

## ORIGINAL ARTICLE

## Lipid Profile and Serum Paraoxonase1 Activity in CRF Patients Pre and Posthemodialysis

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**Abstract:** Chronic renal failure (CRF) is associated with an increased risk of cardiovascular disease (CVD). In the present study, 30 CRF patients undergoing hemodialysis and 50 healthy controls matching in age and sex were included. We have estimated blood urea, serum creatinine, lipid profile and serum Paraoxonase1 (PON1) activity in pre and post hemodialytic samples. HDL-Cholesterol (HDLC) and serum Paraoxonase1(PON1) activity was found to be significantly reduced ( $P<0.001$ ) in CRF patients as compared to that of controls. However HDL-Cholesterol (HDLC) and Paraoxonase1 (PON1) show significant elevation ( $P<0.001$ ) post hemodialysis than prehemodialytic samples of CRF patients. The levels of blood urea, serum creatinine, total cholesterol, LDL-Cholesterol (LDLC) and Triglyceride (TG) were significantly elevated ( $P<0.001$ ) in CRF patients. After hemodialysis these parameters were significantly reduced ( $P<0.001$ ) as compared to pre hemodialytic samples. The low HDL-Cholesterol (HDLC) and serum Paraoxonase1 (PON1) activity may be responsible factor for cardiovascular disease associated with CRF. Therefore, management of CRF patients by Hemodialysis (HD) has beneficial effect.

**Key Words:** Chronic renal failure (CRF), Hemodialysis (HD), HDL-Cholesterol (HDLC), LDL-Cholesterol(LDLC), Triglyceride (TG), Cardiovascular disease(CVD), Paraoxonase1 (PON1).

### Introduction

Chronic renal failure is a devastating disease with clinical, economic and ethical dimensions and is emerging as a major public health problem globally. The renal replacement therapy, the mainstay of the management of chronic kidney disease (CKD) is beyond the reach of a large number of CKD patients in many developing countries, including India. Concerted efforts are needed for the prevention, early diagnosis, and feasible and affordable management of chronic renal failure patients [1]. Cardiovascular diseases are the leading cause of death in hemodialysis patients. Hyperlipidemia is an independent risk factor. Basic research has provided strong evidence that oxidation of low density lipoprotein (LDL) plays an important role in the pathogenesis of atherosclerosis. Oxidative stress, alterations in lipid metabolism, hyperhomocysteinaemia observed in hemodialysis patients could increase LDL oxidation [2]. In the atherosclerotic lesion, which is characterized by the accumulation of cholesterol loaded macrophages, the presence of Paraoxonase1 (PON1) was shown in histological studies. Human serum Paraoxonase1 (PON1) is an HDL - associated esterase, which possesses antiatherosclerotic properties [3]. Biochemical studies of this enzyme indicated that Paraoxonase1 (PON1) could

prevent lipid-peroxide accumulation on LDL. Low activity of this Paraoxonase1 (PON1) can lead to increased LDL oxidation. Oxidation of LDL is recognized as a key stage in the early development of atherosclerosis [4].

Lipid abnormalities and reduction in PON1 activity has been reported in CRF. Maintenance hamodialysis is the support of treatment for the patients with CRF who are waiting for, or who are not suitable to undergo renal transplantation. Adequate dialytic treatment has prolonged the survival of patients with quality of life. This improved quality and extended duration of life, by hemodialysis treatment, may be related to the lowering of risk factors for cardiovascular diseases in CRF patients. With this postulation, we thought it worthwhile to study the lipid fractions and Paraoxonase1 (PON1) in the pre and post hemodialysis samples of CRF patients with a view of observing the beneficial effects of hemodialysis.

### Materials and Methods

In the present study, 30 CRF patients and 50 healthy controls matching in age and sex were included. The patients were admitted in nephrology ward and renal intensive care unit of Wanless Hospital Miraj and Bharti Vidyapeeth University Medical College, Hospital Sangli. The patients were selected on the basis of their estimated glomerular filtration rate (eGFR). According to National Kidney Foundation guidelines, the stages of CRF varied between 1 to 5 depending on eGFR [5] and eGFR was determined by using Modification of Diet in Renal Disease (MDRD) equation [6] displayed on table no.1. All the selected patients were in the 4<sup>th</sup> and 5<sup>th</sup> stages of CRF, undergoing hemodialysis.

Stages of CRF	eGFR (mls/min/1.73m <sup>2</sup> )	Description
1	90+	Normal Kidney function but abnormalities in urine sample
2	60-89	Mildly reduced kidney function
3	30-50	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	14 or less	Very sever or End Stage Renal Disease (ESRD)
Controls	120-125	Normal kidney function

Hemodialysis was carried out three times per week for four hours per session using high flux polysulphone hemodialysis membrane. Heparin (1000IU) was administered during HD process. The patients were on low fat and protein rich diet and were taking statin for lowering blood lipids (30mg/day). These samples were analyzed to observe the immediate effect of hemodialysis process on the biochemical parameter. Upon inclusion of the patients, a record was made of all current medications being taken, risk factor for cardiovascular disease, current and historic relevant data by means of a written evolution and the database of clinical history was updated. Patients afflicted by liver disease, diabetes, infectious disease, or malignancy, were excluded from this study. On the day of dialysis, all patients arrived after overnight

fasting. 8-10 ml of blood was collected from each patient in plain bulb before and after hemodialysis taking aseptic precautions. Separated sera were processed for the assay of biochemical analytes. Blood Urea [7], Serum Creatinine [8], Serum Lipid Profile [9-12] were assayed by colorimetric and spectrophotometric methods. Serum Paraoxonase1 (PON1) activity was measured with phenyl acetate as substrate. The reaction mixture contained 1.25 mM phenyl acetate and 0.9mM CaCl<sub>2</sub> in 9mM/lit Tris (hydroxy methyl) aminomethane/HCL buffer (pH 8.0). The reaction was initiated by adding a 500 µl of 1/100 prediluted serum with normal saline to 2ml of buffer reagent. The initial velocity of phenol formation during the hydrolysis of phenyl acetate was calculated from the increase of A<sub>270</sub> nm recorded on a spectrophotometer [13]. The statistical analysis included 'Z' test and regression analysis for correlation coefficient.

### Results

Laboratory data showed a significant change in the levels of biochemical analytes in both the prehemodialytic and post hemodialytic groups in comparison to normal controls (TableII).

Sr. No.	Biochemical Parameter	Control N=50	Pre HD N=30	Post HD N=30
1	Blood Urea mg%	25.34 ± 6.29	116.57± 39.07 ***	86 ± 31.72 ***
2	Serum Creatinine mg%	1.04 ± 0.27	12.12 ± 3.79 ***	7.10± 2.49 ***
3	Total cholesterol mg %	171.87 ± 19.30	214.8 ± 29.03 ***	191.17 ± 20.08 ***
4	Serum HDLC mg%	49.60 ± 5.14	31.8 ± 7.80 ***	35.17 ± 6.63 ***
5	Serum LDLC mg%	111.82 ± 20.12	126.9 ± 18.10 ***	110.85 ± 10.82 ***
6	Serum TG mg%	130 ± 34.12	134.87 ± 30.31 ***	122.63 ± 26.07 ***
7	Serum Paraoxonase1 (PON1) U/ml	90.01 ± 16.28	58.51 ± 12.94 ***	64.98 ± 9.78 ***
* P>0.05 ---- Not Significant      ** P<0.05 --- Significant *** P<0.001--Highly Significant				

In the present study prehemodialytic samples showed significant rise in blood urea and serum creatinine (P< 0.001) as compared to that of controls. There was significant rise (P<0.001) in total cholesterol, LDLC, and TG of prehemodialytic samples of CRF patients as compared to controls. Mean values of HDLC and serum Paraoxonase1 (PON1) activity was found to be significantly reduced (P<0.001) in prehemodialytic samples as compared to controls.

In post hemodialytic samples mean values of blood urea, serum creatinine, total cholesterol, LDLC and TG fall significantly ( $P<0.001$ ) when compared to prehemodialytic samples. Mean values of serum HDLC and Paraoxonase1 (PON1) activity were significantly increased ( $P<0.001$ ) in post hemodialytic samples as compared to premodialytic samples. In the present study, there was no significant correlation between serum HDLC and serum Paraoxonase1 (PON1) activity samples of chronic renal failure patients.

### **Discussion**

Cardiovascular disease (CVD) is the leading cause of death among patients with chronic and end-stage renal disease (ESRD) [31,32]. This problem has been rather poorly studied in various cardiovascular studies, particularly the pattern of dyslipidemia and the factors contributing to these abnormalities are different among patients with renal insufficiency, severe renal failure, those on hemodialysis and peritoneal dialysis, and those with renal transplants [14]. Abnormal lipid profiles start to appear soon after renal function begins to deteriorate [15]. As the excretory function of kidney is impaired, urea and creatinine excretion is hampered leading to its increased levels in blood, so significant elevation in blood urea and serum creatinine levels are observed in CRF patients before HD in the present study [16]. These parameters show a significant decrease in their mean levels in the post hemodialytic samples. About 25% reduction in mean levels of urea and creatinine are observed in the post hemodialysis treatment. The observation is suggestive of clearance of creatinine and urea from blood during hemodialysis. Hemodialysis is an important treatment modality in most renal units for the management of renal failure and forms the alternative to renal transplantation. The distinctive features of the lipid profile in dialysis patients appear to be the presence of low concentration of HDL cholesterol and increased triglycerides, with elevations of LDL cholesterol. The risks associated with this lipid profile are still not quite clear, due to the relative lack of research in the dialysis population. However, there is growing evidence that a low HDLC concentration imparts additional risk of coronary heart disease [15]. Experimental evidence supports the hypothesis that lipids contribute directly to glomerulosclerosis and tubulointerstitial injury and that, correction of lipid abnormalities associated with renal disease will slow the progression of chronic renal failure [17-18]. Results of earlier studies show that plasma total cholesterol is usually normal or reduced and occasionally elevated in patients with CRF [19]. In our study, we found a significant elevation of serum total cholesterol in CRF patients in spite of statin therapy. Post hemodialytic samples of the CRF patients show a fall in serum total cholesterol, as compared to Pre hemodialytic samples. During dialysis, the high flux biocompatible membrane may be responsible to remove cholesterol in post HD samples [34]. The Post Hemodialytic total cholesterol levels are still high as compared to that of controls. Few factors are responsible to elevate the mean level of serum total cholesterol. Among them upregulation of hepatic enzymes Hydroxy-3-Methylglutaryl- CoA reductase and cholesterol 7 $\alpha$  -hydroxylase are important [19]. Heavy proteinuria in CRF patients can lead to upregulation of HMG CoA reductase. In addition LDL receptor deficiency, may play a central role in the genesis of the associated hypercholesterolemia in CRF patients [19-20].

One of the principal characteristics of lipid metabolism alterations in CRF patients is hypertriglyceridemia, which reflect the balance between removal and production of triglyceride [21]. Along with the generation of oxidized low-density lipoprotein (OX-LDL) it is a common feature of oxidative stress, and high levels of OX-LDL have been recognizably involved in atherogenesis, in quantitative, as well as qualitative alteration [22]. This is probably the result of the increased VLDL catabolism during dialysis or it may be caused by the increase in plasma lipoprotein lipase activity. Lipoprotein lipase bound to the endothelium may be released by heparin which is given during HD process. Also high flux biocompatible dialysis membrane may be responsible for increasing the lipoprotein lipase activity during dialysis [34]. In our study the mean levels of serum TG and LDLC are significantly increased as compared to those of controls. But these increased levels of TG and LDLC still lie in the reference range of the parameters which is quite wide, because the CRF patients were under treatment of lipid lowering drug statin and taking low fat diet as advised by the clinician. The CRF associated HDL abnormalities are marked by reduction of plasma HDL cholesterol relative to the non HDL concentration. Impaired maturation of cholesterol ester-poor HDL-3 to cholesterol ester rich cardioprotective HDL-2 [23] and other factors may be responsible for progressive decline in the level of HDL in CRF. Among them deficiency of lecithin cholesterol acyltransferase (LCAT), hepatic lipase, potential increase of Cholesterol ester transfer protein (CETP) and acyl-CoA: cholesterol acyltransferase (ACAT) activity may contribute for diminished plasma HDL and HDL maturation in CRF [24-26]. ApoA-I and ApoA-II constitute main structural constituents of HDL. In addition, apoA-I serves as the LCAT activator, where as apoA-II hepatic lipase activator. The reduction in plasma concentration of these important constituents can, therefore, contribute to both diminished HDLC concentration and impaired HDLC function in CRF [7,37]. In our study concentration of HDLC is significantly reduced in prehemodialytic samples. After hemodialysis, we found an elevation in HDLC levels. Dialysis characteristics and heparin dose could be responsible factors for effect on HDLC level in hemodialysis [34]. The decrease in Paraoxonase1 (PON1) activity observed in this study could be the result of lower HDL concentration in CRF, given that HDL is the main serum carrier of Paraoxonase1 (PON1). Oxidative modification of HDL has also been shown to impair the ability of the lipoprotein to promote Cholesterol efflux [32]. Paraoxonase1 (PON1) has been shown to inhibit the oxidative modification of LDL during copper oxidation in vitro [29,30], possibly by destroying active phospholipids in minimally oxidized LDL [31]. Human serum Paraoxonase1 (PON1) activity is inversely related to the risk of developing atherosclerotic lesion [33], which contain cholesterol loaded macrophage foam cells. HD seems to be effective also in raising serum Paraoxonase1 (PON1) activity of the patients. Paraoxonase1 (PON1) present in serum is located on HDL, being tightly bound to a HDL subfraction containing apo A -I and culsterin [28]. In CRF, the concentration of middle size and low molecular weight plasma Advanced Glycation End products (AGE) are highly elevated [35]. These AGE residues are formed on long and short lived proteins. Due to low molecular weight, AGE free adducts are easily excreted through the urine. Hence, it acts as a good renal clearance tool which distinctly

declines in CRF, leading to accumulation of plasma AGE free adducts. Retention of AGE free adducts could play a role in decreasing Paraoxonase1 (PON1) activity. During hemodialysis procedure AGE free adducts may be removed, along with the uremic toxins- urea and creatinine. Thus the inhibition of PON1 may be removed. This may result in elevation of PON1 activity after successful HD process. Acrolein, an  $\alpha$ - $\beta$  unsaturated aldehyde is highly elevated in CRF [39]. Paraoxonase1 (PON1) contains two critical cysteine residues in its catalytic hydrophobic pocket [38]. Gugliucci A. et al. have shown that acrolein inactivates PON1 through cysteine modification [37]. Acrolein may be partially removed by hemodialysis and due to this; the level of PON1 may be elevated after dialysis process. The data observed in this study show that patients with CRF present alterations in lipid parameters and serum Paraoxonase1 (PON1) which are minimized after HD treatment. The low HDLC and serum Paraoxonase1 (PON1) activity may be responsible factor for cardiovascular disease associated with CRF. Our results enlight that the process of hemodialysis by highflux polysulphone membrane and use of heparin may have beneficiary effect on the alteration of HDLC, Paraoxonase1 (PON1) activity. Choice of membrane may be a very important criterion during hemodialysis. Our study is based on single episode dialysis trial and further follow up study post HD is needed to draw definite conclusions. In India, many CRF patients do not undergo kidney transplant surgeries, mainly due financial constraints and hemodialysis remains the major therapy. To conclude the management of CRF patients by HD has beneficial effect on lowering the cardiovascular risk factors.

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