

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.

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

Comment [M2]:

Study design: A comparative ~~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

Comment [M3]: This is not a case control study as the outcome in both groups – cases and controls wasn't known

Methodology: A comparative ~~cross sectional study~~ among 138 patients with CKD and 69 healthy consenting hospital staff individuals. ~~They had their estimated Gglomerular 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. All subjects had the homocysteine levels measurements using the enzyme-linked immunosorbent assay. Homocysteine levels were compared between healthy persons and CKD patients as well as within different stages of chronic kidney disease.

Comment [M4]: This is not a case control study as the outcome in both groups – cases and controls wasn't known

Results: Most of the CKD patients (47.8%) were stage 4, followed by (33.3%) stage 3 and stage 5 (14.5%). While stages 1 and 2 were the least (2.2% each). ~~2.2~~ 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% among control subjects with median homocysteine (Hcy) level being $19.1 \mu\text{mol/l}$ (IQR - 13.8) in cases and $8.3 \mu\text{mol/l}$ (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.

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

29 INTRODUCTION

30 Chronic kidney disease (CKD) is a public health problem ~~that is present~~ present
31 worldwide with an incidence that's on the rise, unfavourable outcomes and very high cost
32 [1].

33 Cardiovascular disease (CVD) ~~is a~~ major cause of morbidity and mortality in patients
34 with CKD ~~that~~ ~~which~~ is well documented ~~is cardiovascular disease (CVD)~~ [2]. Although patients
35 with CKD are at risk of ultimately developing end- stage renal disease (ESRD), about 50% of
36 CKD patients die of CVD before commencement of dialysis [3-6]. ~~The morbidity and mortality~~
37 ~~from CVD is extremely high in CKD~~ In patients requiring haemodialysis ~~patients~~ (usually stage
38 5 CKD ~~patients~~) ~~the morbidity and mortality from CVD is extremely high~~ [7]. Based on data
39 from the U.S. Renal Data System Coordinating Center Case-Mix Adequacy Study, clinical
40 coronary heart disease (CHD) is seen in 40% of haemodialysis patients and CVD in them is 10 to
41 30 times higher than in the general population despite stratification by gender, age, race, and the
42 presence of diabetes [7].

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

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

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

59 Hyperhomocysteinemia in itself has also been shown to be an independent
60 cardiovascular risk factor in patients with chronic kidney disease [10, 13] however, the
61 mechanism is not clear. Elevated levels of homocysteine and cardiovascular disease are common
62 in patients with decreased renal function. An increase in ~~Homocysteineey~~ represents a potent
63 risk factor for coronary, cerebrovascular and peripheral arterial disease as well as for deep vein
64 thrombosis [14]. Hyperhomocysteinemia is seen in the early stages of CKD at levels of
65 glomerular filtration rate (GFR) of about 60ml/min and its prevalence increases to about 85-
66 100% ~~+15~~ in ESRD[15]. In the general population moderate hyperhomocysteinemia is seen in
67 approximately 5 – 7 % [16].

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

73 **MATERIALS AND METHODS**

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

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86 **SAMPLE SIZE**

87 Studies on the prevalence of hyperhomocysteinemia revealed a range of 56% - 90% [7, 10, 16,
88 19, 20, 21]. The sample size was extrapolated from the formula for sample size determination in
89 an infinite population [22] using a prevalence of 90% and a degree of precision of 0.05. A
90 sample size of 138 was arrived at. A total of 138 chronic kidney disease patients and 69 controls
91 in a ratio 2:1 were recruited for this study.

Comment [M5]: A ratio 2:1 was used to minimise cost

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

98 measured in the horizontal plane at the level of the natural waist line taken to be at the umbilicus
99 using a non-stretchable tape. All patients and controls were instructed to be observed
100 overnight fast for 8-10 hours before blood ~~the day of~~ sample collection on the next day. About
101 10ml of venous blood was collected, 5ml into a lithium heparin sample bottle for serum
102 creatinine estimation using the Jaffe's reaction and 5ml into an EDTA bottle and centrifuged for
103 15 minutes within 30 minutes of collection and plasma stored at < 20oC for homocysteine level
104 assay using the Axis® Homocysteine FHCY100 Enzyme Immunoassay (EIA) [17].

105 The results were categorized ~~interpreted~~ as follows:- moderate hyperhomocysteinemia -
106 15-30µmol/L, intermediate hyperhomocysteinemia - >30-100µmol/L, and severe
107 hyperhomocysteinemia - >100µmol/L [16].

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

110 Glomerular filtration Rate R was calculated using the Cockcroft-Gault formula to
111 estimate creatinine clearance (CCr): $CCr = (140 - \text{age}) \times (\text{weight})/72 \times \text{SCr} \times (0.83 \text{ if female})$,
112 where SCr is in mg/ dL, weight in kg and age in years and results were stratified according to the
113 KDIGO guidelines [17] into CKD ~~the different~~ stages 1-5 of CKD.

114 SAMPLE SIZE

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116 ~~19, 20, 21]. The sample size was extrapolated from the formula for sample size determination in~~
117 ~~an infinite population [22] using a prevalence of 90% and a degree of precision of 0.05. A~~
118 ~~sample size of 138 was arrived at.~~

119 **DATA ANALYSIS**

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

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

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

132 **RESULTS**

133 ~~A total of 138 chronic kidney disease patients and 69 controls in a ratio 2:1 were~~
 134 ~~recruited for this study.~~ Their ages of patients ranged from 18 to 80 years with the mean age of
 135 the study population being 45.9 years \pm 16.4 for cases and 42.2 years \pm 14.6 for control (P
 136 $=0.119$). The cases consisted of 89 males (64.5%) and 49 females (35.5%) while controls were
 137 made up of 42 males (60.9%) and 27 females (39.1%), $P = 0.610$

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

140 **TABLE 1:** SOCIODEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION

	CASES	CONTROLS	t	P -value
CHARACTERISTICS	n = 138	n = 69		
	<u>(Mean \pm S.D)</u>	<u>(Mean \pm S.D)</u>		

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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
	<u>n = 138(%)</u>	<u>n = 69(%)</u>	χ^2	
Sex				
Male	89 (64.5%)	42 (60.9%)	0.260	0.610
Female	49 (35.5%)	27 (39.1%)		
Marital status				
Unmarried	34 (24.6%)	15 (21.7%)	0.214	0.6440.188
Married	104 (75.4%)	54 (78.3%)		

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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 ($P = 0.0001$), while the estimated GFR was significantly lower in cases.

145 | **TABLE 2H:** CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF STUDY
 146 | POPULATION

VARIABLE	CASES (n=138)	CONTROLS (n=69)	TEST STATISTICS†	P- value
BMI (kg/m ²)	23.71 ± 4.94	26.7 ± 3.6	0.303*	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
	<u>Median (IQR)</u>	<u>Median (IQR)</u>	<u>Wilcoxon</u>	<u>P- value</u>
Estimated GFR (mg/dl)	29.9(IQR-18.9)	96.7(IQR-16.7)	187.00‡	<0.01
Homocysteine level(μmol/L)	19.0(IQR- 13.8)	8.3(IQR - 2.9)	1820.00‡	<0.01

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147 | * t-test

148 | † Wilcoxon

149 | BMI – Body Mass Index, GFR – Glomerular filtration rate

150 |

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

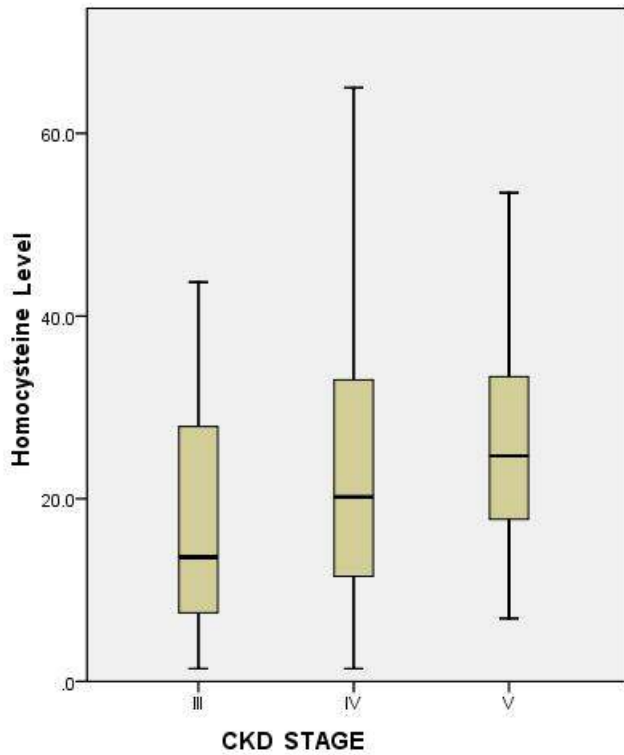
154 | The [frequencyprevalence](#) of hyperhomocysteinemia was 57.9% among CKD patients and
 155 | 4.3% among the controls $P < 0.01$, OR = 30.34 (95% CI = 9.09,- 101.29). Forty-two percent of
 156 | the CKD patients had normal levels of homocysteine, 30.4% moderate hyperhomocysteinemia
 157 | and 27.5% intermediate hyperhomocysteinemia. None had severe hyperhomocysteinemia as
 158 | depicted in table [3III](#).
 159 |

160 | **TABLE 3III: COMPARISM OF PREVALENCE OF HOMOCYSTEINEMIA (CASES**
 161 | **VERSUS CONTROLS)**

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

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

163 | The median values of Hcy increased from stage 1 to 5. There was a significant correlation
 164 | between the stages of CKD and the degree of homocysteine levels (spearman's rho = 0.204, $P =$
 165 | 0.016-) as shown on figure 1.
 166 |



167

168 Fig 14: Boxplot showing correlation between CKD stage and median homocysteine levels

169 **DISCUSSION**

170 Increased cardiovascular morbidity and mortality in CKD patients has been associated
 171 with elevated homocysteine levels which is a common finding in them [10,11]. This study
 172 shows that hyperhomocysteinemia is significantly higher among CKD patients. Using
 173 the classification by Kang et al, 57.9% of the CKD patients had hyperhomocysteinemia. This is
 174 comparable with 56% reported by Menon Vandana et al in England in which baseline
 175 homocysteine was measured in 2 different populations; population A with estimated GFR

176 25 – 55 ml/min had a prevalence of hyperhomocysteinemia of 56% [21]. It is, however, lower
177 than that reported by Ajith *et al* in New York [23], in which 147 patients; 85 males and 62
178 females aged 58 ±15 years requiring hemodialysis had a prevalence of 82%. The difference in
179 prevalence rate may be due to the fact that the index study assessed the prevalence in both
180 dialyzing and non-dialyzing patients.

181 Cases with moderate and intermediate hyperhomocysteinemia were 30.452.5%, and
182 247.5% respectively, none had severe hyperhomocysteinemia, which is not surprising as cases of
183 severe hyperhomocysteinemia usually due to homozygous defects in genes encoding for
184 enzymes of homocysteine metabolism leading to accumulation of Hcy in blood and urine, are
185 extremely rare. An example of this is a disorder caused by homozygosity for a defective gene
186 which encodes for cystathionine beta-synthase. In this condition Hcy can be as high as
187 400umol/L [24]. In the index study, however, a majority of the patients with
188 hyperhomocysteinemia had moderate hyperhomocysteinemia which may be due to deficiencies
189 in vitamins that play major roles in Hcy metabolism, depending on the arm of the 2 metabolic
190 pathways that is defective, that is vitamin B12 and folate deficiency in the remethylation
191 pathway. These vitamins may be lacking in CKD patients as renal disease results in a catabolic
192 state, a syndrome of malnutrition, inflammation and atherosclerosis with reduced intake and
193 minimal absorption usually prevalent in patients especially those in end-stage [25].

194 The A-significant difference ~~was noticed~~ in ~~tHcy homocysteine levels between~~ cases
195 and control ~~in this study which was higher in the cases than control, which is also~~ in keeping
196 with the findings by Muhammad *et al* in Pakistan [26]. The mean tHcy value of the controls 8.3
197 ± 2.85 is also comparable to that reported by Okubadejo *et al* amongst their control subjects –
198 10.1 ± 7.7 [27] and also with that reported by Osunkalu amongst otherwise healthy subjects with

199 a mean tHcy of 9.5 ± 2.4 [28]. This is expected as the kidneys help in clearing homocysteine,
200 and with kidney disease the renal clearance of homocysteine is impaired.

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

Comment [M6]: Estimated GFR is used in the staging of CKD,

211 | There is good evidence that normal kidneys play a major role in amino acid and Hcy
212 | clearance and metabolism however it may be difficult to identify the source of clearance defect
213 | of Hcy owing to the lack of data on Hcy extraction and metabolism by diseased kidneys. The
214 | existence of Hcy-metabolizing enzymes and uptake systems in renal tubular cells has been
215 | confirmed, and Hcy extraction studies in animal kidneys documented significant Hcy uptake [33,
216 | 34]. Logically the loss of metabolically active kidney tissue normally involved in Hcy handling
217 | should decrease Hcy clearance and increase plasma levels. The inverse relationship between Hcy
218 | levels and GFR, which is consistent throughout the different stages of CKD, supports the fact
219 | that it is reduced renal function, not the accumulation of uremic toxins that causes Hcy levels to
220 | increase.

221

222

223 **CONCLUSION**

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

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