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Homocysteine and Atherogenic Lipid Profile as Markers for Atherotherombatic Disease among Sudanese Patients with Chronic Kidney Disease

[1]Mohammed M. Ahamed, [2]Mariam A. Ibrahim, [1]Emaduldeen M. Khalil, [1]Abdelaziz H. Al Haj [1]Ayman M. Al Gassim
[1]Al-Neelain University, Khartoum. Sudan
[2]Sudan University of Science and Technology, Khartoum. Sudan

Abstract

Background: Hyperhomocystinemia is frequently encountered in renal failure. This suggests that the kidney's function is crucial. Chronic kidney disease (CKD) is an abnormality of the kidney structure or function, and persisting for greater than 3 months. The risk factors for cardiovascular disease in chronic kidney disease were a mixture of traditional and specific CKD factors.

Objective: To assess the significance of homocysteine (Hcy) and atherogenic lipid profile as markers for atherotherombatic disease among Sudanese patients with chronic kidney disease.

Materials and methods: A case control study conducted, included 60 patients (53% male, 47% female) and 40 apparently healthy individuals (57%female, 43% male) age was matched. Atherogenic lipid profile and serum creatinine were measured by Biosystem350, and Hcy was detected qualitatively in urine by using Speath Barber modification method Silver-Nitroprusside test.

Results: Statistical analysis was done by SPSS and revealed that 19% of patients had Hcy +ve while 41% had Hcy -ve. The Creatinine, T. cholesterol ,LDLc ,were significantly increased p. value (0.000,0.000,0.000) respectively While HDLc was insignificantly decreased and GFR was significantly decreased p.value (0.224,0.00) respectively, there is a significant positive correlation between creatinine and duration (r=0.814,p=0.000), there is a significant positive correlation between T. cholesterol and duration (r=0.649,p=0.000), and a significant positive correlation between LDL and duration (r=0.822,p=0.000), there is a significant positive correlation between T. cholesterol and creatinine (r=0.723,p=0.000), there is a significant positive correlation between LDL and creatinine (r=0.883,p=0.000) ,there is a significant positive correlation between HDL and GFR (r=0.64,p=0.000). But there is a significant negative correlation between HDL and duration (r=0.718,p=0.000), there is a significant negative correlation between GFR and duration

(r=-0.573,p=0.000), there is significant negative correlation between HDL and Creatinine (r=0.808,p=0.000), there is significant negative correlation between GFR and creatinine (r=-0.834,p=0.000),there is a significant negative correlation between T. cholesterol and GFR (r=-0.621,p=0.000),and significant negative correlation between LDL and GFR(r=-0.655,p=0.000).

Conclusion: Chronic kidney disease accompanied by +ve Hcy may lead to atherothrombatic disease.

Key words: Chronic kidney disease, Atherotherombatic disease, Hyperhomocystinemia.

Introduction

Studies identifying the incidence, causes, and complications of CKD had focused on advanced disease and kidney failure. Markers used to identify CKD includes plasma creatinine, estimated GFR(glomerular filtration rate), and measured creatinine. Homocysteine (Hcy) is a sulfur containing amino acid produced in metabolism of the essential amino acid methionine. To convert homocystine back to methionine and trans-sulfuration subsequently converting the homocystine to cysteine and tauriner-acquired folate and vitamin B12[1].
Normal homocystine level ranges between 5μmol/L and 15μmol/L. 16-30 μmol/L, 31-100μmol/L and >100μmol/L are classified as mild, moderate, and severe homocysteinemia, respectively. Deficiency of folic acid, vitamin B6, and vitamin B12 are responsible for the majority of cases of elevated homocystine in the general population. Increase in homocystine level 3μmol/L above the normal is associated with 49 higher risk of ischemic heart disease and reversed by taking folic acid and vitaminB12. Hyperhomocystinemia may have an effect on endothelial function and integrity. It is a risk factor for atherosclerosis, morbidity, and mortality. It is commonly associated with cardiovascular diseases[2].
Hyperhomocystinemia is a result of either impaired enzymatic function (cystathionine Beta synthase) or a deficiency of essential vitamins (folic acid, B6, B12) or both. Hence, it can be treated with vitamin supplements. Available data suggested that elevated plasma Hcy concentrations lead to oxidation of LDL cholesterol, potentially caused atherosclerosis. An interaction with lipoprotein which promotes binding with fibrin has also been reported. Hyper-homocystinemia is frequently associated with renal failure. This suggests that the kidney function is crucial for Hcy catabolism[3].
Hcy is ultra filtrated in glomeruli, almost completely absorbed in tubuli, and degraded in kidney tissue by trans-methylation and trans-sulphuration in the activated methyl cycle. It has been shown that metabolism in kidney tissue accounts for a major fraction of total renal clearance of plasma Hcy and the loss of capacity for Hcy degradation might explain the increase in plasma Hcy seen in end stage renal disease. Increase in plasma Hcy leading to the transient decrease in plasma cysteine, which is a substrate for nitric oxide (NO) production, may decrease the availability of endothelium-derived relaxing factor and the function of S-nitrosocysteine, a more potent vasodilator than endothelium derived relaxing factor[4].

In addition hyper-homocystinemia (HHcy) may directly impair the NO pathway reducing NO elaboration by endothelial cells and increase oxidative degradation of NO. HHcy is recognized as a risk factor for vascular disease, detecting HHcy in patients at high139 risk of cardiovascular disease may be of clinical importance. Many studies have shown that there has been an inverse correlation between HHcy, vitamin B12, and folic acid levels which is stronger for folic acid than vitamin B12. One etiology of mild HHcy is insufficient intake of vitamins B6, B12 and folic acid, therefore an effective treatment of deficiency of these vitamins may be beneficial. The HHcy may be readily reversed by folic acid either alone, or in combination with vitamins B12 and B6. Recently, folic acid supplementation to bread has been started in some countries[5].

It has been suggested that if enhancing oxidative stress is an effect of HHcy on the endothelial cells, dietary antioxidants such as vitamin E may also help reduce the risk of vascular disease associated with Hcy. But further studies are needed to test the effect of such treatments on the primary and secondary prevention of cardiovascular diseases. The mechanisms by which HHcy promotes atherosclerosis are not fully understood. Among the possible causes: induction of *cyclin A* gene expression in vascular smooth muscle cells; endothelial dysfunction; reduction of protein C levels; inhibition of von Wile brand factor processing and secretion; enhancement of lipid per oxidation; direct endothelial damage caused by Hcy, due to accumulation in endothelial cells; interaction between nitric oxide (NO) and Hcy; and finally reduction in serum anti thrombin activity, with a reduction of thrombomodulin have been revealed[6].

Available data suggest that elevated plasma Hcy concentrations lead to oxidation of LDL-cholesterol, potentially causing atherosclerosis. An interaction with lipoprotein (a) which promotes binding with fibrin has also been reported. Although several explanations have been proposed for Hcy mediated vascular disease, there is growing evidence that endothelial dysfunction is an initiating event. Evidence that Hcy has a direct toxic effect on endothelial cells has been derived both from inventor studies in human cell cultures and from in vivo animal models. Short terminator venous infusion of Hcy results in desquamation of endothelial cells and has been associated with arterial damage similar to early human atherosclerosis[7].

The redox property of the sulfhydryl group of Hcy, leading to the formation of reactive oxygen species, such as superoxide and hydroxyl radicals, is believed to play a pivotal role. On the other hand, increase in plasma Hcy leading to the transient decrease in plasma cysteine, which is a substrate for NO production, may decrease the availability of endothelium derived relaxing factor and the function of S-nitrosocysteine, more potent vasodilator than endothelium derived relaxing factor. In addition Hcy may directly impair the NO pathway reducing NO elaboration by endothelial cells and increase oxidative degradation of NO[8].

Materials and methods

This was a cross-sectional, case control study conducted at the Academy Charity Teaching Hospital, Khartoum (Sudan) during the period from February to May 2017. The study population was 60 Sudanese chronic kidney disease patients (53% males and 47% females), and 40 apparently healthy, control individuals (57 % females and 34% males). Ages of all participants ranged from

20 to 67 years. Inclusion criteria were patients with CKD and apparently healthy, control participants. Exclusion criteria were patients on folic Acid, B6, and B12 supplements, and patients with acute renal failure, cancer, cardiac disorders or anti-hypertensive drugs abusers. Approval to conduct the study was obtained from Al Neelain University (Khartoum); and permission to collect the specimens was taken from the Academy Charity Teaching Hospital, (Khartoum). Informed consent was obtained from each participant prior to sample collection. Data processing and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) program (version 14). The results were expressed as frequency, percentage, and mean ±SD. Independent T-test was used to compare the study prevalence rate in patients and control group. Also Chi-square was used to compare the percentage of Hcy in patients and control participants. Pearson 's correlation was used to study the relation between study parameters and study variables. P - value < or = 0.05 was considered significant. Normal and pathological control sera were analyzed to check the accuracy and precision of the results. 5ml venous blood were collected from each participants into plain containers, and serum was separated by centrifugation at 3000-4000 rpm. Urine specimens were collected in urine containers following the standard microbiological procedures. Clinical and demographical data were collected using a structural questionnaire. Atherogenic lipid profile and serum creatinine were measured by the Biosystem 350, Spain machine. Homocysteine was detected qualitatively in urine using the Speath Barber modification silver-nitroprusside test (detectable 2 mg/100 mL or 75 µmol/L is considered positive). The glomerular filtration rate (GFR) was calculated by the formula:
GFR (ml/min/1.73m^2) = 175 x creatinine (mg/dl)$^{-1.154}$ x (age)$^{-0.203}$ x 1.210 (♂) and x 0.742 (♀).

Results

Furthermore, the study showed insignificant gender distribution between the levels of creatinine, total cholesterol, LDLc, and HDLc; while GFR was significant. At the same time, there was a significant positive correlation between disease duration and creatinine, total cholesterol, and LDL. On the other hand, there was a significant negative correlation between disease duration and HDL. Also there was a significant negative correlation between GFR and disease duration. However, there was a significant positive correlation between total cholesterol, creatinine, and LDL. Also there was a significant negative correlation between total cholesterol and GFR, and a significant negative correlation between LDL and GFR; and a significant positive correlation between HDL and GFR (Fig. 1).

Furthermore, there was a significant negative correlation between HDL and creatinine; and a significant negative correlation between GFR and creatinine (Fig. 2).

19% of patients were homocystine positive, and 41% were homocystine negative (Table 1). Positive homocystine was found accompanied by an increase in GFR. The study also showed increased levels of serum creatinine, total cholesterol, LDLc, and decreased levels of HDLc, and GFR (Table 2).

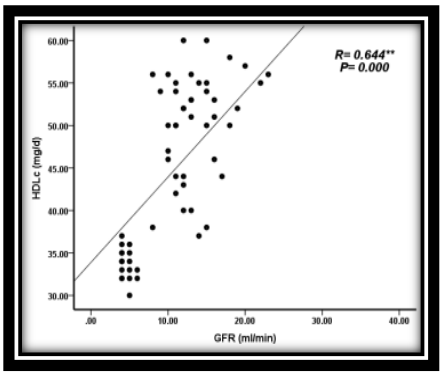

Fig. 1: Correlation between HDL and GFR

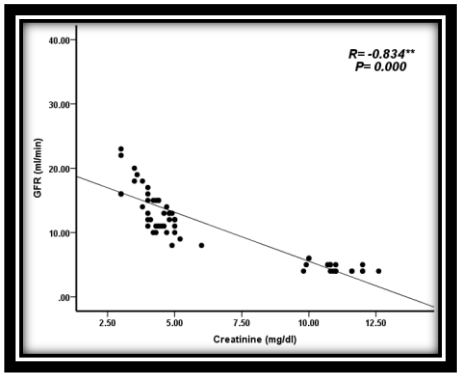

Fig.2: Correlation between creatinine and GFR

Table (1)**:** Frequency rate of homocystine among the study and control groups

Groups	Homocystine		Total
	Positive	Negative	
Study	19 (19.0%)	41 (41.0%)	60 (60.0%)
Control	0 (0.0%)	40 (40.0%)	40 (40.0%)
Total	19 (19.0%)	81 (81.0%)	100 (100.0%)
P = 0.000			

Discussion

Kidney function is crucial for Hcy catabolism. Hyperhomocysteinemia can have an effect on endothelial function and integrity, so it is a risk factor for atherosclerosis. In the current study 19% of patients were homocystine positive, while 41% of patients were Hcy negative. In USA (2006) Hcy was found to promote atherosclerosis and renal impairment. The study results agreed with that

Table (2): Frequency rate of creatinine, lipid profile and GFR among the study and control groups

Parameters	Study group (mean± SD)	Control group (mean± SD)	p-value
Creatinine	6.28±3.22	0.635±0.168	0.000
Total cholesterol	199.05±21.15	178.45±6.41	0.000
LDLc	97.77±15.22	75.85±6.31	0.000
HDLc	45.15±9.21	46.80±4.02	0.224
GFR (ml/min)	11.20±5.89	129.85±19.62	0.000

reported by Robinson and his colleagues [9].
Another study performed by Paul and his co-workers in Saudi Arabia (2015) reported that HHcy may be a predictor for cardiovascular disease (CVD) in patients with renal failure[10] . Hcy correlated significantly with increasing severity of coronary artery disease ($p < 0.001$). Also Chao and his co-authors in Taiwan (2014) reported that both HHcy and CKD increase the risk of CVD; and both the GFR and creatinine level may not be accurate methods for renal failure prediction [11.]
Our study evaluated Hcy as a predictive and prognostic marker for end stage renal disease (ESRD) and no significant evidence ($p = 0.06$) was detected. Oluseyi and his colleagues in Nigeria (2016) reported that HDLc level was significantly lower in CKD patient ($p = < 0.001$). They also reported that creatinine and LDLc were significantly higher in CKD group as compared with the control group ($p = < 0.001$)[12]
On the other hand, Hatem and his co-workers in Egypt (2016)showed that Hcy level was significantly increased during mild kidney impairment; then it is significantly decreased until the patient reaches severe impairment; while it shows significant elevation in the last stage of chronic renal disease (Hcy and creatinine p - value = < 0.001)[13]
The present study revealed that the creatinine, total cholesterol, and LDLc levels were significantly increased in CKD patients compared to control group; while HDLc and GFR levels were significantly decreased in CKD patients as compared to control group**.**
In the current study, there was no statistical difference found in the markers tested according to

gender variation among the patients investigated; however, GFR was significantly affected (p = 0.004). These findings disagreed with Ashish and his co-authors in India (2016) who noted that GFR was significantly increased from the baseline value (52.3 ±17.59) to (65.01 ±17.9) at the end of 3 months treatment (p = < 0.05)[14]
The present context showed positive correlation between the disease duration and creatinine, total cholesterol, and LDLc; while negative correlation was with disease duration, HDLc, and GFR. There was also a positive correlation between creatinine and total cholesterol, and LDLc; while negative correlation was found with creatinine and HDLc. Also our study results revealed a positive correlation between HDL and GFR; while a negative correlation was detected with GFR and creatinine, total cholesterol, and LDLc.
Conclusion: Chronic kidney disease accompanied by +ve Hcy may lead to atherothrombatic disease.

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