Original Research Paper

Volume-9 | Issue-6 | June-2019 | PRINT ISSN No. 2249 - 555X

Biochemistry

LIPID PROFILE AND OXIDATIVE STRESS MARKER IN PRE -& POST-HEMODIALYSIS

Dr. Shilpa Kasat	Assistant professor, Dept of Biochemistry, GMC, Akola, Maharashtra
Dr. C. R. Mallikarjun*	Professor and Head, Department of Biochemistry, S.S. Institute Of Medical College, Davangere (karnataka) *Corresponding Author
Dr. K.S. Ghorpade	Professor and Head, Department of Biochemistry, GMC, Akola, Maharashtra
Dr Kumud N Harley	Associate professor, Department of Biochemistry, GMC, Akola, Maharashtra
Dr. Narendra Dange	Associate professor, Department of Biochemistry, GMC, Akola, Maharashtra
Dr. prerna Nandedkar	Assistant professor , Department of Biochemistry, GMC, Akola, Maharashtra

ABSTRACT

INTRODUCTION: Dyslipidemia and increased oxidative stress are the causes for accelerated atherosclerosis with high cardiovascular morbidity and mortality in Chronic renal failure (CRF) patients.

OBJECTIVES: 1) To evaluate oxidative stress and lipid profile in pre- and post hemodialysis CRF patients.

METHODOLOGY : 40 CRF cases undergoing hemodialysis were selected . Serum lipid parameters like Triglycerides, Total cholesterol , HDL –Cholesterol were estimated by commercially available kits & Serum Malondialdehyde (MDA) was estimated by Thiobarbituric acid method.

RESULT: Significantly reduced HDL-C (P>0.001) & increased blood urea, serum creatinine, Total cholesterol, LDL –cholesterol, Triglycerides (P>0.001) in pre as compared to post dialysis CRF patients were observed. As Compared to pre serum MDA significantly increased in post-hemodialysis.

CONCLUSION: Hemodialysis leads to oxidative stress with increased generation of oxidized low density lipoproteins and chronic deficiency of antioxidants which in turn accelerate the process of atherosclerosis resulting in cardiovascular complication.

KEYWORDS : chronic renal failure (CRF), Malonadialdehyde, Oxidative stress

INTRODUCTION

Chronic Renal Failure (CRF) is a pathophysiological process with multiple etiologies, resulting in the inexorable attrition of nephron number and function and frequently leading to End Stage Renal Disease (ESRD).[1]

CRF leads to many complications over a period of time. The most common cause for mortality in these patients includes cardiovascular, cerebrovascular and peripheral vascular diseases. Death due to cardiovascular complication is 4 to 20 fold higher in CRF patients than any other cause in general population.[2] 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) < 50 mL/min]. Most characteristic lipid abnormality is increased serum Triglycerides (TG), Very Low Density Lipoproteins (VLDL-C), Intermediate Density Lipoprotein (IDL-C) and low levels of High Density Lipoproteins (HDL-C).[3]

CRF patients are subjected to an enhanced oxidative stress due to reduced antioxidant systems and increased pro-oxidant activity.[4] Oxidative stress is generally expressed as an outcome of oxidative damage to biologically important molecules. During this process Polyunsaturated Fatty Acids (PUFA), present in cell membranes are oxidized in vivo to form aldehydes of variable chain length like Malondialdehyde (MDA). This lipid peroxidation product can structurally alter DNA, RNA, body proteins and other biomolecules.[5] It has been reported that in patients undergoing hemodialysis, due to oxidative stress, there is an increased generation of oxidized low density lipoproteins and chronic deficiency of antioxidants which in turn accelerate the process of atherosclerosis resulting in cardiovascular complication[6]

According to the Markus Daschner et al [5] the oxidative stress marker MDA was decreased in post- hemodialysis as compared to pre-hemodialysis, which is in contrast to other studies.

The present study is thus planned to assess the alterations in serum lipid profile which include serum TC, TG, VLDL-C, LDL-C and HDL-C along with the oxidative stress marker, serum MDA among CRF patients during pre and post dialysis sessions.

OBJECTIVES

1. To evaluate the Serum Lipid Profile in CRF patients during pre- and post- hemodialysis sessions
2. To estimate activity of serum MDA in CRF patients during pre- and post- hemodialysis sessions

MATERIALS AND METHODS

Study Type: Hospital based case-control study

Study design: Study designed to evaluate alterations in serum lipid parameters and serum MDA levels in post patients during pre and post dialysis sessions.

Study Site: Nephrology Unit, S.S.Institute of Medical Sciences & Research Centre, Davangere

Sample population size: 40 clinically diagnosed CRF patients

Selection criteria:

Inclusion Criteria: Clinically diagnosed CRF cases with age group between 20-60 years

Exclusion criteria: The following patients were excluded from the study.

Patients with
1. Liver diseases
2. Infectious diseases
3. Familial hyperlipoproteinemia
4. Malignancies
5. Hypolipidemic drugs.

Sample Collection:
After obtaining ethical approval from Institute and receiving informed and written consent in regional language from all the study subjects. Approximately 5 ml of blood sample was collected in a sterile vacutainer from CRF patients during pre- and post- hemodialysis sessions. Serum was separated by centrifugation and following biochemical parameters were processed in biochemistry department by using commercially available kits.

1) Total cholesterol (TC) by modified Roeschlau's method (CHOD-PAP method)[7]
2) Triglycerides (TG) by GPO-PAP Trinder method[8]
3) High density lipoprotein (HDL) by phosphotungstic acid method, End point[9]
4) Low density lipoprotein (LDL) ,Very low density lipoprotein (VLDL) were calculated by using Friedwald formula.[10]
5) Serum Malondialdehyde (MDA) by thiobarbituric acid method[11]

STATISTICALANALYSIS
Data was entered in SPSS/ graphed software and results were expressed as mean ± SD by applying student's unpaired 't'test. For all the tests, a p-value of 0.05 or less was considered as statistical significance.

RESULTS
In present study, we analysed Serum lipid parameters (TC, TG, HDL,VLDL) and MDA activity among 40 CRF patients undergoing hemodialysis (HD) (pre –post dialysis sessions) at tertiary care hospital.

Table 1: Comparison Of Serum Lipid Profile Among The Cases Of Crf During Pre- Hemodialysis And Post-hemodialysis Sessions

Biochemical Parameters	Pre-Hemodialysis N=40	Post-Hemodialysis N=40	Mean Difference	P* Value, Significance
	Mean ± SD	Mean ± SD		
Total Cholesterol (TC) mg/dL	184.60 ± 35.60	172.40 ± 22.90	12.20	0.001 HS
Triglycerides (TG) mg/dL	206.20 ± 73.0	137.90 ± 49.20	68.30	0.001 HS
HDL-C mg/dL	41.20 ± 0.50	48.40 ± 3.20	7.20	0.001 HS
LDL-C mg/dL	100.20 ± 19.0	90.58 ± 14.0	9.62	0.003 S
VLDL-C mg/dL	41.20 ± 15.0	32.90 ± 10.80	8.30	0.001 HS
TC/HDL	4.20 ± 0.50	3.50 ± 0.40	0.70	0.001 HS

Student's paired't' test ;p<0.003 S-significant; p<0.001HS-highly significant

Table 1 depicts comparison of serum lipids and their ratios among CRF during pre- and post- hemodialysis sessions. The mean concentrations of TC, TG, LDL-C, VLDL-C were significantly increased with mean ± SD of 184.60 ± 35.60, 206.20 ± 73.0, 41.20 ± 0.50, 100.20 ± 19.0, 41.20 ± 15.0, respectively during pre-hemodialysis as compared to post- hemodialysis sessions where mean ± SD (TC 172.40 ± 22.90), (TG-137.90 ± 49.20), (LDL-C 90.58 ± 14.0), (VLDL-C ±32.90 ± 10.80) was observed respectively. However, in pre-dailysis, HDL concentration was significantly low with p =0.001 HS and mean ± SD 41.20 ± 0.50 was observed as compared to post-dialysis with mean ± SD 48.40 ± 3.20.

Table -2 Comparison Of Concentrations Of Blood Urea, Serum Creatinine And MDA Levels Among The Cases Of Crf During Pre-Hemodialysis And Post- Hemodialysis Sessions

Biochemical Parameters	Pre-Hemodialysis N=40	Post-Hemodialysis N=40	Mean Difference	P* Value, Significance
	Mean ± SD	Mean ± SD		
Blood urea mg/dL	69.50 ± 16.50	29.75 ± 9.71	39.75	0.001 HS
serum creatinine mg/dL	7.60 ± 2.80	3.68 ± 1.30	3.92	0.001HS
serum MDA nmol/L	3.68 ± 1.30	6.32 ± 1.09	0.58	0.009S

Student's paired't' test ; p<0.001 HS-Highly significant; p>0.009S-Significant

Table 2 shows comparison of different variables among pre-and post dialysis CRF patients. Blood urea (mean ± SD : 69.50 ± 16.50) & serum creatinine (mean ± SD :7.60 ± 2.80)were significantly raised (p=0.001)during pre- hemodialysis as compared to post-dialysis session with Blood urea (mean ± SD : 29.75 ± 9.71) and & serum creatinine (mean ± SD : 3.68 ± 1.30) Whereas MDA levels during pre-hemodialysis was comparatively significantly lower (p = 0.009) and (mean ± SD 3.68 ± 1.30)than and post- hemodialysis sessions with(mean ± SD 6.32 ± 1.09)

DISCUSSION
The high incidence of premature cardiovascular disease in patients with chronic kidney disease (CKD) has been well documented. Despite advances in the delivery of renal replacement therapy and the decline in mortality from cardiovascular disease in the general population, ischaemic heart disease presents a major source of morbidity, and remains one of the leading causes of death in patients with ESRD.[12]

It is well known fact that CRF is associated with dyslipidemia (Table 1) associating , hypercholesterolemia, hypertriglyceridemia, elevated LDL ,VLDL cholesterol, HDL metabolism is impaired and HDL-3 are not maturated into HDL-2 due to a lecithin-cholesterol acyl-transferase (LCAT). Earlier study stated that [13,14] renal dysfunction is associated with many perturbations in lipoprotein metabolism leading to dyslipidemia and accumulation of atherogenic particles. Since hepatic lipase has an important function in the removal of the triglyceride content of the IDL cholesterol and conversion of IDL cholesterol to LDL cholesterol, hepatic lipase deficiency in nephrotic syndrome leads to increased serum levels of atherogenic IDL cholesterol and triglyceride enrichment of the LDL cholesterol [13-16]

After dialysis, post heparin plasma lipoprotein lipase activity and hepatic lipase activity have been reduced, whereas the apo CII/apo CIII ratio is decreased. Disturbance in these enzymes, along with an increase in apo CIII in VLDL, leads to prolonged half-life of the VLDL particles, which eventually produced hypertriglyceridemia in CRF patients . However, the effects of long term hemodialysis on lipolytic activities are not be clarified. Limitated data showed about the effect of hemodialysis duration on dyslipidemias generated by CRF. [17]

On the contrary, low HDL –C results were obtained in present study in pre as compared post dialysis which are accordance with the studies of Asfaq Altaf, et al[18] and Nitin S Nagane, et al [19]. HDL functions as the transport of surplus cholesterol from the arterial wall to the liver for excretion by process, called 'reverse cholesterol transport', is critical for cellular cholesterol homeostasis and protection against atherosclerosis.[20] Patients with impaired renal function usually exhibit decreased levels of apolipoprotein AI and AII, diminished activity of LCAT as well as increased activity of cholesteryl transfer protein that facilitates the transfer of cholesterol esters from HDL to TG-rich lipoproteins thus reducing the serum concentrations of HDL-C. Hemodialysis procedure , type of membrane used may also have a contributory role in the reduced HDL-C levels of dialysis patients. [20]

Increased pre dialysis TC than in post and reverse in case of TC/HDL-C changes has also been documented in HD similar to the results observed by Asfaq Altaf, et al [18] and Nitin S Nagane, et al[19,21]

There was an improvement in lipid profile during post- hemodialysis session. 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.[22]

Present study (Table 2) showed about 54% and 52% reduction in the mean concentrations of blood urea and serum creatinine, respectively during post as compared to pre-hemodialysis CRF patients and was statistically highly significant (p<0.001HS). This is in accordance with studies of Meerashivshankar, et al [23] and Nitin S Nagane.[19]

Serum levels of malondialdehyde was elevated in post dialysis patients in present study(Table 2), suggesting increased lipid peroxidation in the presence of renal failure which was further exacerbated by dialysis. MDA is a sensitive marker of lipid peroxidation. ESRD patients are subjected to enhanced oxidative stress and increased levels of MDA.

A pro-inflammatory state and oxidative stress in patients on hemodialysis which is mediated through the production of ROS &

produce deleterious effect on the endothelium. Oxidation of PUFA within LDL cholesterol is the reponsible factor contributes to release of short chain aldehydes such as MDA.[7] Previous studies are in line with the our results shown by A Marjani,[24] Meerashivshankar, et al[23] and SatishKumar D, et al.[25]

It has been stated that hemodialysis (HD) represents a state of chronic stress for the patients where the oxidative reactions are mainly due to bioincompatibility of components of dialysis apparatus leading to the production of ROS by inflammatory cells. There is substantial evidence that those oxLDL accumulate, especially in HD patients .All these changes relate to oxidative stress and increased cardiovascular mortality in CRF patients.[26]

Recently it has been reported that there is an impairment of the antioxidant defenses in hemodialysis patients caused by diffusion and loss of hydrophilic antioxidants during the dialysis session.[27] When cells come in contact with the dialysis membrane they cause sensitization of cell membrane components leading to complement activation resulting in formation of other ROS which will initiate peroxidation of PUFA.[28]

Although hemodialysis leads to improvement of several biochemical parameters like creatinine, urea and plasma lipid patterns as seen in our study also (table 2), the superoxide radicals can initiate lipid peroxidation of fatty acid and can react with nitric oxide to form peroxynitrite radicals. The peroxynitrite radical can also get converted to hydroxyl radical and nitrates. The hydroxyl radicals can perpetuate lipid peroxidation.[22,23]

CONCLUSION

CRF patients with and without HD are at high risk of development of dyslipidemia. Other important mechanism considered is oxidative stress and inflammation. The association between oxidative stress and inflammation is crucially involved in promoting cardiovascular morbidity/mortality in CKD patients along with dyslipdemia. Keeping these alterations in mind, we conclude that there is a need of assessing marker that can identify extent of oxidative stress . Also would suggest strict monitoring of lipid profile parameters among these patients which will provide clinicians insight to alter the management modalities and nutritional supplementation strategy. So as to improve the general well being patients and can reduce the morbidity and mortality rate and will also improve the quality of life and lower the cost of health care in of CRF patients.

REFERENCES

1) Bargman JM, Skorecki K. Chronic Kidney Diseases. In: Fauci, Braunwald, Kasper, Hauser, Longo, Jameson, et al. Editors. Harrison's Principles of internal medicine vol 2. 18th ed. New York: Mc GrawHill;2012 p.2308-10
2) Oda H, Keane WF. Lipid abnormalities in end stage renal disease. Nephrol Dial Transplant 1998;13(1):45-49.
3) Wanner C. Importance of Hyperlipidemia and therapy in renal patients. Nephrol Dial Transplant 2000;15(5):92-96.
4) Locatelli F, Canaud B, Eckardt KW, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant 2003;18:1272-1280.
5) Daschner M, Lenhartz H, Botticher D, Schaefer F, Wollschlager M, Mehls O et al. Influence of Dialysis on Plasma Lipid Peroxidation products and antioxidant levels. Kidney Int1996;50:1268-72.
6) Spittle MA, Hoenich NA, Handelman GJ, Adhirkarla R, Homel P, Levin NW. Oxidative stress and inflammation in hemodialysis patients. American Journal of Kidney Diseases 2001;
7) Allan CC, Poon LC, Chan CSG, Richmond W, Fu PC. Enzymatic Determination of total serum cholesterol. Clin Chem 1974;20(4):470-475.
8) McGowan MW, Artiss JD, Strandbergh DR, Zak B. A Peroxidase- coupled Method for the Colorimetric Determination of Serum Triglycerides. Clin Chem 1983;29(3):538-542.
9) Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Journal of lipid Research 1970;(11).38(6).
10) Nader R, Warnick GR. Lipid, lipoproteins, apolipoproteins and other cardiovascular risk factors. In : Burtis CA, Ashwood ER and Bruns DA, eds. Tietz text book of clinical chemistry and molecular diagnostics, 6th edn. New Delhi: Elsevier Co., 2006;p.411-412.
11) Dillard, Kunert KJ, Tappel. Effects of vitamin E, Ascorbic acid and Mannitol on Alloxan induced lipid peroxidation in rats. Arch Biochem Biophysics 1982;216(1):204-12
12) C M . Loughrey et al Q J Med 1994; 87:679-683, Oxidative stress in haemodialysis,
13) Vaziri ND: Role of dyslipidemia in impairment of energy metabolism, oxidative stress, inflammation and cardiovascular disease in chronic kidney disease. Clin Exp Nephrol 2014;18:265–268].
14) K Amin, et al. Pattern of Dyslipidemia in patients with CRF. Professional Med J Mar 2006; 13(1): 79-84.
15) 4.K Janicki, et al. Abnormal lipoprotein metabolism in hemodialysis patients ,AnnalesUniversitatisMariae Curie - Skłodowska Lublin– Polonia 2007; Vol. LXII, N 1, 58 Section D.
16) E. P. Reddy, et al. 'Dyslipidemia: End Stage Renal Disease and Hemodialysis'. The Internet Journal of Nephrology 2009 : Volume 5 Number 1)
17) A Study of Lipid Profile in Chronic Renal Failure Patients Undergoing Hemodialysis Dr. Lokesh Rao Magar.S1, Dr.Anwar Miya Mohammad 2, Dr. Sandhya Anil.S IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 6 Ver. II (June. 2016), PP 01-03)
18) Altaf A, Halim A, Khan DA, Khalid M, Zuhra FT, Saif I. Assessment Of Lipid Dysfunction In Patients on Maintenance Haemodialysis. J Ayub Med Coll Abbottabad 2007;19(4).
19) Nagane NS, Ganu JV. Lipid Profile and serum Paraoxonase activity in CRF patients Pre and Pot-hemodialysis. Al Ameen J Sci 2011;4(1):61-68.
20) Tsimihodimos V, Mitrogianni Z, Elisaf M. Dyslipidemia Associated with Chronic Kidney Disease. The open Cardiovascular Medicine Journal 2011;(5):41-48.
21) Kwan BCH, Krononberg F, Beddhu S, Cheung AK. Lipoprotein Metabolism and Lipid Management in Chronic Kidney Disease. J Am Soc Nephrol 2007;18:1246-1261.
22) Sumathi M E, Tembad M, Murthy J, Preethi B P. Study of Lipid Profile and Oxidative Stress in CRF. Biomed Res 2010;21(4):451-6.
23) Meerashivashekar, William WE, Revathi R, Padmanbhan. Effect of Oxidative stress in Pre and Post Hemodialysis in Chronic renal Failure Patients. Int j Biol Med Res 2012;3(1):1335-1337
24) Marjani A. Clinical Effect of Haemodialysis on plasma lipid peroxidation and erythrocyte antioxidant enzyme activities in Gorgan (south east of Caspian sea). Indian J Nephrol 2005;15:214-217.
25) Satishkumar D, Vishali V, Indumati V, Kodliwadmath MV, Dverannavadgi BB, Candrakanth KH. Oxidative stress & Antioxidant in CRF patients before and after Dialysis. J Clinical diagnostic Research 2010; 4:2752-56.
26) Cohen DE, Fisher EA: Lipoprotein metabolism, dyslipidemia, and nonalcoholic fatty liver disease. Semin Liver Dis 2013;33:380– 388.)
27) Reddy PE, Manohar SM, Reddy SV, Bitla AR, Vishnubhotla S, Narsimha SPVL. Ferric Reducing Ability of Plasma and Lipid Peroxidation in Hemodialysis Patients: Intradialytic Changes. Int J Nephrol Urol 2010;2(3):414-421.
28) Patel ML, Rekha S, Srivastava AN. Dyslipidemia and oxidative stress in Maintenance Hemodialysis Patient – An Emerging Threat to Patient. International journal of Scientific and Research Publication 2012 April;2(4).