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Estimation of Homocysteine and Lipid Profile in Sudanese Patients with End Stage Renal Diseases

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Abstract

Previous studies showed the link between hyperhomocysteinemia (HHcy), dyslipidemia, and Atherosclerosis among End stage renal disease. However, there was limited epidemic data concerning Sudanese patient and risk associated with hyperhomocsteinemia. This study aims to investigate the association of plasma homocysteine (Hcy) level with lipid profiles in End stage renal disease compare to control group. A total of 120 Sudanese subjects from a cohort of the Khartoum state were included in the analysis. Plasma total Hcy, serum lipid files including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) were measured to evaluate associations of Hcy and lipid profiles in ESRD and control group. Subjects were 47.18 ± 8.91 years old, and 58.33% were male. Median Hcy was 21.85 ± 1.8 µmol/L for patients, and 12.11± 0.9 for healthy individuals. HHcy was associated with increasing risk of low HDL-C (HDL-C < 50 mg/dL; and hypertriglyceridemia (TG ≥ 150 mg/dL, there were significant different association between HHcy and TC or LDL-C. The present study showed that HHcy was associated with hypertriglyceridemia and low levels of HDL-C in end stage renal disease compare to healthy individual group, which provides evidence that Hcy levels might affect HDL-C and TG metabolism and may increase risk factor for developing cardiovascular diseases.

Key words: Homocysteine, Lipid Profile, Sudanese Patients, End Stage Renal Disease

Introduction

Homocysteine (Hcy) is nonprotein amino acid derived from dietary methionine after trans-methylation of biologically important molecules. Hcy is found in the plasma of all mammals, and the term “total homocysteine” (tHcy) is used to describe a composite of free (sulfhydryl), protein-disulfide bound, and homocysteine-cysteine and other mixed disulfide species (Mudd *et al.*, 2000).

Disturbances in intracellular homocysteine metabolism may lead to elevated plasma homocysteine concentrations and its multifactorial including genetic background, such as an inherited enzyme deficiency, a variation in the genes encoding these enzymes or an environmental aetiology, such as diet and lifestyle factors, which could lead to depletion of important cofactors or substrates involved in homocysteine metabolism or even inhibited enzyme activity (Kwok *et al* 2001).

Elevated plasma tHcy (hyper-homocysteinemia) is almost universal among patients who have a renal disease and require haemodialysis (Friedman 2002). Recent studies have reported a reversed epidemiology in chronic kidney disease (CKD) patients where low, rather than high plasma Hcy is an indicator of poor outcome (Suliman et *al.*, 2007). However, this association remains unclear because wasting and inflammation seem to share the responsibility for this reverse association, as they both decrease serum

albumin levels, which are a major determinant of Hcy levels (Suliman *et al.,* 2007). In addition. they are risk factors for increased morbidity and mortality (Kalantar-Zadeh, *et al.,* 2003).

Homocysteine levels tend to increase with age and are higher in men than in women. High levels of homocysteine can be very damaging to the kidneys and the vascular system (Dierkes *et al.,* 1999, Marangon *et al.,* 1999 and Levin *et al.,* 2002).

Moreover, hyper-homocysteinemia has been associated with the development of cardiovascular disease (CVD), which is common in patients with chronic kidney disease (CKD) and is responsible for the majority of morbidity and mortality in these patients (Levin *et al 2002*).

The homocysteine theory remains unproved in dialysis patients, and its evaluation requires a safe, effective therapy for long-term normalization of tHcy (Bradley *et al 2008*).

In Sudan, hyperhomocystenemia, is an independent risk factor for atherosclerotic vascular disease which has been described in the last ten years, with the increasing number of end stage renal disease (ESRD) and its association with other cardiovascular risk factors necessitate the study of hyper-homocystenemia in hemodialysis patients.

An emerging strategy to lower total homocysteine is to increase its free fraction within the blood, thereby improving its removal by dialysis. This can be achieved by the addition of a pharmaceutical agent that is capable of forming a sulfhydryl within the plasma and that will exchange with Cys^{34}-albumin bound homocysteine, allowing homocysteine to pass through the dialytic membrane.

Strategies for normalizing plasma tHcy concentration in ESRD have also included increasing dialysis membrane pore size and pharmacologic doses of water-soluble vitamins, including folic acid, vitamin B_6, and vitamin B_{12} (Bostom *et al* 2000). Although vitamin therapy has been shown to lower plasma tHcy by 15 to 47% (Bostom *et al.,* 1995), the majority of investigations failed to normalize plasma homocysteine to levels observed in healthy control subjects with normal kidney function (Bostom *et al* 1995). In the previous decades, many studies were performed to evaluate the effect of hyperhomocysteine on the serum lipid profile in patients with kidney disease; however, there is no similar studies in Sudan.

Despite the increasing number of renal failure patients in Sudan and despite the routine prescription of folic acid, the weight of hyperhomocysteinemia is still unstudied in Sudan in spite its known risk factor among End Stage Renal Disease (ESRD). Cardiovascular diseases are the most common causes of death among hemodialysis patients, they account for about 40% of hospitalized patients and 50% of deaths, yet the risk factors of these events have not been well established.

The objectives of the studies to evaluate the plasma homocysteine level in Sudanese ESRD patients, assess the correlation between the homocysteine levels in patients and controls. And the association between high levels of plasma homocysteine and lipid profile (total cholesterol, LDL, HDL, VLDL and Triglycerides) in ESRD patients.

Materials and Methods

This study was conducted in three haemodialysis centres in Khartoum state (Ribat University Hospital Dialysis center, Al-Amal Hospital Dialysis center and Bahri Dialysis center). All patients were confirmed with renal failure and receiving regular hemodialysis. Patients (120 samples) of either sex, male and female, aged >18 years old matched controls (60 samples).

Collection of samples

Venous blood samples were collected in two different sets of blood containers, one of them contains EDTA anticoagulants and the others doesn't contain any anticoagulants, after that the samples were immediately placed on ice, then plasma and serum were separated

within 1 hour by centrifugation (3000rpm) and stored at –20°C.

Biochemical analysis:

Total plasma homocysteine concentration was measured using commercial ELISA kit purchased from (IBL International GmbH Company, Hamburg, Germany).

Lipids profile (Triglycerides (TG), Total Cholesterol and (TC) HDL-cholesterol), were measured by (Spectrophotometer, JENWAY 6305-UK) using chemical reagent kits provided by (Bio system company- Germany). LDL-cholesterol and VLDL-cholesterol were measured using friedwalds equation.

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version l6. The differences between the groups were tested for significance by student's t-test, Onaway ANOVA test and chi-square test. Data were expressed as the mean ± SD. P-values < 0.05 are considered statistically significant.

Results:

Demographic Characteristics of Participant

This study included 120 patients with ESRD (50 women, 70 men), all patients was above 18 years and the Average was 47.18.

Serum levels of Homocysteine

The mean value of serum homocysteine in patients was 21.85 µ/L and showed significant increase compared to control group 12.11 µ/L.(figure 1)

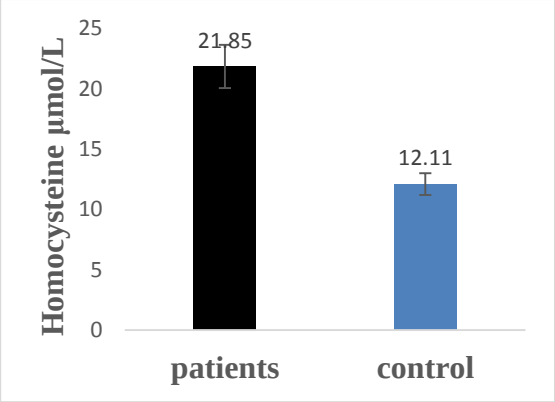

Figure (1): showing the serum homocysteine concentration in patients and control.

Lipid profile

lipid profile showed significant increase in cholesterol (figure 2), LDL (table 1), TG (table 2), and VLDL (figure 3) in hemodialysis patients compare to control groups, while the mean values of HDL-cholesterol (figure 4) showed a significant decrease in patients compared to controls ($p<0.05$).

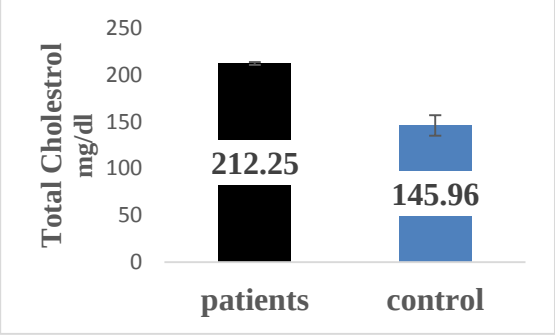

Figure (2): showing the concentration of serum cholesterol in patients and control.

Table 1: showing the concentration of serum LDL in patients and control.

Group	LDL mg/dl
Patients	160.26 ± 12.3
Control	95.75 ± 7.5

Table 2 : The concentration of serum triglycerides in patients and control.

Group	Triglycerides mg/dl ±SD
Patients	186.87 ± 1.2
Control	93.08 ± 8.2

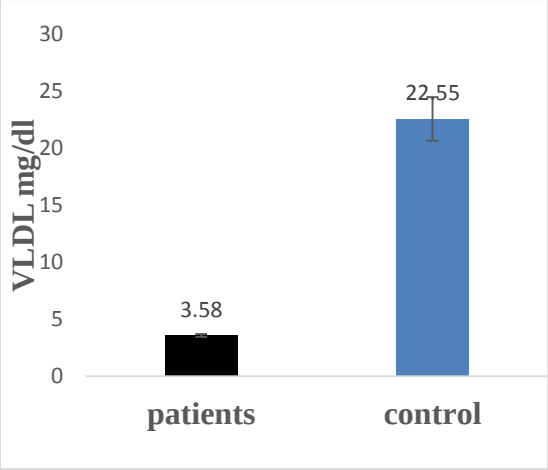

Figure (3): showing the concentration of serum VDL in patients and control.

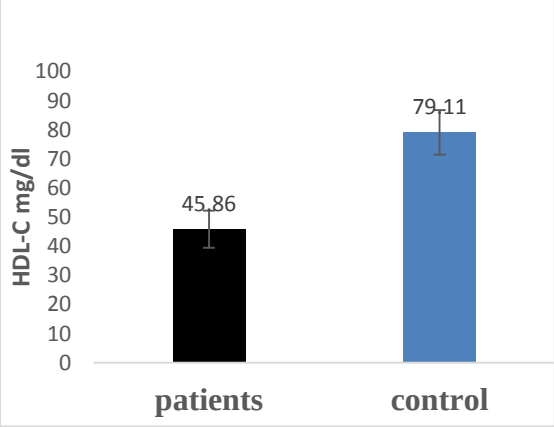

Figure (4): showing the concentration of serum HDL in patients and control.

Discussion

Homocysteine level in patients are within the normal range but significantly different from the control group According to Gascon et al. (2010) who classify the homocysteine level. In our studies Hcy status is associated with higher Total Cholesterol, Triglycerides, LDL, and VLDL but lower HDL-C Compare to control group. the lipid profile of Patients with End Stage Renal Disease showed remarkable increased except HDL-C. our finding were compatible with other studies found The link between Hcy and HDL-C using experimental animal, showed significant and negative correlation was found, and positive correlation to LDL-C (Watanabe et al., 1995; Namekata et al., 2004; Wang et al., 2003; Mikael et al., 2009).

Durdi et al., 2001 evaluate the lipid profile for in 126 patients with myocardial infraction. they found positive correlation between Hcy and LDL-C, and negative correlation with HDL-C. another studies conducted in india include 300 patients were diagnosed as coronary heart disease, Hcy was found to be positively correlated with TG and VLDL-C, and negatively with HDL-C (Mahalle, *et al*., 2013).

Shakiba et al., 2019 showed that sodium valproate leads to increases serum homocysteine levels in migraine patients and increase the The mean serum levels of TC, HDL, and TG.

Anan et al. reported similar results to our present study that Hcy is associated with TG and HDL-C, but not with TC nor LDL-C in 40 Japanese patients with diabetes [24], Rosa et al. found that Hcy correlates negatively with ApoA-I and with HDL-C in elderly rural subjects from Sicily [25]. Other studies from china conducted in 2058 Chinese consecutive coronary artery angiographic patients, Hcy was found to be negatively correlated with HDL-C ($r = -0.148$, $P < 0.001$) [4]. In northern Chinese subjects, the prevalence of HHcy in the combined hyperlipidemia (highTG)

group has been reported to be significantly higher than that in the control group.

Healthy kidney plays a significant role in homocysteine clearance and metabolism, and other amino acids. The major mechanism for hyper-homocysteinemia in renal failure is a decrease of elimination homocysteine in urine from the body (9, 12). However, the full mechanism is unclear it may be caused by diminished uptake by the kidney, an organ in which the transsulfuration pathway is developed fully, or by disturbance in the remethylation and/or trans-sulfuration pathways, which progressively develop along with the decrease in renal function. Despite the lack of evidence for a role for the human kidney in homocysteine metabolism, it has been demonstrated that in hemodialysis patients, the remethylation of homocysteine is decreased by 30%.17.

Conclusion

We have observed serum homocysteine levels were significantly increased in patients of end stage renal failure compare to control group. the results of this study which point of the fact that serum homocysteine, total cholesterol, Triglyceride, LDL, VLDL values are significantly higher and HDL–C are lower in patients with ERSD, in comparison to the population with regular renal function, suggest that increased homocysteine level are risk factor for CHD. It can be concluded that the determination of homocysteine and Lipid profile parameters would be advisable to complete risk assessment of premature atherosclerotic development, but also of possible further progression of ESRD.

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