www.jmscr.igmpublication.org
Index Copernicus Value: 79.54
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v7i5.57

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Lipid Profile and Lipid Peroxidation Levels of Chronic Kidney Disease Patients (CKD) in Sokoto

Authors
C.A. Otitolaiye[1*], A.M. Makusidi[2], A. Onu[3], N.B Muhammad[1], H.B. Zainab[1]
[1]Department of Biochemistry, Sokoto State University, Sokoto
[2]Department of Medicine, Usmanu Danfodiyo University Teaching Hospital, Sokoto
[3]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto
*Corresponding Author
Catherine A. Otitolaiye
Department of Biochemistry, Sokoto State University, Sokoto
Mobile: 08067301981, Email: *catherineotits@yahoo.com*

Abstract
Introduction: *Chronic kidney disease (CKD) is a common and serious problem that adversely affects human health, limits longevity and increases costs of health-care systems worldwide. This study investigated lipid profile and lipid peroxidation levels as risk factors for cardiovascular disease among CKD patients.*
Method: *The study group consisted of 67 patients attending Nephrology Units of Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Specialist Hospital, Sokoto. The total cholesterol, triglycerides, HDL-C, LDL-C and VLDL-C were analyzed using standard procedures. Atherogenic Index (AIX) was calculated from the ratio of LDL-C to HDL-C. The markers of lipid peroxidation analysed were malondialdehyde (MDA) and oxidised-Low density lipoprotein (ox-LDL).*
Results: *The levels of LDL-C, VLDL-C and LDL-C/HDL-C ratio were significantly higher ($p<0.05$) while the level of HDL-C was significantly lower ($p<0.05$) among the CKD patients when compared to the healthy controls. The correlation of HDL with LDL/HDL ratio (Atherogenic index) showed a significant ($p = 0.0001$) negative correlation coefficient while LDL and LDL/HDL ratio showed a significant positive correlation coefficient. The plasma levels of malondialdehyde and oxidized LDL were significantly ($p<0.05$) higher in CKD patients than controls. There was a progressive increase in the plasma levels of both MDA and ox-LDL with advancing CKD. The correlation of eGFR with markers of lipid peroxidation indicated a significant ($p<0.0001$) negative correlation coefficient.*
Conclusion: *This study showed an increase in atherogenic index and lipid peroxidation products (MDA and ox-LDL) but a decreased HDL-c of the CKD patients, thereby increasing the cardiovascular risk of the CKD patients as CKD advances in stages.*
Keywords: *chronic kidney disease, malondialdehyde, atherogenic index, estimated glomerular filtration rate.*

Introduction

The main functions of the kidney include elimination of waste products, regulation of water, electrolyte and acid base balance, and the synthesis and regulation of hormones (Chung *et al.*, 2012). Kidney damage is defined as structural or functional abnormalities of the kidney, initially without decreased GFR which overtime can lead to decreased GFR (George and Neilson, 2008; NKF-KDOQ1, 2002). Kidney damage can be

acute or chronic. Markers of kidney damage include abnormalities in the composition of the blood or urine or abnormalities in imaging tests as well as GFR less than 60 ml/min/1.73m^2 for more than 3 months (NKF-KDOQI, 2002).

Lipid peroxidation is a crucial step in the pathogenesis of diseases including chronic kidney disease and cardiovascular diseases. Lipid peroxidation is a free radical related process which is harmful to the body and can cause distruption of membranes, lipids, proteins and other cell components. A lot of oxygenated compounds particularly aldehydes such as malondialdehyde, are produced during the attack of free radicals to membranes, lipoprotein and polyunsaturated fatty acids (Mahbood and Rahman, 2005). In addition, excess production of free radicals can alter the levels of antioxidants in the body and consequently result in lipid peroxidation products and oxidized low density lipoprotein. Oxidized LDL, a by-product of oxidative damage plays a pivotal role in the pathogenesis of atherosclerosis and thus cardiovascular disease (Ehara *et al*., 2012).

Cardiovascular disease is the leading cause of morbidity and mortality in CKD patients, occurring even at the earliest stages of CKD without manifesting vascular disease (Kooman *et al.*, 2014; Di-Angelantonio *et al.*, 2010; USRDS, 2009). Exposure to cardiovascular risk factors such as hypertension, dyslipidemia, diabetes mellitus, obesity, lack of physical exercise, smoking, genetics, increased age, family history and even depression, contributes to CVD in these patients (Ardhanari *et al.*, 2014; Dutta *et al.*, 2012).

This study investigated the levels of lipid peroxidation markers and the lipid profile of CKD patients in Sokoto, Nigeria.

Materials and Method

This study group consisted of 67 patients attending Nephrology Units of Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Specialist Hospital, Sokoto. The consent of the patients were obtained and questionnaires administered as approved by the Ethical Committees of Usmanu Danfodiyo University Teaching Hospital Sokoto and Specialist Hospital Sokoto.

Blood samples were collected from each patient using a lithium heparin vacutainer tube after an overnight fast. The blood samples were centrifuged at 5000rpm for 5mins, and the supernatant removed using a Pasteur pipette into labeled specimen tubes and then used to analyze creatinine, total cholesterol, triglycerides and HDL-C. The concentration of creatinine was determined using colorimetric method reported by Haeckel (1981). Creatinine reacts with picric acid to produce a coloured compound, creatinine alkaline picrate. The change in absorbance is directly proportional to the creatinine concentration. Cholesterol was determined by enzymatic hydrolysis and oxidation using the method of Flegg (1973).The indicator red quinone is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The absorbance of the dye is measured spectrophotometrically at 540nm.

Triacyglycerol was by the method of Schettler and Nussel, 1975. Triacylglycerols are estimated after an enzymatic hydrolysis with lipases. The indicator is quinoneimine formed from H_2O_2 and 4-aminoantipyine under the catalytic influence of peroxidase (POD). HDL-C was estimated by the method reported by Gordon *et al*., (1977).

Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C)was calculated according to the Friedewald Formula (Friedewald*et al*., 1974).

$$\mathrm{LDL-C} = \mathrm{TC} - (\mathrm{HDL-C} + {}^{\mathrm{TAG}}/_{2.2})$$

$$\mathrm{VLDL-C} = \frac{\mathrm{TAG}}{2.2}$$

Atherogenic Index (AIX)was calculated from the ratio of LDL-C to HDL-C (Ranjna, 1999).

$$\mathrm{AIX} = \frac{\mathrm{LDL-C}}{\mathrm{HDL-C}}$$

The estimated glomerular filtration rate (eGFR) of each participant was calculated from plasma creatinine utilizing the modification of diet in renal disease (MDRD) 4 variable equation (Levey *et al.*,1999).

$eGFR = 186.3 \times creatinine^{-1.154}) \times (age^{-0.203}) \times 1.212$ (if patient is black) x 0.742 (if female)

Serum creatinine is measured in mg/dl, age is in years, and GFR is expressed in ml/min per 1.73 m^2.The eGFR was used to assign CKD patients to each stage of CKD according to the NKF-KD0Q1 (2000) CKD classification method.

Stage 1 = Kidney damage with increased or normal eGFR (eGFR>90 ml/min/1.73m^2); n = 15

Stage 2 = Kidney damage with mild decreased eGFR (eGFR = 60 - 89 ml/min/1.73m^2); n =15

Stage 3 = Moderate decreased eGFR (eGFR 30-59 ml/min/1.73m^2); n = 10

Stage 4 = Severe eGFR (eGFR 15 - 29 ml/min/1.73m^2); n = 11

Stage 5 = Kidney failure (eGFR <15ml/min/1.73m^2); n = 16

Statistical Analysis

The results were expressed as Means ± SEM. Student's t-test was used to compare the healthy control and the CKD subjects. ANOVA was used to compare between the different stages and with the control with Dunnet post test. Pearson's correlation was used to determine the relations between the different variables. $P < 0.05$ was considered as statistically significant. Statistics was by Graph pad instat3 version 3.02, USA.

Result

The plasma creatinine level and eGFR of the CKD patients and apparently healthy control subjects are compared in Table 1. There was no statistical difference ($p<0.05$) in the age of the CKD patients and healthy control subjects. The mean plasma creatinine level of the patients was significantly increased ($p=0.0001$) and consequently, the eGFR of the patients was significantly decreased ($p=0.0001$) in comparison to apparently healthy control subjects.

Table 1: Plasma Levels of Creatinine and eGFR of CKD Patients

Parameters	Control (n = 15)	CKD Patients (n = 67)
Creatinine (mg/dl)	0.79 ± 0.05^a	3.55 ± 0.49^c
eGFR (ml/min/1.73m^2)	117.53 ± 7.99^a	49.97 ± 4.69^c

Values are mean ± standard error of means. Values with different superscript on the same row are statistically different at $p < 0.05$. eGFR = Estimated Glomerular Filtration Rate

The levels of the plasma creatinine and eGFR of CKD patients, at various stages of the disease are shown in **Fig 1.** The mean concentrations of creatinine steadily increased while the levels of eGFR decreased with advancing CKD.

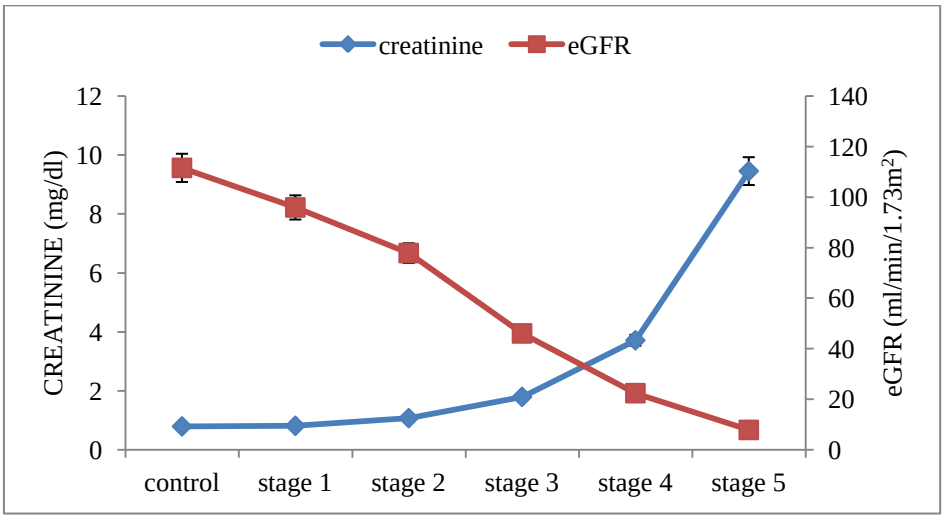

Fig 1: Plasma Levels of Creatinine (mg/ml) and eGFR (ml/min/1.73m^2) at Various Stages of CKD.

The plasma levels of malondialdehyde and oxidized LDL were significantly higher in CKD patients than controls ($p < 0.05$).

Table 2: Plasma Levels of MDA and ox-LDL in CKD Patients and Apparently Healthy Subjects

Parameters	Control (n = 15)	CKD Patients (n = 67)
Malondialdehyde (nmol/ml)	0.52 ± 0.03[a]	0.81 ± 0.04[c]
ox-LDL (ng/ml)	35.98 ± 4.11[a]	88.81 ± 6.38[c]

Values with different superscript on the same row are statistically different at $p < 0.05$.
MDA =Malondialdehyde, ox-LDL = Oxidized Low Density Lipoprotein.

Fig 2 shows progressive increase in the plasma levels of both MDA and ox-LDL with advancing CKD

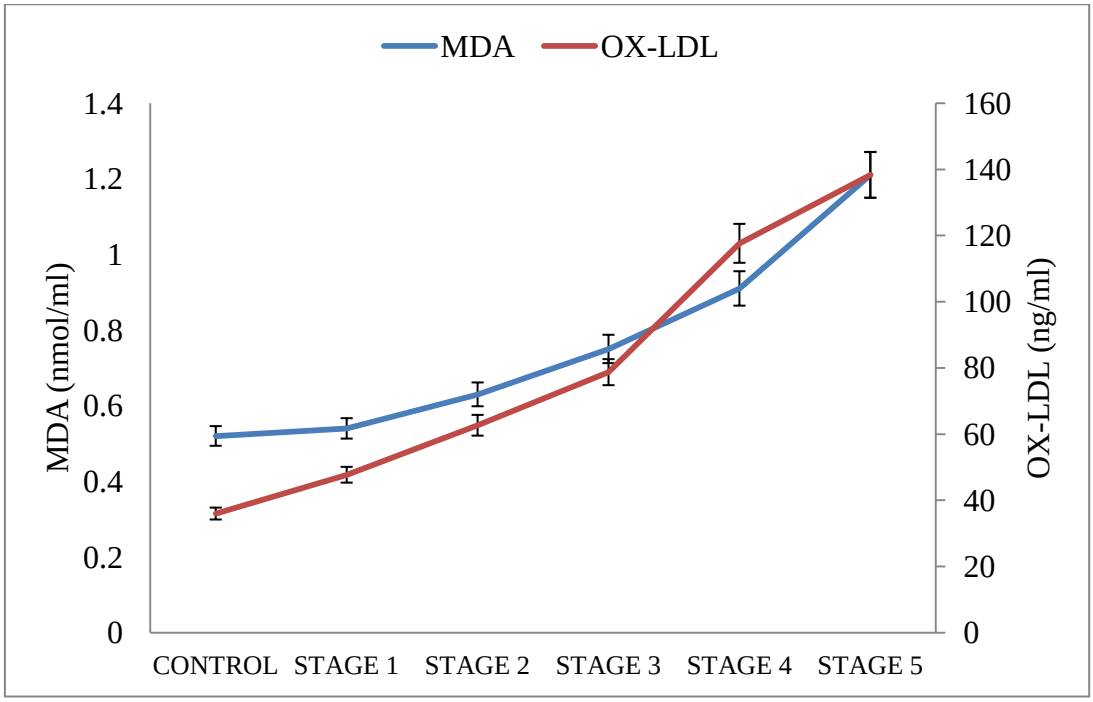

Fig 2: Plasma Levels of MDA and OX-LDL from stage 1 to 5 of CKD

MDA= Malondialdehyde. OX-LDL= Oxidised Low Density Lipoprotein.

The plasma lipid profiles of CKD patients when compared with control subjects are summarized in Table 3. The CKD patients had non-significantly ($p > 0.05$) higher level of total cholesterol and triglycerides when compared to the controls. There was however a significantly higher ($p < 0.05$) levels in LDL-C, VLDL-C and LDL/HDL ratio (atherogenic index), while a significantly lower ($p < 0.05$) levels in HDL-C of the CKD patients when compared to the controls.

Table 3: Plasma Lipid Profile of CKD Patients and Apparently Healthy Subjects

Lipid Profile	Control (n = 15)	CKD Patients (n = 67)
Total Cholesterol (mmol/L)	4.26 ± 0.19	4.78 ± 0.21
Triglycerides (mmol/L)	1.33 ± 0.12	1.65 ± 0.11
HDL-C (mmol/L)	1.69 ± 0.07 [a]	1.33 ±0.06[c]
LDL-C (mmol/L)	1.83 ± 0.16 [a]	2.66 ± 0.18 [c]
VLDL (mmol/L)	0.60 ± 0.05 [a]	0.75 ± 0.05 [b]
LDL/HDL RATIO	1.11 ± 0.11 [a]	2.29 ± 0.19 [c]

Values with different superscript on the same row are statistically different at $p < 0.05$ HDL-C = High Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol, VLDL-C = Very Low Density Lipoprotein Cholesterol, LDL/HDL Ratio = Atherogenic index.

The plasma lipid profile of CKD at various stages of the disease are presented in Fig 3. There was a steady decrease in total cholesterol and triglycerides from stage 1 to stage 5 CKD.

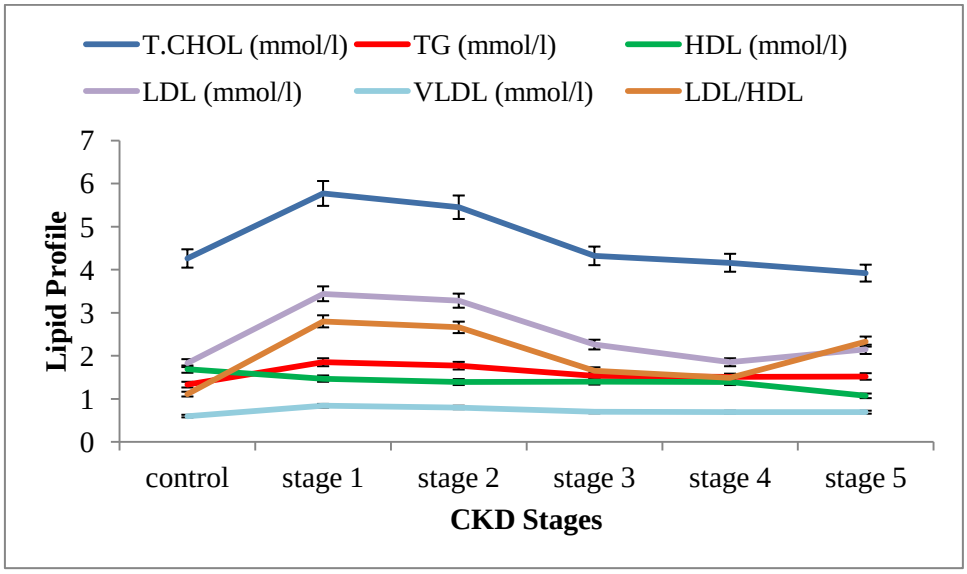

Fig 3: Plasma Lipid Profile at Various Stages of CKD and Apparently Healthy Controls in Sokoto

The correlation of eGFR with markers of oxidative stress indicated a significant ($p < 0.05$) negative correlation coefficient as shown in Fig 4a and 4b below.

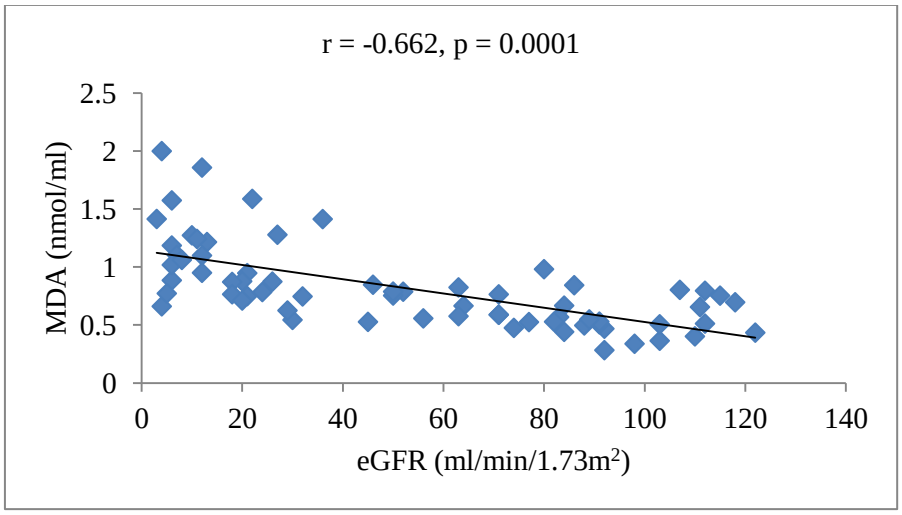

Fig 4a: Correlation Graph of eGFR and MDA Levels in CKD Patients

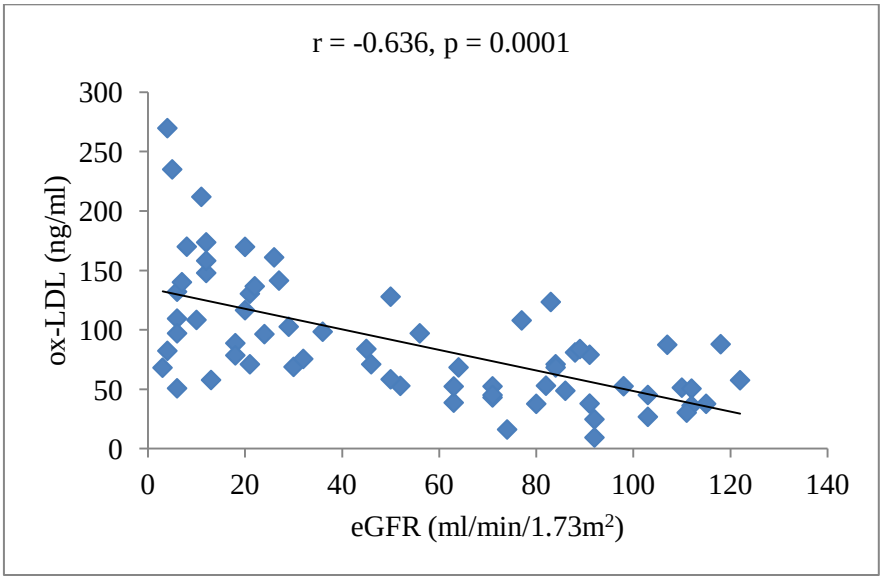

Fig 4b: Correlation Graph of eGFR and ox-LDL Levels in CKD Patients

HDL and LDL/HDL ratio (Atherogenic index) showed a significant negative correlation coefficient as shown in Fig 5a. LDL and LDL/HDL ratio showed a significant positive correlation coefficient (Fig 5b).

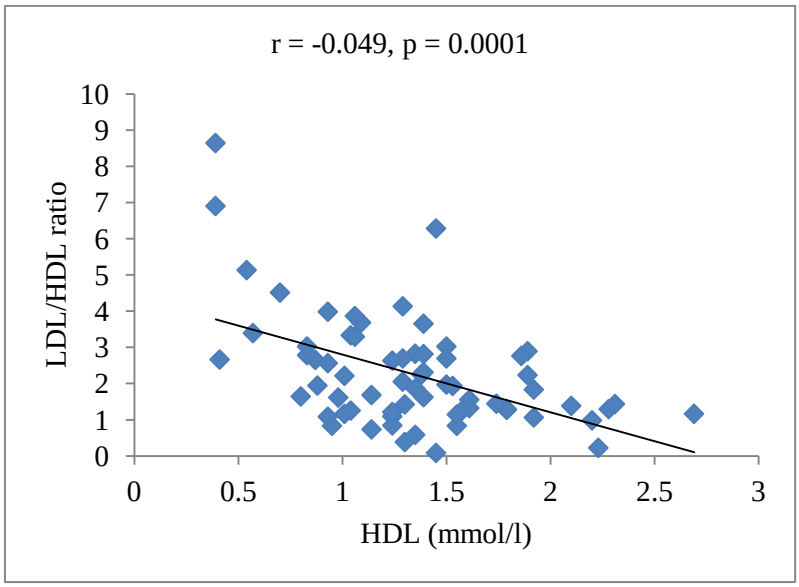

Fig 5a: Correlation Graph of HDL and LDL/HDL Ratio in CKD Patients

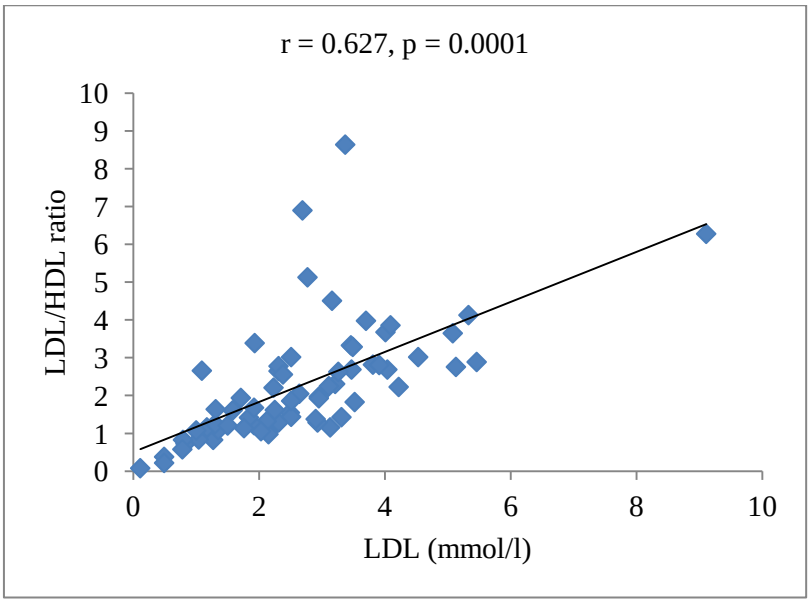

Fig 5b: Correlation Graph of LDL and LDL/HDL Ratio in CKD Patients

Discussion

Cardiovascular disease constitutes the major risk of morbidity and mortality in CKD patients (USRDS, 2009). Elevated lipid peroxidation markers (Malondialdehyde and Oxidized-LDL) and dyslipidemia are cardiovascular risk factors examined in this study. MDA (Malondialdehyde) is a low molecular weight hydro-soluble molecule, which can be cleared by the kidneys. It is increased in kidney dysfunction. This study showed the highest increase in MDA and ox-LDL was at CKD stage 5 (hemodialysis group). Oxidized-LDL (ox-LDL) is a highly atherogenic molecule which could initiate an inflammatory process in blood vessels and consequently result in myocardial infarction and stroke (Putri and Thaha, 2014; Ross, 1999). The increased levels of the lipid peroxidation markers at stage 1 CKD shows the process could occur at early stages of the CKD.

According to Ardhanari (2014) Dyslipidemia (higher LDL and lower HDL- C) is associated

with atherosclerotic vascular disease and is an increased risk of cardiovascular events. Likewise, National Cholesterol Education Program (NCEP) guidelines have identified dyslipidemia, and in particular, elevated levels of low density lipoprotein cholesterol (LDL-C) as the risk factor for cardiovascular events in the general population (NCEP, 2001). An increased levels of total cholesterol, triglycerides, LDL and 2- fold increase in LDL/HDL ratio was observed in this study and are characteristics of dyslipidemia (NCEP, 2001). There was an elevation of total cholesterol, triglycerides and LDL-C in patients at stages 1 and 2 but as the CKD advances to kidney failure, there was decrease in the mean cholesterol, triglycerides and LDL-C. This could be due in part to malnutrition (Weiner and Sarnak, 2004) or effect of lipid lowering drugs on the CKD patients. The increased LDL in CKD could result into an influx of LDL into the endothelial wall where it is being oxidized by free radicals, to initiate a cascade of events leading to increased inflammation and endothelial injury (Putri and Thaha, 2014). Increase in LDL and atherogenic (LDL/HDL) ratio makes the CKD patients highly susceptible to cardiovascular disease.

High levels of LDL cholesterol and low levels of HDL cholesterol could be independently associated with endothelial dysfunction and inflammation (Mineo *et al*., 2006). This occurs by changes in vascular permeability that leads to the entry of LDL cholesterol into intimal layer leading to oxidized LDL. As such, the circulating levels of ox-LDL depend on the degree of both oxidative stress and the number of LDL particles. Oxidation can further cause LDL not to be recognized by LDL receptors on the cell thereby causing less deposition of LDL in intracellular pools. According to Bowden and Wilson (2010), less deposition can lead to the same amount of circulating LDL cholesterol being associated with smaller and denser LDL particles which carry more risk for CVD. Excess LDL cholesterol is then removed from circulation primarily by being engulfed by macrophages which in turn can lead to more atherogenic foam cells (Bowden and Wilson, 2010), thereby increasing the CVD risk in the CKD patients.

In this current study, there was a significant negative correlation between MDA and HDL-C. This implies that elevated malondialdehyde could decrease the high density lipoprotein cholesterol, thereby decreasing the transportation of cholesterol from the peripheral tissue to the liver for degradation and removal, and consequently increasing the risk for cardiovascular disease. This study has shown a highly significant ($p = 0.0001$) negative correlation between HDL-C and LDL-C/HDL-C ratio, a highly significant ($p = 0.0001$) positive correlation between LDL-C and LDL-C/HDL-C. These suggest reduced levels of HDL and increased levels of LDL-C could increase the atherogenicity of the CKD patients, thereby predispose them to cardiovascular burden. The results have shown that decreased HDL-C and consequent elevated LDL-C/HDL-C ratio could increase the risk of CVD mortality among the CKD patients.

Conclusion

This study investigated lipid peroxidation levels and the lipid profile of CKD patients as risk factors for cardiovascular disease. The patients were shown to have increased lipid peroxidation products (MDA and ox-LDL), elevated LDL-C/HDL-C and decreased HDL-C as the CKD advanced thereby increasing their cardiovascular burden.

References

1. Ardhanari S., Alpert M.A. and Aggarwal K. (2014) Cardiovascular disease in CKD: Risk factors, Pathogenesis, and Prevention. *Advances in Peritoneal dialysis*. Vol 30;40-53
2. Bowden and Wilson
3. Chung S., Koh E. S., Shin S. J. and Park C. W. (2012) Malnutrition in patients with chronic kidney disease. *Open Journal of Internal Medicine*.2: 89-99
4. Di-Angelantonio E., Chowdhury R., Sarwar N., Aspelund T., Danesh J., and

Gudnason V. (2010) Chronic kidney disease and risk of major cardiovascular disease and non-vascular mortality: prospective population based cohort study. *British Medical Journal* 341:c4986

5. Dutta D., Calvani R., Bernabei R., Leeuwenburgh C. and Marzetti E. (2012) Contribution of impaired mitochondrial autophagy to cardiac aging: mechanisms and therapeutic opportunities. *Circulation Research.* 110(8): 1125-38
6. Ehara H., Yamamoto-Honda R., Kitazato H., Takahashi Y., Kawazu S., and Akanuma Y. (2012) ApoE isoforms, treatment of diabetes and the risk of coronary heart disease. *World Journal of Diabetes*. 15;3(3):54-9.
7. Flegg H.M. (1973) An investigation of the determination of serum cholesterol by an enzymatic method. *Annals of Clinical Biochemistry* 10: 79-84
8. Friedewald W.T., Levy R.I. and Fredrickson D.S. (1994). Estimation of the concentration of Low-Density Lipoprotein in plasma, without the use of preparative ultracentrifuge. *Clinical Chemistry* 18(6): 499-502.
9. George A, and Neilson E. (2008) Disorder of the kidney and urinary tract. In: Fauci A, eds. Harrison's principle of internal medicine. Birmingham: McGraw-Hill Professional p. 1741-75
10. Gordon T., Castelli W.P., Hjortland M.C., Kannal W.B. and Dawber T.R. (1977) High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *American Journal of Medicine* 62(5):707-714
11. Haeckel R. (1981) Assay of creatinine in serum, with use of fuller's earth to remove interference. *Clinical Chemistry* 27(1): 179-183
12. Kooman J.P., Kotanko P., Schols A.M., Shiels P.G. and Stenvinkel P. (2014) Chronic kidney disease and premature ageing. *Nature Reviews Nephrology* 10: 732-742
13. Levey A.S., Coresh J., Balk E., Kausz A.T., Levin A., Stett M.W., and Eknoyan G. (1999) National Kidney Foundation Practice guidelines for chronic kidney disease: Evaluation, clasification and stratification. *Annals of Internal Medicine* 139(2): 137-147
14. Mahbood M.F. and Rahman P.G. (2005) Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic subjects. *Singapore Medical Journal*. 46: 322-324.
15. National Cholesterol Education program NCEP (2001) Executive summary of the third report of the National Cholesterol Education Program: Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment panel III). *The Journal of the American Medical Association* 285: 2486-2497
16. National Kidney Foundation-KD0Q1 (2000) Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification.Part 4: Definition and classification of Stages of Chronic Kidney Disease.
17. www2.kidney.org/professionals/KDOQ1/guidelines_ckd/p4_class_g1.htm
18. National Kidney Foundation-KD0Q1 (2002) Clinical practice guidelines for chronic kidney disease: Evaluation, classification and stratification. Part 4, guideline 1. *American Journal of Kidney Disease* 39: S1-S1000
19. Putri A. Y. and Thaha M. (2014) Role of oxidative stress on chronic kidney disease progression. Acta Medica Indonesiana-*The Indonesian Journal of Internal Medicine.* Vol 46, no 3: 244-252
20. Ross R. (1999) Atherosclerosis, an inflammatory disease. *New England Journal of Medicine* 340: 115-126

21. Schettler G. and Nussel E. (1975) Determination of triglycerides. *Arab Medical Sozial Medical Journal* 502(10):25-8
22. US Renal Data System, USRDS (2009) "Atlas of end-stage renal disease in the United States," Annual Data Report, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Md, USA.
23. Weiner D.E. and Sarnak M.J. (2004) Managing dyslipidaemia in chronic kidney disease. *Journal of General Internal Medicine* 19:1045.