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Biochemical Assessment of Atherosclerosis in Sudanese Patients with Chronic Renal Failure on Hemodialysis Maintenance Therapy

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OFI is the supervisor of the study. Author SA managed the analyses of the study. Author AEAA is the co-supervisor of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Renal manifestations are becoming a very important health problem in the developing world. In Sudan, the new cases account for about 70-140 thousand of inhabitants per year. Leading to reduced quality of life, these manifestations have negative social and economic impact on the population.
Atherosclerosis is a very frequent complication in uremia due to the coexistence of hypertension,

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hyperhomocysteinemia, inflammation, malnutrition, increased oxidative stress and hyperlipidemia.
Alteration in structural and functional ability of high-density lipoprotein cholesterol (HDL), Low-density lipoprotein cholesterol (LDL), apolipoprotein (A), apolipoprotein (B), and Lipoprotein (a) were also associated with atherosclerosis.

Purpose: The aim of this study was to determine serum level of total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, lipoprotein (a), fibrinogen and homocysteine as markers for atherosclerosis and to measure plasma homocysteine to assess endothelial Cell Dysfunction and vascular disease.

Materials and Methods: A total of one hundred patients with Chronic Kidney Disease on Dialysis and one hundred sex and age-matched healthy control subjects were enrolled in this study; after informed consent six milliliters of venous blood were collected after hemodialysis session from each patient and healthy subjects after an overnight fasting in a plain container from which serum is separated and then stored in Eppendorf tubes at -80C°. Before analysis, frozen specimens were thawed and allowed to reach room temperature for various measurements.

Results: The results showed that there was increased in the mean level of serum triglycerides (277.45±59.47) (P. value >0.001), Lp(a) (60.29±20.66) (P. value >0.001), Homocysteine (24.30±6.37) (P. value >0.001)and Fibrinogen (498.66±56.48) (P. value >0.001) among the Chronic Kidney Disease patients on maintenance hemodialysis therapy when compared to the control group.

The results also showed that there was a reduced level of HDL-cholesterol (24.76±6.46) (P. value >0.001) among the Chronic Kidney Disease stage on dialysis when compared to the control group.

Furthermore, the results showed that there was positive correlation between serum Lipoprotein (a) with plasma fibrinogen level and homocysteine

Conclusion: In conclusion, the significant increase in Plasma triglyceride concentration, Lp (a), Homocysteine and Fibrinogen level, as well as decreased HDL-Cholesterol level among CKD patients on regular hemodialysis maintenance therapy place them at risk of developing cardiovascular disease.

This risk is also evident in the positive correlation between serum Lipoprotein (a) with plasma fibrinogen level and homocysteine and these findings showed that homocysteine enhanced the binding of Lipoprotein (a) to Fibrin and this interaction linked between Thrombosis and Atherosclerosis.

Keywords: CKD: Chronic kidney disease; ESRD: End stage renal disease; HD: Hemodialysis atherosclerosis and biochemical assessment.

1. INTRODUCTION

Cardiovascular disease is the leading cause of death on dialysis patients, followed by infections, myocardial infarction, strokes and ischemic events of the limbs [1]. The risk of cardiovascular disease is significantly increased if GFR has fallen below approximately 75 ml/min and the damage is progressed when patients reach end-stage renal disease [2]; thus, evaluation of traditional risk factors, including lipid profile, blood pressure, smoking, and stable lifestyle, is essential [3]. Atherosclerotic heart disease accounts for approximately 55% of mortality in CKD patients and furthermore, it contributes to a 20-fold increase in ischemic heart disease and to a 10-fold increase in the risk of stroke among HD patients with ESRD [4].

Atherosclerosis is a very frequent complication in uremia due to the coexistence of hypertension, hyperhomocysteinemia, inflammation, malnutrition, increased oxidative stress and hyperlipidemia.

Alteration in structural and functional ability of high-density lipoprotein cholesterol (HDL), Low-density lipoprotein cholesterol (LDL), apolipoprotein (A), apolipoprotein (B), and Lipoprotein (a) were also associated with atherosclerosis [5].

1.1 Serum Lipids in Patients with MIA Syndrome

Lipid abnormalities are common in patients with CKD on hemodialysis therapy which participates to the high incidence of Ischemic heart disease which is the major cause of death in HD patients, furthermore, the incidence of cardiovascular complications is also abnormally elevated in predialysis patients with CKD [6].

Lipid metabolism influenced by the severity of renal dysfunction and the patients with chronic kidney disease characterised by an elevated level of triglycerides due to increased production of very-low-density lipoproteins (VLDL) in the liver and decreased activity of hepatic lipase and peripheral lipoprotein lipase [7]. These abnormalities, successively, may be due to an inhibitory effect of hyperparathyroidism which in turn leads to accumulation of the calcium in islet cells resulting in Hyperinsulinemia which consider as the main factor increasing synthesis of triglycerides and directly decreases the activity of lipoprotein lipase [8]. The most distinguished changes in lipid metabolism are increased serum triglyceride levels and low levels of high-density lipoprotein (HDL) cholesterol. Low-density lipoprotein (LDL) cholesterol levels are often normal, but the cholesterol may be slightly elevated derived from the atherogenic small and dense LDL subclass [8].

1.2 Lipoprotein (a) [Lp(a)]

Various cell types in the kidney express the LDL receptor-related protein, consider to have a role in the catabolism of Lp(a) [9]. Lp (a) may have participated in the pathogenesis of kidney disease. Fragments of apolipoprotein (a) are present in human atheroma and their probable bioactivity has been suggested by in vitro and cell culture studies [10]. It was observed that elevation of Lp (a) in CKD patients was dependent on apo (a) phenotype.Only renal patients with high molecular weight apo(a) phenotypes expressed higher median Lp (a) concentrations [11]. Lp(a) may be involved in the pathogenesis of kidney disease [12].

Studies considering the apo (a) size polymorphism concluded that the apo (a) gene locus determines the risk for cardiovascular disease through its allelic control of Lp (a) concentrations [13]. Dialysis patients with low molecular weight apo (a) phenotypes have a two–to–three fold higher prevalence as well as incidence of major cardiovascular events than those with high molecular weight apo (a) types [14].

1.3 Fibrinogen

Fibrinogen is a positive acute phase protein and plasma fibrinogen levels were associated with elevated CRP and with the presence of abnormalities of cardiovascular system including left ventricular hypertrophy, arterial stiffness or systolic myocardial dysfunction. Fibrinogen levels were consistently elevated in patients with CKD and in dialysis patients [15].

1.4 Homocysteine

Increased homocysteine levels in patients on dialysis therapy may contribute to the excess vascular morbidity [16]. Hyperhomocysteinemia is an independent risk factor for vascular disease in both healthy population and dialysis patients [17]. Elevated homocysteine and Lp (a) levels in CKD patients might be particularly atherogenic because of the potential biochemical interactions between these two risk factors. It is postulated that Lp (a) may compete with plasminogen for binding to fibrin and homocysteine may enhance the binding of Lp(a) to fibrin, thereby potentiating the atherogenecity of Lp(a) [18].

1.5 Objective

The main goal of this study is to detect atherosclerosis in Sudanese patients with chronic renal failure on maintenance hemodialysis therapy at the biochemical and immunological level.

1.6 Hypotheses

Patients with CKD on maintenance hemodialysis therapy will show evidence of increased risk of atherosclerosis.

2. MATERIALS AND METHODS

This is a case control study conducted at Ahmed Gasem Hospital, Omudrman Teaching Hospital, Khartoum Teaching Hospital and Ibn Sina Speacialized Hospital from December 2014 to December 2016, these hospitals represent all the three cities of tricapital Khartoum. Sudanese patients with CKD whom routinely attend to dialysis centre at all above-mentioned hospitals during the period of the study were randomly recruited for this study.

The study included one hundred CKD patients on regular hemodialysis maintenance therapy and one hundred age and sex matched healthy controls, the sample size was derived by using the Fleiss formula for a case control study using the following information [19]: Confidence interval = 95%, power of study = 80%, ratio of cases to control of 1:1, percentage of control exposed: 8.7% and percentage of cases exposed: 26%, this formula gave a minimum sample size of 75 for cases and 75 for control.

None of the patients suffered from any symptoms of infections or presented with clinical signs of infection (Hepatitis B, Hepatitis C and HIV), malignancy and active immunological disorders. Furthermore the patients did not receive any medications known to affect immune functions or drugs like steroids and antioxidant which were known to alter serum lipid levels.

All participants gave informed consent before enrolling in the study and the study was approved by Alneelain University Ethical Committee.

The patients' information such as age, sex and clinical history were recorded.

Blood samples (6 ml) were collected after the hemodialysis session from patients in plain containers after an overnight fasting from which serum is separated and then stored in Eppendorf tubes at -80C°. Before analysis, frozen specimens were thawed and allowed to reach room temperature for various measurements.

Serum triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol were measured by enzymatic method using Cobas C311 analyser (Roche Diagnostics, Germany).

Serum Lp (a), Homocysteine and Fibrinogen were measured by sandwich Enzyme-Linked Immunosorbent Assay using Stat Fax Microstrip Reader (Awareness Technology, USA).

2.1 Statistical Analysis

The t-test was employed to compare differences between the mean concentrations of study parameters. P-value 0.05 was considered statistically significant. Data were analysed by SPSS (Version 16.0; SPSS Inc).

3. RESULTS

This is a case–control study, included 100 patients with CKD stage 5 patients on regular hemodialysis maintenance therapy, 54% of them were males and 46% were females, their ages ranged between 16-81 years and the mean age was 43 years.

The results obtained revealed that hypertension was the primary cause of CKD (35.0% of respondents) while diabetes mellitus accounted for 30.0% of cases. However, UTI and glomerulonephritis were identified to be the primary cause in 8.0% and 7.0% of cases, respectively. Other identified causes, were Lupus Nephritis (4.0%), Autosomal Dominant Polycystic Kidney Disease (3.0%), Gout (4.0%), Renal Stone (5.0%) and Obstructive Uropathy (4.0%) (Fig. 1).

The results in Table 1 showed that the mean level of triglycerides (277.45 mg/dl) was higher in CKD patients compared to the control group (120.90 mg/dl) with significant differeces between the two groups (P. value >0.001).

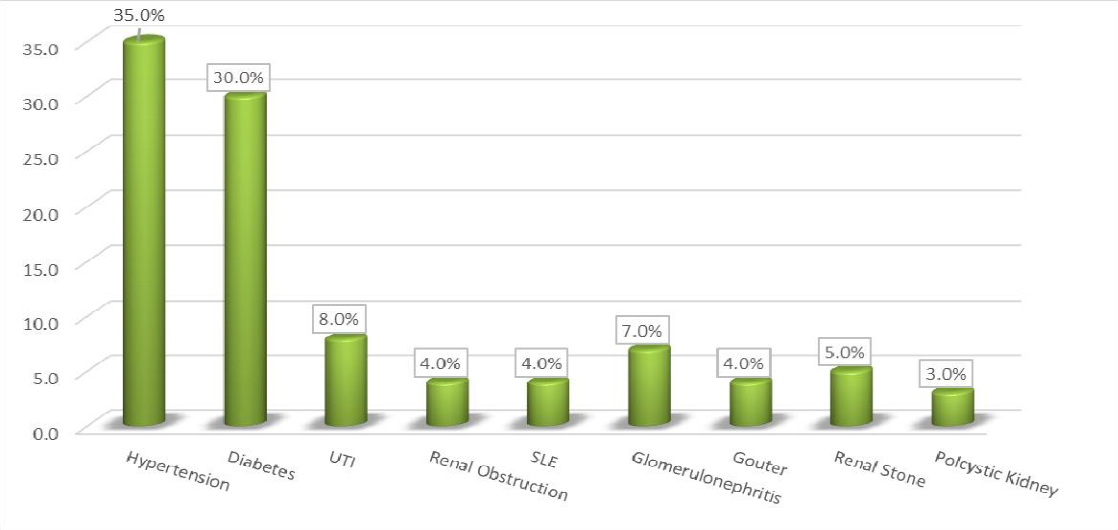

Fig. 1. The main causes of chronic kidney disease in Sudan

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The results in Table 1 revealed that the mean level of total cholesterol (137.26 mg/dl) was higher in CKD patients compared to the control group (133.31 mg/dl) with significant different between the two groups (P. value 0.143).

The results in Table 1 displayed that the mean level of HDL-cholesterol (24.76 mg/dl) was lower in CKD patients compared to the control group (86.28 mg/dl) with significant different between the two groups (P. value >0.001).

The results in Table 1 showed that the mean level of LDL-cholesterol (58.13 mg/dl) was higher in CKD patients compared to the control group (55.14 mg/dl) with significant differences between the two groups (P. value 0.304).

The results in Table 1 revealed that the mean level of Lp (a) (60.29 mg/dl) was higher in CKD patients compared to the control group (7.57 mg/dl) with significant different between the two groups (P. value >0.001).

The results in Table 1 displayed that the mean level of Homocysteine (24.30 umole/l) was higher in CKD patients compared to the control group (7.49 umole/l) with significant different between the two groups (P. value >0.001).

Furthermore, the mean level of Fibrinogen (498.66 mg/dl) was higher in CKD patients compared to the control group (284.98 mg/dl) with significant different between the two groups (P. value >0.001).

4. DISCUSSION

Chronic kidney disease is a dynamic disease governed by multiple factors that affect its progression and prognosis. The prevalence of CKD in Sudan in different communities is 0.7% of the adult population, In Sudan, the estimated incidence of new cases of CKD patients is 70–140 per million inhabitants/year [20].

The principal aim of the present study was to determine the association of atherosclerosis with chronic kidney disease patients on maintenance hemodialysis.

The present study revealed that the most common quantitative lipid abnormalities in CKD patients were increased triglycerides level, as well as low levels of high-density lipoprotein (HDL) cholesterol, while the Levels of total cholesterol, and low-density lipoprotein (LDL-C) cholesterol, however, were usually not elevated. These findings were in agreement with the results reported by Nosratola et al. [21] who showed that plasma total cholesterol and LDL cholesterol concentrations were usually normal in CDK patients on maintained hemodialysis while plasma triglycerides and very low-density lipoprotein (VLDL) levels were elevated, Also his findings showed that The other major CKD-induced lipid disorder is significant reduction in serum apoA-1 and high-density lipoprotein (HDL) cholesterol concentration.

Table 1. The level of total-cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, lipoprotein (a), homocysteine and fibrinogen in CKD patients group compared to the control group

Study groups / Parameters	CKD patients (Case group)		Healthy individuals (Control group)		P. value
	Mean	SD	Mean	SD	
Total cholesterol mg/dl	137.26	19.865	133.31	18.118	0.143
Triglycerides mg/dl	277.45	59.474	120.90	51.254	>0.001
HDL-cholesterol mg/dl	24.76	6.458	86.28	13.680	>0.001
LDL-cholesterol mg/dl	58.13	20.774	55.14	20.249	0.304
Lp (a) mg/dl	60.29	20.663	7.57	5.585	>0.001
Homocysteine umole/l	24.30	6.378	7.49	3.102	>0.001
Fibrinogen mg/dl	498.66	56.478	284.98	41.463	>0.001

Table 2. Pearson correlations of serum Lp(a) with the level of serum fibrinogen

Thrombogenic marker fibrinogen / Lipoprotein (a)	Fibrinogen mg/dl	
	Pearson correlation (r)	P. value
Lp(a) mg/dl	0.810	>0.001

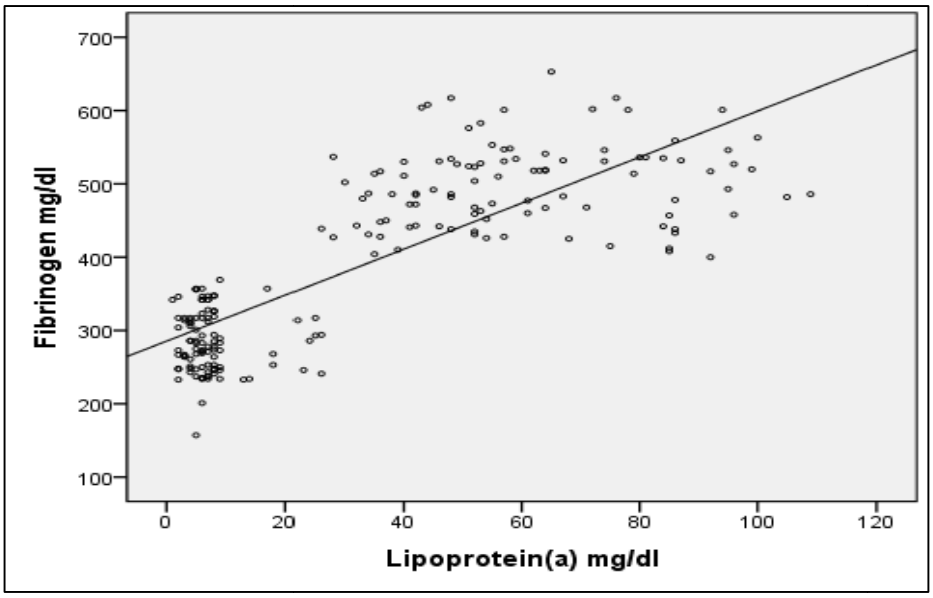

Fig. 2. Correlation between serum lipoprotein (a) and plasma fibrinogen levels in CKD patients group (n=100, r=0.810, p<0.02)

Table 3. Pearson correlations of Serum Lp(a) with the level of serum homocysteine

Atherogenic marker homocysteine / Lipoprotein (a)	Homocysteine umole/l	
	Pearson correlation (r)	P. value
Lp(a) mg/dl	0.763	>0.001

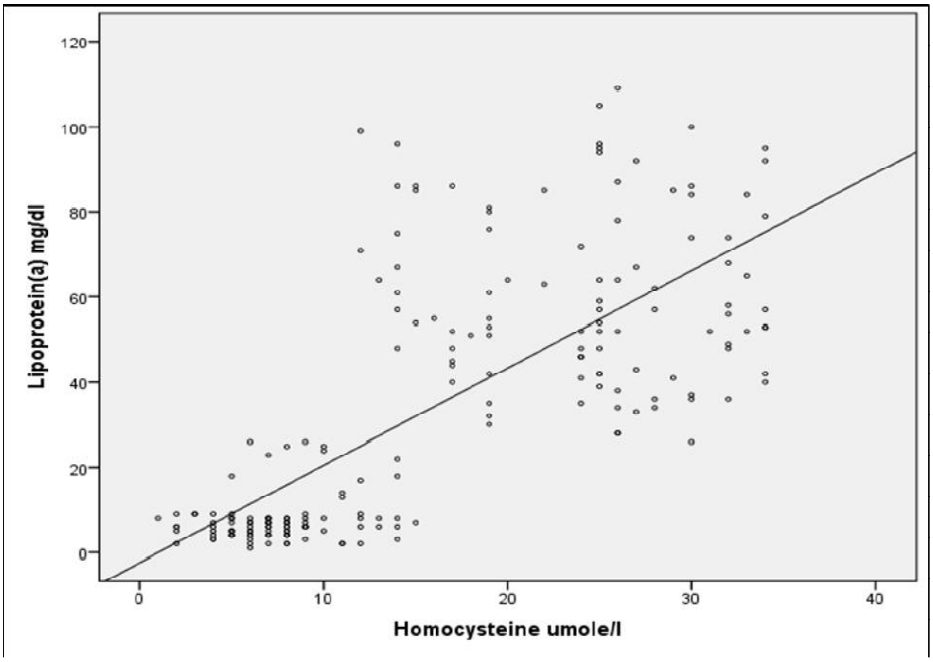

Fig. 3. Correlation between serum lipoprotein (a) and plasma fibrinogen levels in CKD patients group (n=100, r=0.810, p<0.02)

The present study disagreed with study done by Fathia et al. [22], she conducted study in Sudanese patients and reported that all chronic renal failure patients had normal triglyceride, total cholesterol, LDL-cholesterol and HDL-cholesterol levels, the variation of the results could be attributed to the differences in diagnostic precision, assay sensitivity and the small sample size used her that study.

When plasma Lipoprotein (a) was analyzed, the result obtained revealed that the mean Lp(a) level in CKD patients was significantly high than those obtained among the control group, these findings was agreement with the result reported by Valdete et al. [23] who reported that Lp(a) level started to increased early during the course of CKD and it was an independent risk factor for atherosclerotic cardiovascular disease in patients on chronic hemodialysis.

In this, study the mean plasma fibrinogen level was significantly increased in CKD patients than those obtained by the control group, which is in agreement with Bostom et al. [24], who showed Hyperhomocysteinemia, hyperfibrinogenemia and lipoprotein(a) excess were common in maintenance dialysis patients.

Furthermore, in the present study, the mean plasma homocysteine level was significantly increased in CKD patients than those obtained by the control group, which in agreement with finding obtained by Friedman et al. [25] who reported that hyperhomocysteinemia was common in patients with chronic renal failure.

In this study, we investigated any possible correlation between Lipoprotein (a) which recognised as an atherogenic and thrombogenic lipoprotein and fibrinogen which act as atherothrombosis proteins. We found that elevated serum Lipoprotein (a) levels were positively correlated with increased plasma fibrinogen level and these relations between those atherothrombogenic acute-phase proteins were contributed to the increased incidence of atherosclerotic cardiovascular disease (CVD) and its complications in CDK patients. These findings were agreed with the results reported by Bostom et al. [24], who showed Hyperhomocysteinemia, hyperfibrinogenemia and lipoprotein (a) excess were common in maintenance dialysis patients.

Furthermore, we investigate any possible correlation between Lipoprotein (a) and homocysteine in CKD patients. We found that elevated serum Lipoprotein (a) level was positively correlated with increased plasma homocysteine and these findings were consistent with the results reported by Harpel et al. [18] who conducted a study on USA CKD patients and his findings showed that homocysteine and other sulfhydryl compounds enhanced the binding of Lipoprotein (a) to Fibrin and this interaction linked between Thrombosis and Atherosclerosis.

5. CONCLUSION

In conclusion, the significant increase in Plasma triglyceride concentration, Lp (a), Homocysteine and Fibrinogen level, as well as decreased HDL-C level among End Stage Renal Disease patients on regular hemodialysis maintenance therapy place them at risk of developing cardiovascular disease.

This risk is also evident in the positive correlation between serum Lipoprotein (a) with plasma fibrinogen level and homocysteine and this findings showed that homocysteine and enhanced the binding of Lipoprotein (a) to Fibrin and this interaction linked between Thrombosis and Atherosclerosis.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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