from February 2007 to February 2008. Both controls and cases of CRF attending OPD and dialysis units were selected from Bapuji hospital and Chigateri general hospital. Both these hospitals are attached to teaching institute

J.J.M medical college, Davangere. Each gave an informed consent and this study was approved by the ethical and research committee of J.J.M Medical college to use human subjects in the research study.

A total number of 110 subjects were participated in the study, of which 60 clinically diagnosed cases of chronic renal failure > 20 years of age were included. Among 60 CRF cases, 30 were on maintenance hemodialysis for a period of 5 months to 3 years. These patients were undergoing hemodialysis for 3-4 hours three times a week. Remaining 30 patients were on conservative line of treatment.

Control group consists of 50 age and sex matched normal healthy adults without any major illness. Patients with diabetes mellitus, familial hyper lipoproteinemia and who are on hypolipidemic drugs were excluded from the study by asking family and past history.

About 5ml of venous blood was drawn under aseptic pre

cautions in a sterile bulb from selected subjects after a period of overnight fasting of 12 hrs, serum was separated by centrifugation and used for analysis. Serum lipid profile which includes triglycerides (TG), total cholesterol (TC), high density cholesterol (HDL-C) were estimated by enzymatic method and serum low density cholesterol (LDL-C) and very low density cholesterol (VLDL-C) were calculated by using Friedwald formula.[7] Serum MDA (malondialdehyde) by thiobarbituric acid (TBA) method [8] and Superoxide dismutase by Marklund and marklund method.[9]

Lipid profile was analyzed by using ERBA kits in micro-lab semi-autoanalyzer of MERCK Company. All the reagents used in the estimation were of analytical grade.

### Statistical Analysis

Independent T test was done to compare means and Pearson's correlation was done to correlate lipid profile and oxidative stress parameters. All the statistical analysis was done by using SPSS software.

## Results

Table 1 shows comparative analysis of serum lipid profile between controls and cases. The mean values of TC, TG, LDL-C, VLDL-C, TC/HDL-C and LDL/HDL ratio are increased in cases when compared to controls except HDL-C.

Table 2 shows the comparative analysis of serum lipoprotein between conservative management and hemodialysis patients. When compared to hemodialysis patients, the mean values of TC, TG, HDL-C, LDL-C and VLDL-C are increased in conservatively managed patients and this difference is statistically significant ( $p < 0.001$ ) except LDL-C which was not significant ( $p < 0.05$ ). The mean values of TC/HDL-C and LDL-C/ HDL-C ratios are increased in hemodialysis patients when compared to cons-

ervatively managed patients and it is statistically significant.

Table 3 shows the comparative analysis of serum MDA between controls and cases. When compared to controls, both conservatively managed and hemodialysis patients showed significant increased levels of MDA ( $p < 0.001$ ) and the levels of MDA are significantly increased in hemodialysis patients when compared to conservatively managed patients ( $p < 0.001$ ).

Table 4 shows comparative analysis of serum SOD levels between controls and cases. Mean values of SOD are decreased in both conservatively managed patients and hemodialysis patients when compared to controls. SOD level is decreased markedly in hemodialysis patients when compared to conservatively managed patients ( $p < 0.001$ ).

**Table 1.** Comparison of serum lipid profile between controls and cases

Particulars		TC mg/dl	TG mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL- C mg/dl	TC/HDL-C	LDL/HDL-C Ratio
Controls (n=50)	Mean $\pm$ SD	182.1 $\pm$ 22.3	115.0 $\pm$ 30.2	44.3 $\pm$ 5.0	115.4 $\pm$ 23.4	23.0 $\pm$ 6.1	4.2 $\pm$ 0.69	2.64 $\pm$ 0.64
Cases (n=60)	Mean $\pm$ SD	205.7 $\pm$ 24	219.28 $\pm$ 37.82	36.1 $\pm$ 5.3	125.4 $\pm$ 20.4	43.8 $\pm$ 7.7	5.8 $\pm$ 1.0	3.55 $\pm$ 0.85
Control v/s cases	t value	5.29	15.75	8.27	2.38	15.53	9.88	6.14
	p value	< 0.001	< 0.001	< 0.001	< 0.05	< 0.001	< 0.001	< 0.001

\*Unpaired t test > 0.05 – Not significant,  $p < 0.05$  – Significant,  $p < 0.001$  – Highly significant

**Table 2.** Comparison of serum lipid profile between conservatively managed and hemodialysis patients

	Particulars	TC mg/dl	TG mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	TC/ HDL-C	LDL/ HDL-C ratio
Conservatively managed patient (n=30)	Mean±SD	213.8±22.9	242.4±36.5	40.0±4.1	125.2±18.4	48±7.7	5.39±0.73	3.16±0.58
Hemodialysis patients (n=30)	Mean±SD	197.6±22.7	196.83±21.83	32.2±3.1	125.5±22.4	39.2±4.4	6.2±1.03	3.93±0.91
Conservatively managed V/S hemodialysis patients	t value	2.74	5.95	8.3	0.041	5.71	3.44	3.89
	p value	<0.05	<0.001	<0.001	>0.05	<0.001	P=0.001	<0.001

\* unpaired t test

P &gt; 0.05 – Not significant

p &lt; 0.05 – Significant, p &gt; 0.001 – Highly significant

**Table 3.** Comparison of serum MDA between controls, conservatively managed and hemodialysis patients

Groups	Controls			Conservatively managed patients			Hemodialysis patients	
Number	50			30			30	
Serum MDA nmol/ml	3.0 ± 0.5			4.8 ± 1.0			6.6 ± 1.0	
Difference between the groups	Control	V/s	conservatively managed patients	Control	V/s	Hemodialysis patients	Conservative	V/s Hemodialysis patients
t-value	11.15			21.4			7.27	
p-value	< 0.001			< 0.001			< 0.001	

Unpaired t test, p &lt; 0.001 – highly significant

**Table 4.** Comparison of serum SOD between controls, conservatively managed and hemodialysis patients

Groups	Controls			Conservatively managed patients			Hemodialysis patients	
Number	50			30			30	
Serum SOD U/ml	9.6 ± 2.2			4.6 ± 1.3			3.7 ± 1.3	
Difference between the groups	Controls	V/s	conservatively managed patients	Control	V/s	Hemodialysis patients	Conservative	V/s Hemodialysis patients
t-value	15.74			13.32			2.79	
p-value	< 0.001			< 0.001			< 0.05	

Unpaired t test; p &lt; 0.05 – Significant, p &lt; 0.001 – Highly significant

**Table 5.** Correlation between lipid profile and oxidative stress parameters

		T.C. mg/dl	T.G mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	Serum MDA nmol/ml	Serum SOD U/ml
Serum MDA nmol/ml	r value	0.294	0.339	-0.681	0.217	0.554	1	-0.708
	P value	<0.001	<0.001	<0.001	<0.05	<0.001		<0.001
Serum SOD U/ml	r value	-0.373	-0.382	0.513	-0.186	-0.669	-0.708	1
	P value	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	

r value: pearson correlation coefficient, p value: &gt; 0.05 significant

hemodialysis patients when compared to controls. SOD level is decreased markedly in hemodialysis patients when compared to conservatively managed patients ( $p < 0.001$ ).

## Discussion

Disorders of lipoprotein metabolism, imbalance between generation of free radicals and antioxidant defense system during uremia and dialysis are important mechanisms of atherogenesis in CRF.

The mean value of triglycerides is significantly increased in cases when compared to controls. This result is accordance with studies done by S M Alam et al [10], Bharat Shah et al [11], P Lee et al [12] and Ziad A Massy [13]. Hypertriglyceridemia is a common feature of CRF. Presence of insulin resistance in renal failure activates hormone sensitive lipase causing increased FFA which stimulates the production of apoB-100 containing lipoproteins like VLDL leading to hypertriglyceridemia. Several authors also suggested that hypertriglyceridemia in CRF may be due to defective metabolism of TG rich lipoproteins by lipoprotein lipase (LPL) and hepatic lipase [14,15,16].

The mean value of TC is significantly increased in cases when compared to controls. This is in accordance with the study of M.M. Avram et al [17], P.O Attman et al [18] and Mayumi Tsumura et al [19]. Many studies have reported variable results. A study done by B.S Das et al., [20] observed decreased levels of TC in CRF patients. The reason for this decrease may be due to reduced food intake. CRF is associated with hypercholesterolemia which is due to associated proteinuria and renal insufficiency per se. Proteinuria leads to alteration in gene expression for HMG-CoA reductase resulting in increased activity of HMG-CoA reductase leading to hypercholesterolemia. [14].

In our study mean value of HDL-C is significantly decreased in CRF patients when compared to controls. Many studies conducted by Ziad A Massy et al [13], B.S. Das et al [20] and Tetsuo Shoji et al [21] have also observed the same results. The reason for decreased concentration of HDL-C in CRF is not fully understood. It may be due to decreased activities of LPL, hepatic triglyceride lipase (HTGL), lecithin cholesterol acyl transferase (LCAT) and increased concentration of cholesterol ester transfer protein (CETP) and decreased apolipoprotein concentrations. [14]

This study included 30 conservatively managed patients and 30 hemodialysis patients. The mean values of TC, TG, LDL-C, VLDL-C, TC/HDL-C and LDL/HDL-C in conservatively managed patients are significantly increased when compared to hemodialysis patients except for LDL-C. This is in accordance with the study of SM Alam et al [10], Bharat Shah et al [11] and Mayumi Tsumura et al [19]. The cause for the decrease in lipoprotein concentrations could be due to removal of lipoproteins by repeated dialysis and decreased peripheral resistance to insulin after initiation of dialysis [11].

MDA is a lipid peroxidation product which is formed during oxidation process of PUFA by reactive oxygen species. MDA is the sensitive marker of lipid peroxidation. MDA level is significantly elevated in hemodialysis patients when compared to conservatively managed patients. This is in accordance with study of C.M Loughrey et al [22], A. Marjani [23] and Talia Weinstein et al [24]. Although hemodialysis leads to improvement of several biochemical parameters like creatinine, urea levels and plasma lipid patterns, but it can cause harmful atherogenic effects. The increase in lipid peroxidation resulting from hemodialysis could be provoked by bio incompatibility of dialysis membrane. When cells come in contact with the dialyzer membrane leads to sensitization of cell membrane components leading to complement activation which cause formation of other reactive oxygen species which will initiate peroxidation of PUFA. [23,24]

SOD functions as a scavenger of superoxide radical in the body. Mean value of SOD is significantly decreased in hemodialysis ( $p$  value  $< 0.05$ ) patients when compared to conservatively managed patients. This is in accordance with the study of A. Marjani [23], M Sasikala et al [25] and M. Nouri et al [26]. Mechanisms involved in decreased serum SOD activity in CRF patients may be due to increased production of ROS such as  $H_2O_2$  which is known to suppress SOD activity. Decreased SOD activity among hemodialyzed patients could be due to decreased levels of  $Cu^{2+}$  and  $Zn^{2+}$  as they are cofactors of cytoplasmic SOD. Increased lipid peroxidation causes consumption of antioxidant enzymes, particularly in hemodialysis patients, may be also one of the reasons for decreased SOD levels. [27]

We also compared the serum lipids with oxidative stress parameters by using pearson correlation. We observed that serum MDA positively correlates with TC, TG, LDL, VLDL and negatively correlates with HDL and SOD. With respect to SOD it is vice versa. Thus dyslipidemia and oxidative stress may act in synergy leading eventually to accelerated atherosclerosis.

## Conclusion

This study suggests that there is increased risk of cardiovascular complications in CRF patients particularly in patients who are on hemodialysis. The altered concentration of serum lipoproteins, MDA and SOD leads to accelerated atherosclerosis in CRF patients. Hence by advising proper diet or drugs lipid abnormalities will be brought to normal levels.

## Acknowledgement

It is a great honor to express my gratitude to my beloved teacher Dr. D.S JAYAPRAKASH MURTHY,<sup>B.Sc.,MD.,</sup> Professor and Head, Department of Biochemistry, for his suggestion and constant source of inspiration throughout my post graduation course. It is with this sense of heart-felt gratitude and appreciation to my guide and teacher, Professor . Manjunath M. Tembadi, Department of Biochemistry, J.J.M. Medical College, Davangere for the preparation of this work.

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