Original Research ~~Article~~.

HYPERHOMOCYSTEINEMIA IN CHRONIC KIDNEY DISEASE PATIENTS IN A TEACHING HOSPITAL IN NIGERIA

ABSTRACT

Aim: To determine the prevalence ~~prevalence~~ of hyperhomocysteinemia amongst chronic kidney disease (CKD) patients at a Teaching Hospital in Nigeria and to determine its relationship with the severity of kidney disease.

Study design: A cross sectional study ~~cross-sectional study~~ carried out in the department of Medicine in a Teaching Hospital in Nigeria between April 2012 and May 2013

Methodology**:** One hundred and thirty-eight patients with CKD and 69 healthy controls were recruited. ~~They had their estimated~~ Glomerular filtration rates was estimated for both patients and controls~~assessed~~, using measured serum creatinine in the Cockcroft-Gault formula and the patients were ~~were~~ grouped into the different stages of chronic kidney disease. They also had homocysteine levels measured using the enzyme-linked immunosorbent assay and these ~~Homocysteine levels~~ were also compared in the different chronic kidney disease stages.

Results: A hundred and thirty-eight chronic kidney disease- patients participated in this study, of which 3 each (~~2.~~2.2 ~~2~~%) were in CKD stages 1 and 2, 46 (33.3%) in stage 3, 66 (47.8%) in stage 4 and 20 (14.5%) in stage 5. The ~~patients~~ mean age of the patients was 45.9 ± 16.5 years and 42.3 ± 14.7 years for control subjects. The prevalence of hyperhomocysteinemia was 57.9% amongst cases and 4.3% amongst control subjects with median homocysteine (Hcy) level being 19.1 µmol/l (IQR - 13.8) in cases and 8.3 µmol/l (IQR - 2.9) in controls, this was significant (*P*<.001) with the chronic kidney disease patients having higher median homocysteine levels as the degree of kidney disease worsened.

Conclusion: The prevalence ~~prevalence~~ of hyperhomocysteinemia is high in CKD patients compared to controls and it ~~s prevalence and severity~~ increases as CKD progresses.

Comment [M1]: The term prevalence was used because this was a cross sectional study.

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Comment [M3]: This is not a case control study as the outcome in both groups – cases and controls wasn't known It is a Comparative cross-sectional stud It is not cross sectional because the recruited person was predefined as 'a CKD or healthy ,which is more to the side of case control stud.Therfore you can use the Comparative cross-sectional stud

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Keywords: chronic kidney disease, homocysteine, hyperhomocysteinemia, cardiovascular disease

INTRODUCTION

Chronic kidney disease (CKD) is a public health problem that is present ~~present~~ worldwide with an incidence that's on the rise, unfavourable outcomes and very high cost [1].

Cardiovascular disease (CVD) ~~A~~is a major cause of morbidity and mortality in patients with CKD that~~which~~ is well documented ~~is cardiovascular disease (CVD~~ [2]. Although patients with CKD are at risk of ultimately developing end- stage renal disease (ESRD), about 50% of CKD patients die of CVD before commencement of dialysis [3-6]. ~~The morbidity and mortality from CVD is extremely high in CKD~~ In patients requiring haemodialysis ~~patients~~ (usually stage 5 CKD patients) the morbidity and mortality from CVD is extremely high~~—~~ [7]. Based on data from the U.S. Renal Data System Coordinating Center Case-Mix Adequacy Study, clinical coronary heart disease (CHD) is seen in 40% of haemodialysis patients and CVD in them is 10 to 30 times higher than in the general population despite stratification by gender, age, race, and the presence of diabetes [7].

CKD patients usually develop accelerated atherosclerosis and the risk of premature death from CVD is higher ~~in them~~. The predisposition of these patients to atherosclerosis ~~is increased and~~ is driven mainly by inflammation, dyslipidemia and oxidative stress, and these are features associated usually with CKD [2]. Chronic inflammation is prevalent in CKD patients and numerous studies have demonstrated that chronic inflammation may be a contributor to the morbidity and demise among end stage renal disease (ESRD) patients [8, 9]. The increased cardiovascular morbidity and mortality among CKD patients may not be fully explained by conventional risk factors.

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A number of abnormalities in the metabolism of protein and amino acid are seen in patients with CKD especially ESRD, an increase in the plasma concentration of homocysteine, a sulphur containing amino acid is one of such abnormalities that may be seen[10].

Homocysteine is associated with inflammation and is been considered as an inflammatory marker [11,12]. It acts as an atherogenic and thrombophilic agent which potentiates hypertension and smoking as well as other risk factors that predispose to peripheral arterial disease [10].~~,~~ ~~all~~All of which increase morbidity as well as mortality in CKD patients. Hence homocysteine is attracting a lot of attention among renal patients.

Hyperhomocysteinemia in itself has also been shown to be an independent cardiovascular risk factor in patients with chronic kidney disease [10, 13] however, the mechanism is not clear. Elevated levels of homocysteine and cardiovascular disease are common in patients with decreased renal function. An increase in Homocysteine~~cy~~ represents a~~n~~ potent risk factor for coronary, cerebrovascular and peripheral arterial disease as well as for deep vein thrombosis [14]. Hyperhomocysteinemia is seen in the early stages of CKD at levels of glomerular filtration rate (GFR) of about 60ml/min and its prevalence increases to about 85-100% ~~[15]~~ in ESRD[15]. In the general population moderate hyperhomocysteinemia is seen in approximately 5 – 7 % [16].

The burden of CKD in terms of morbidity and mortality is huge, worse in developing countries where access to, and affordability of care is not readily available. A major factor contributing to this burden is CVD. To reduce this burden, cardiovascular risk factors ~~i~~in CKD patients must be sought and addressed. The paucity of data on hyperhomocysteinemia in chronic kidney disease in a developing country like Nigeria~~ours~~ necessitated this study.

MATERIALS AND METHODS

This was a hospital-based cross sectional ~~ross-sectional comparative~~ study carried out in the Nephrology unit, in a tertiary hospital in Nigeria. Patients presenting at the Nephrology clinic/Dialysis/Accident and Emergency units who met the inclusion criteria -adults above 18 years of age in CKD stages 1 – 5, on conservative or renal replacement therapy and who gave their consent, were consecutively recruited. Exclusion criteria were patients below 18 years of age, non-consenting patients, patients on methotrexate, phenytoin or theophylline therapy, menstruating women, patients with acute kidney injury, hypothyroidism, acute lymphoblastic leukemia and cancers of the breast, ovaries and pancreas. Controls were recruited from amongst hospital staff (doctors and nurses) who were apparently healthy and not on medication for any acute or chronic ailments. Approval for the study was obtained from the Ethics and Research Committee of the hospital. All participants gave written informed consent.

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SAMPLE SIZE

Studies on the prevalence of hyperhomocysteinemia revealed a range of 56% - 90% [7, 10, 16, 19, 20, 21]. The sample size was extrapolated from the formula for sample size determination in an infinite population [22] using a prevalence of 90% and a degree of precision of 0.05. A sample size of 138 was arrived at. A total of 138 chronic kidney disease patients and 69 controls in a ratio 2:1 were recruited for this study.

Comment [M4]: A ratio 2:1 was used to minimise cost

Upon recruitment, a researcher administered questionnaire was completed. Data on physical characteristics such as weight, height, hip and waist circumference and blood pressure were recorded. Weight was measured in kilograms using hospital health scale ZT-120 with patients putting on light clothing without foot wears. The height was measured in meters using the same scale. The body mass index BMI defined as weight in kilogram divided by the square of patient's height in meters was calculated. The waist circumference in centimetres was measured in the horizontal plane at the level of the natural waist line taken to be at the umbilicus

using a non-stretchable tape. All patients and controls were instructed to be on~~observe an~~ overnight fast for 8-10 hours before blood~~the day of~~ sample collection on the next day. About 10ml of venous blood was collected, 5ml into a lithium heparin sample bottle for serum creatinine estimation using the Jaffe's reaction and 5ml into an EDTA bottle and centrifuged for 15 minutes within 30 minutes of collection and plasma stored at < 20oC for homocysteine level assay using the Axis® Homocysteine FHCY100 Enzyme Immunoassay (EIA) [17].

The results were categorized ~~interpreted~~ as follows~~-~~: moderate hyperhomocysteinemia - 15-30µmol/L, intermediate hyperhomocysteinemia - >30-100µmol/L, and severe hyperhomocysteinemia - >100µmol/L [16].

Fasting blood sugar (FBS) was measured using an Accucheck glucometer and strips.~~ The controls had same done.~~

Glomerular filtration ~~F~~rate ~~R~~ was calculated using the Cockcroft-Gault formula to estimate creatinine clearance (CCr): CCr = (140 - age) × (weight)/72 × SCr x (0.83 if female), where SCr is in mg/ dL, weight in kg and age in years and results were stratified according to the KDIGO guidelines [17] into CKD ~~the different~~ stages 1-5~~of CKD~~.

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DATA ANALYSIS

Data entry, storage, and management were performed on the ~~statistical software package for Social Sciences (~~SPSS~~)~~ version 17.

~~Results were presented in tabular and graphical forms. A univariate analysis describing the baseline socio-demographic characteristics of participants and prevalence rate were done.~~ ~~Continuous variables that are~~ Normally distributed continuous variables were presented as means and standard deviation (SD) and skewed data as medians with inter-quartile ranges (IQR) or standard deviation while d~~s. D~~iscrete variables were presented as percentages. The student t-test was employed for comparing the means of data with normal distribution while Wilcoxon Signed Ranks was used for skewed data in cases versus controls. Chi-square was used in testing for significant differences between proportions and frequencies of cases and controls that had hyperhomocysteinemia.

The confidence interval was set at 95% limit, with a level of significance, $P < 0.05$.

RESULTS

~~A total of 138 chronic kidney disease patients and 69 controls in a ratio 2:1 were recruited for this study.~~ The~~ir~~ ages of patients ranged from 18 to 80 years with the mean age of the study population being 45.9 years ± 16.4 for cases and 42.2 years ± 14.6 for control (P =0.119). The cases consisted of 89 males (64.5%) and 49 females (35.5%) while controls were made up of 42 males (60.9%) and 27 females (39.1%), $P = 0.610$

There was no statistically significant difference between mean ages, sex and marital status of cases and controls as shown in table I.

TABLE 1: SOCIODEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION

CHARACTERISTICS	**CASES** **n = 138** **(Mean ± S.D)**	**CONTROLS** **n = 69** **(Mean ± S.D)**	**t** **P –value**

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	~~(Mean ± S.D)~~	~~(Mean ± S.D)~~		
~~Age~~	~~45.9 ± 16.4~~	~~42.2 ± 14.6~~		~~0.119~~
Age	45.9 ± 16.4	42.2 ± 14.6	1.570	0.119
	n = 138(%)	**~~n = 69(%)~~**	***X*²**	
Sex				
Male	89 (64.5%)	42 (60.9%)	0.260	0.610
Female	49 (35.5%)	27 (39.1%)		
Marital status				
Single	34 (24.6%)	15 (21.7%)	-	~~0.188~~
Married	92 (66.7%)	53 (76.8%)		
Divorced	9 (6.5%)	1 (1.4%)		
Widowed	3 (2.2%)	0 (0.0%)		

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Table II shows the mean BMI, waist-hip ratio (WHR), FBS, estimated GFR and homocysteine levels in cases and controls. The homocysteine and FBS levels were significantly higher in the patients ($P < .001$), while the estimated GFR was significantly lower in cases.

TABLE 2~~I~~: CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF STUDY POPULATION

VARIABLE	CASES (n=138)	CONTROLS (n=69)	~~TEST~~ STATISTICS~~t~~	*P*-value
BMI (kg/m^2)	23.71 ± 4.94	26.7 ± 3.6	4.987~~*~~	0.762
Waist-Hip Ratio	0.92 ± 0.07	0.90 ± 0.45	0.562~~*~~	0.575
Fasting Blood Sugar (mg/dl)	107.2 ± 34.0	92.6 ± 9.9	4.637~~*~~	< 0.01
	Median (IQR)	**Median (IQR)**	**Wilcoxon**	***P*-value**
Estimated GFR (mg/dl)	29.9(IQR-18.9)	96.7(IQR-16.7)	187.00~~ǂ~~	<0.01
Homocysteine leveL(µmol/L)	19.0(IQR- 13.8)	8.3(IQR - 2.9)	1820.00~~ǂ~~	<0.01

~~* t test~~

~~ǂ Wilcoxon~~

BMI – Body Mass Index, GFR – Glomerular filt~~e~~ration rate

Three (2.2 %) of the patients with CKD were in stage 1, another three (2.2%) were in stage 2, 46 (33.3%) in stage 3, 66 (47.8%) in stage 4 and 20 (14.5%) in stage 5. ~~The majority of the case population were in stage 4 (47.8%).~~

The frequency~~prevalence~~ of hyperhomocysteinemia was 57.9% among CKD patients and 4.3% among the controls *P* <0.01, OR = 30.34 (95% CI = 9.09,- 101.29). Forty-two percent of the CKD patients had normal levels of homocysteine, 30.4% moderate hyperhomocysteinemia and 27.5% intermediate hyperhomocysteinemia. None had severe hyperhomocysteinemia as depicted in table 3~~III~~.

TABLE 3~~III~~: COMPARISM OF PREVALENCE OF HOMOCYSTEINEMIA (CASES VERSUS CONTROLS)

	CASES	**CONTROL**
Elevated	80(57.97%)	3(4.35%)
Normal	58(42.03%)	66(95.65%)
Total	**138(100%)**	**69(100%)**

df = 2, *P*- value <0.01, OR = 30.34 (95% CI = 9.09,- 101.29)

The median values of Hcy increased from stage 1 to 5. There was a significant correlation between the stages of CKD and the degree of homocysteine levels (spearman's rho = 0.204, *P* = 0.016-) as shown on figure 1.

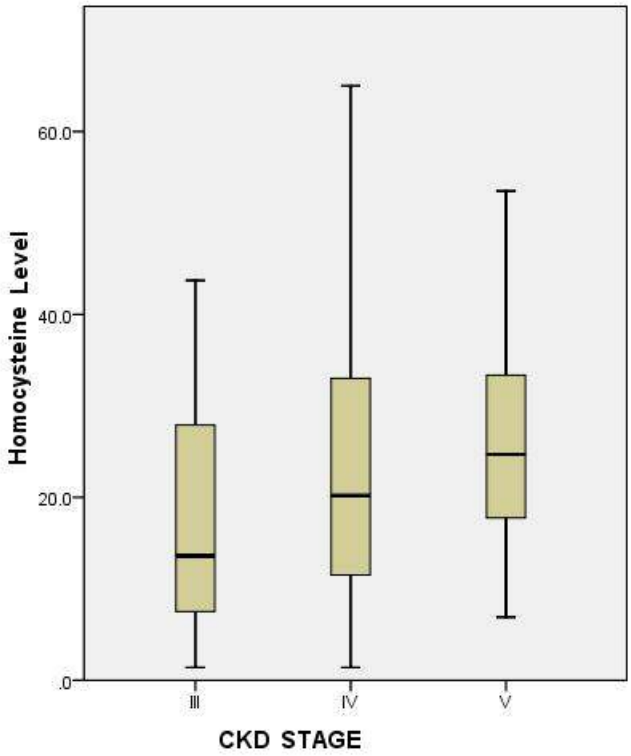

Fig I~~1~~: Boxplot showing correlation between CKD stage and median homocysteine levels

DISCUSSION

Increased cardiovascular morbidity and mortality in CKD patients has been associated with elevated homocysteine levels which is a common finding in them [10,11]. This study shows that hyperhomocysteinemia is significantly higher~~common~~ among CKD patients. Using the classification by Kang et al, 57.9% of the CKD patients had hyperhomocysteinemia. This is comparable with 56% reported by Menon ~~Vandana~~ *et al* in England in which baseline

homocysteine~~tHcy~~ was measured in 2 different populations; population A with estimated GFR 25 – 55 ml/min had a prevalence of hyperhomocysteinemia of 56% [21]. It is, however, lower than that reported by Ajith *et al* in New York [23], in which 147 patients; 85 males and 62 females aged 58 ±15 years requiring hemodialysis had a prevalence of 82%. The difference in prevalence rate may be due to the fact that the index study assessed the prevalence in both dialyzing and non-dialyzing patients.

Of the number of cases with hyperhomocysteinemia, 30.4~~52.5~~%, and 2~~4~~7.5% had moderate and intermediate hyperhomocysteinemia respectively, none had severe hyperhomocysteinemia, which is not surprising as cases of severe hyperhomocysteinemia usually due to homozygous defects in genes encoding for enzymes of homocysteine metabolism leading to accumulation of Hcy in blood and urine, are extremely rare. An example of this is a disorder caused by homozygousity for a defective gene which encodes for cystathionine beta-synthase. In this condition Hcy can be as high as 400umol/L [24]. In the index study, however, a majority of the patients with hyperhomocysteinemia had moderate hyperhomocysteinemia which may be due to deficiencies in vitamins that play major roles in Hcy metabolism, depending on the arm of the 2 metabolic pathways that is defective, that is vitamin B12 and folate deficiency in the remethylation pathway. These vitamins may be lacking in CKD patients as renal disease results in a catabolic state, a syndrome of malnutrition, inflammation and atherosclerosis with reduced intake and minimal absorption usually prevalent in patients especially those in end-stage [25].

The ~~A~~ significant difference ~~was noticed~~ in ~~tHcy~~ homocysteine levels between~~of~~ cases and control in this study ~~which was higher in the cases than control, which~~ is also in keeping with the findings by Muhammad *et al* in Pakistan [26]. The mean tHcy value of the controls 8.3 ± 2.85 is also comparable to that reported by Okubadejo *et al* amongst their control subjects –

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10.1 ± 7.7 [27] and also with that reported by Osunkalu amongst otherwise healthy subjects with a mean tHcy of 9.5 ±2.4 [28]. This is expected as the kidneys help in clearing homocysteine, and with kidney disease the renal clearance of homocysteine is impaired.

This study also revealed an association between the severity~~degree~~ of kidney disease and the level of hyperhomocysteinemia as it shows a steady rise in the prevalence rate of total hyperhomocysteinemia from CKD stage 1 through to 5. This association was significant; and is in concordance ~~keeping~~ with findings from other studies [10, 29, 30, 31]. Shankar *et al* found that higher plasma homocysteine levels were seen in patients with CKD, independent of BMI, smoking, diabetes mellitus, hypertension, cholesterol levels, and other confounders. Pooled data from 41 trials and 27,000 patients show that homocysteine levels are significantly inversely correlated with estimates of GFR [32], with higher levels of homocysteine seen in patients with reduced GFR.~~,~~ This inverse correlation is even more robust when using clearance methods in measuring GFR [32].

There is good evidence that normal kidneys play a major role in amino acid and Hcy clearance and metabolism however it may be difficult to identify the source of clearance defect of Hcy owing to the lack of data on Hcy extraction and metabolism by diseased kidneys. The existence of Hcy-metabolizing enzymes and uptake systems in renal tubular cells has been confirmed, and Hcy extraction studies in animal kidneys documented significant Hcy uptake [33, 34]. Logically the loss of metabolically active kidney tissue normally involved in Hcy handling should decrease Hcy clearance and increase plasma levels. The inverse relationship between Hcy levels and GFR, which is consistent throughout the different stages of CKD, supports the fact that it is reduced renal function, not the accumulation of uremic toxins that causes Hcy levels to increase.

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CONCLUSION

This study has shown that hyperhomocystienemia is prevalent in CKD patients and worsens as renal function declines.

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