Original Research Article

Homocysteinaemia in Heart Failure Patients in North Eastern Nigeria

ABSTRACT

Aims: The study was aimed at determining the relationship between plasma homocysteine level and indices of severity in heart failure patients seen at a referral teaching hospital in Gombe, Northeast Nigeria.
Study design: It was a hospital based cross-sectional study conducted on newly diagnosed heart failure patients managed by the cardiology unit of the Federal Teaching Hospital, Gombe, between May 2015 and December 2015..
Methodology: Ninety newly diagnosed Heart Failure patients who presented to the hospital along with 90 age and sex matched controls were recruited. All the subjects had clinical and Echocardiography evaluations. Homocysteine was assayed using enzyme-linked Immunosorbent Assay (ELISA) Kit for homocysteine designed by Cloud-clone Corp. Data was analyzed using the Statistical Package for Social Sciences for Windows (SPSS), Version 20.
Results: The mean left ventricular ejection fraction (LVEF) of the patients was 35.4±9.81% while that of the control group was 62.1±7.04% (P <0.001). The mean HCY of patients (11.61 ± 8.00umol/l) was higher than that of controls (10.24±6.98umol.l), though not significantly; P=0.225. The 90th percentile of the homocysteine (HCY) levels in control was 20.9umol/l. There was no significant relationship between plasma HCY level and the NYHA class of the patients, though post hoc analysis shows HCY level significantly increased from class I to other classes (P=0.034, P=0.020 and P=0.047 respectively). The bivariate correlation between plasma HCY and Echocardiographic LV indices revealed no statistically significant relationship especially with the LVEF (r-0.149, p=0.160).However, the plasma HCY of the heart failure patients increased with increasing left ventricular mass index (LVMI) (r=0.246, p=0.019).
Conclusion: The study found that homocysteine levels are not significantly elevated in heart failure patients compared with healthy matched controls. However, elevated values were found with increasing left ventricular mass index. This might suggest a possible role of homocysteine in cardiac remodeling**.**
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Keywords: Heart Failure; Homocysteine; Hyperhomocysteinaemia; left ventricular mass index (LVMI); *North-Eastern Nigeria,*

1. INTRODUCTION

Heart Failure (HF) is a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood. [1] Heart failure is a progressive syndrome with high morbidity and mortality despite significant advancement in its treatment.[2] It is also a major public health problem world-wide that imposes a huge burden on the health care system.[3]

About 20% of adults above 40 years of age have a lifetime risk of developing HF. [2, 4] Worldwide, over 23 million individuals have HF and the prevalence continues to rise. Approximately 5.8 million persons in the United States have clinically manifest HF.[5, 6] Although large scale studies on the prevalence of HF are scarce in Africa, including Nigeria, several studies have revealed a hospital admission rate of 3–7% for HF in general medical wards and over 30% in specialized cardiovascular units across the continent.[7-9]. Unachukwu et al, found heart failure to be the third commonest noncommunicable cause of admission in a teaching hospital in Port Harcourt, Nigeria.[10]

Hypertension, smoking, ischemic heart disease, rheumatic heart disease, diabetes mellitus, obesity, advancing age, among others, have been identified as the most important risk factors that predict the incidence of HF as well as its severity.[9, 11-15] Therefore, identification of risk factors and risk indicators could help to prevent heart failure and ease the global burden of the disease. In the last decade, Plasma Homocysteine (HCY) has emerged as a major vascular disease risk factor. [16-17]

Hyperhomocysteinaemia (HHCY) is also commonly known as an independent risk factor for various cardiovascular diseases like endothelial dysfunction, vascular inflammation, atherosclerosis, hypertension, cardiac hypertrophy, neuro-degenerative and pregnancy associated disorders. [18, 19]

An increased incidence of HF was observed in individuals with elevated homocysteine (HCY) levels without prior Myocardial infarction (MI), suggesting a direct relationship between HHCY and HF.[20] Some of the mechanisms adduced for this relationship include; 1) increased cardiac fibrosis and increased activation of matrix metallo-proteinases, which in turn promote left ventricular remodeling and then HF; 2) activated matrix metallo-proteinases also causes endothelial and structural vascular dysfunction; 3) Hyperhomocysteinaemia induced direct negative inotropic and coronary vasodilatory effect; 4) Increased oxidative stress which is known to promote myocardial dysfunction.[21, 22]

However, there is paucity of information on the relationship between plasma homocysteine levels and HF in Nigeria, especially in North-eastern Nigeria. Hence this study sets out to fill this gap by determining whether plasma total homocysteine levels are related to heart failure from all causes, and to severity of HF as measured by clinical and echocardiographic indices.

2. MATERIALS AND METHODS

The study was a hospital based cross-sectional analytical study conducted on newly diagnosed heart failure patients managed at the cardiology unit of the Federal Teaching Hospital, Gombe in North eastern Nigeria. The hospital is a tertiary health centre serving communities from all the states North East part of Nigeria. The study subjects included ninety newly diagnosed heart failure patients who presented to the Accident & Emergency Department and/or Cardiology Clinic of the hospital. Heart failure was defined using the Framingham Criteria [23].

Age and sex-matched controls that fulfilled the inclusion criteria were also included in the study. The controls were selected mainly from willing patients'relatives who shared the same diet and environmental conditions with the patients. Patients receiving Vitamin B derivatives, folic acid, anticonvulsants, Theophylline and Methotrexate were excluded from the study.

An informed written consent was obtained from the participants using a consent form which stated in clear and simple terms the purpose and importance of the study including the likely involved procedures.

Demographic and relevant clinical information were obtained from the patients and controls. A thorough physical examination with emphasis on the cardiovascular system was performed. Clinical diagnosis of HF was based on the Framingham's criteria of the concurrent presence of two major or one major with two minor criteria. Conventional trans-thoracic echocardiography (TTE) was used to evaluate subjects. Two-dimensional guided M-mode measurements were made according to the recommendations of the American Society of Echocardiography (ASE) convention. [24-25] Measurements were obtained in up to three cardiac cycles, according to the ASE convention and the average recorded.

Total plasma HCY was determined on overnight fasting samples drawn after about 8-10hrs of fasting. All blood samples were centrifuged within 1 hour of collection. About 2mls of plasma was collected using micropipette, transferred into new plain sample bottles and frozen at minus 20°C.Homocysteine was assayed using enzyme-linked Immunosorbent Assay (ELISA) Kit for homocysteine designed by Cloud-clone Corp. Abnormal total plasma HCY was defined as any level above the 90th percentile in the distribution of HCY in the control population.

Data was analyzed using the Statistical Package for Social Sciences for Windows (SPSS), version 20. Demographic variables like age, sex and marital status were summarized using the mean for continuous variables, and proportions for categorical variables. Homocysteine levels in the subjects and control group were presented as mean and standard deviations. Mean homocysteine level was compared between subjects and control using independent sample t test. Patients were divided into four severity groups according to NYHA classification and mean homocysteine was compared across the groups using analysis of variance (ANOVA).The relationship between left ventricular mass index (as an index of severity) and homocysteine was examined using Pearson's correlation. All the analyses were Two-Tailed, with statistical significance put at probability value of ≤ 0.05.

3. RESULTS

A total of 180 subjects (90 patients and 90 age and sex matched controls) were recruited into this study and all of them were included in the analysis. The mean age of the patients was 44.4±18.6 years and was not significantly different from the mean age of the controls (P=0.467) Table 1.The patient group comprised 31 males (34.4%) and 59 females (65.6%) while the control group consisted of 34 males (37.8%) and 56 females (62.2%); (P=0.642). The mean weight of the patients (62.30±10.8kg) was significantly lower than that of the controls (66.8 ±13.4kg; P=0.013) though no significant difference was observed in the height (Table 1)

The mean LVEF of the patients was 35.4±9.81% while that of the control group was 62.1±7.04% (P <0.001). This is shown in Table 2. Most of the patients presented with ejection fraction between 30% and 39%. figure 1

Table 1. SOCIO-DEMOGRAPHIC CHARACTERISTICS AND CLINICAL FINDINGS BETWEEN PATIENTS AND CONTROLS

Variables	**Patients n = 90 (%)**	**Control n = 90 (%)**	**t/χ^2**	**p value**
Age (yrs.) Mean ± SD	44.42 ± 18.59	46.26±14.92	0.730	0.467
Male	31 (34.4)	34 (37.8)	0.217	0.642
Female	59 (65.6)	56 (62.2)		
BMI Mean ± SD	22.80 ±3.83	24.43 ± 5.29	-2.365	0.019*
Height (cm) Mean ± SD	165.3±7.3	165.8±8.1	0.398	0.691
Weight (kg) Mean ± SD	62.3±10.8	66.3± 13.4	2.513	0.013*

Plasma HCY Mean± SD	11.61±8.00	10.24±6.98	1.218	0.225

*Key: t: Independent sample t-test; χ^2: Chi square; *: Statistically significant (i.e. P value < 0.05): SD=Standard deviation, BMI= body mass index,*

FIGURE 1: PIE CHART SHOWING CLASSIFICATION OF PATIENTS BASED ON EJECTION FRACTION

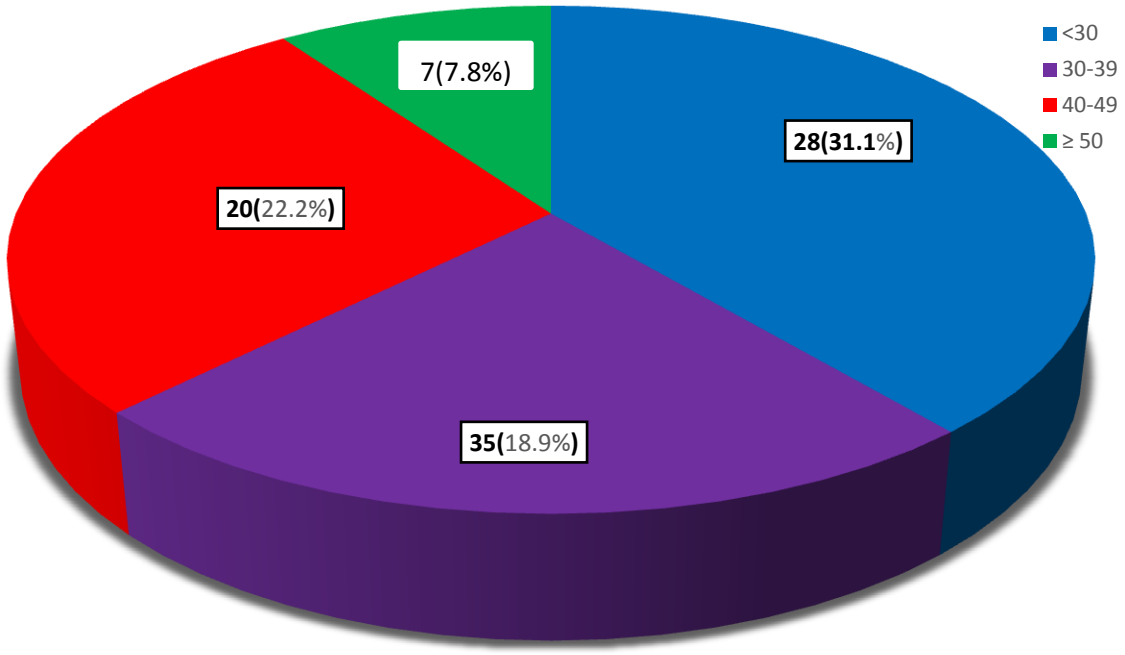

Table 2. COMPARISON OF ECHOCARDIOGRAPHIC FINDINGS BETWEEN PATIENTS AND CONTROLS

Variables			**t**	**p value**
	Subject	Control		
	n=90	n=90		
LVIDD(cm)	5.99 ± 0.99	4.54 ± 0.44	12.670	< 0.001*
PWTD(cm)	0.99 ± 0.30	0.87 ± 0.14	3.409	0.001*

IVSTD(cm)	1.05 ± 0.29	0.90 ± 0.14	4.268	< 0.001*
LVIDS(cm)	4.90 ± 1.00	3.00 ± 0.45	16.377	< 0.001*
LVEF (%)	35.41 ±9.82	62.06 ± 7.04	-20.924	< 0.001*
LVM(g)	257.34 ± 95.08	134.08 ± 33.01	11.618	< 0.001*
LVMI (g/m^2)	184.80 ± 74.46	88.89 ± 21.93	11.721	< 0.001*
ARD(mm)	22.84 ± 11.08	27.78 ± 3.61	-4.018	< 0.001*
LAD(mm)	34.34 ± 17.67	29.40 ± 4.49	2.571	0.011*
E/A ratio	1.69 ± 0.79	1.47 ± 0.31	2.477	0.014*

*Key: t: Independent sample t-test; *: Statistically significant (i.e. p value < 0.05).LVIDd = left ventricular internal dimension in diastole, LVIDs = left ventricular internal dimension in systole, IVSTd=interventricular septal thickness in diastole, EF = Ejection fraction, LVM = left ventricular mass, LVMI = left ventricular mass index, LVEF= left ventricular ejection fraction, E/A = ratio of early ventricular inflow velocity to late ventricular inflow velocity, SD=Standard deviation, CM=Centimeter, gm. =Gram*

The mean HCY in patients with heart failure (11.61±8.00umol/l) was higher than that of the controls (10.24±6.98umol/l), though not significantly (P=0.225) (Table 1).. The 90th percentile of the HCY levels in the control was 20.9umol/l. There was no significant difference in HHCY in patients and control.

No significant relationship was observed between plasma HCY level and the NYHA class of the patients though post hoc analysis showed HCY level significantly increased from class I to other classes (P=0.034,P=0.020 and P=0.047 respectively).(Table 3)

TABLE 3: COMPARISON OF PLASMA HOMOCYSTEINE LEVELS WITH SEVERITY OF HEART FAILURE (NYHA)

Variables	**NYHA**				**F**	**P value**
	Class I n=3	**Class II n=10**	**Class III n=32**	**Class IV n=45**		
Plasma Homocysteine						
Mean ± SD	9.30 ± 1.91	12.76 ±6.42	12.08 ± 8.63	11.08 ± 7.74	0.495	0.687

Post Hoc Tests showing the mean difference between classes

Variables	NYHA	Within NYHA	Mean Difference	*p* value
Plasma Homocysteine				
	Class I	Class II	-11.123	0.034*
		Class III	-11.296	0.020*
		Class IV	-9.444	0.047*
	Class II	Class 1	11.123	0.034*
		Class III	-0.174	0.952
		Class IV	1.678	0.543
	Class III	Class I	11.296	0.020*
		Class II	-0.174	0.952
		Class IV	1.852	0.311
	Class IV	Class I	9.444	0.047*
		Class II	-1.678	0.543
		Class III	-1.852	0.311

*F: One Way Anova; *: Statistically significant (i.e. p value < 0.05), NYHA=New York Heart Association*

The bivariate correlation between plasma HCY and Echo LV indices revealed a statistically significant positive correlation between plasma HCY and PWTD (r= 0.255, P=0.015), IVSTD (r= 0.295, P=0.005) and LVMI (r =0.246, P=0.019).Table 4. Further comparison between HHCY and LVEF group showed no significant relationship. However, all those with LVEF greater than 40 had plasma HCY levels below 21umol/L which was used as the cut off. (Table 5)

TABLE 4: CORRELATION BETWEEN PLASMA HOMOCYSTEINE LEVELS AND ECHOCARDIOGRAPHIC FINDINGS IN PATIENTS WITH HEART FAILURE

Variable	r	*p* value
Plasma Homocysteine		
LVIDD(cm)	-0.005	0.962
PWTD(cm)	-0.255	0.015*
IVSTD(cm)	-0.297	0.005*
LVIDS(cm)	0.069	0.517

LVEF (%)	-0.149	0.160
LVM(gm.)	0.295	0.005*
LVMI (g/m^2)	0.246	0.019*
ARD(mm)	0.474	< 0.001*
LAD(mm)	0.456	< 0.001*
E/A ratio	0.024	0.825

*Key: r: Pearson correlation coefficient; *: Statistically significant (i.e. p value < 0.05),LVIDD= left ventricular internal dimension in diastole, PWTD= Posterior wall thickness in diastole, IVSTD= Interventricular septal thickness in diastole, LVEF = Ejection fraction, LVMI = left ventricular mass index, LVM= left ventricular mass, E/A ratio = ratio of early to late ventricular inflow velocity*

TABLE 5: HOMOCYSTEINE AND EJECTION FRACTION IN SUBJECTS

Variables	**Homocysteine Group**		**χ^2**	***p* value**
	< 21	**≥ 21**		
	n=83 (%)	**n=7 (%)**		
Ejection Fraction				
< 30	26 (31.4)	2 (28.6)	4.359	0.225
30 – 39	30 (36.1)	5 (71.4)		
40 – 49	20 (24.1)	0 (0.0)		
≥ 50	7 (8.4)	0 (0.0)		

χ^2: Chi square

4. DISCUSSION

Plasma homocysteine (HCY) has been suggested has an elevated marker in predicting the occurrence and severity of heart failure irrespective of the aetiology.[26] This index study showed a slightly higher but statistically insignificant mean HCY level among heart failure patients when compared to the healthy matched controls. The mean plasma HCY level is similar to what was reported by Okubadejo et al,[27] in stroke patients and by Ebesunun et al[28] amongst patients with Diabetes (10.2 ± 4.6umol/L and10.5±3.9umol/L respectively). Also, no signicant difference was found by Okubadejo et al when compared with matched control. Glew et al in an earlier study of stroke and MI patients in Gombe has also shown no significant difference between patients and controls. [29] This is however in contrast to what was reported by Herrmann et al when he compared 95 patients with systolic chronic HF and 12 healthy persons without cardiac diseases. He reported a mean HCY of 17.1 umol/L (± 20) and 9.6 umol/L (± 2.6) in HF patients and control respectively (P< 0.01).[30] This difference could be due to the fact that their controls,though small in number, were not matched for age and sex. Moreso, non fasting HCY was used in the study and this could have inadvertently elevated the mean HCY. [31]

Hyperhomocysteinaemia (HHCY), HCY level greater than 90th percentile of control was present in 7.8% of the patients and 5.6% of the control (P=0.55). A similar non significant HHCY level was also reported

by Okubadejo et al, among stroke patients and control, though with a lower cut off (HCY level >14.6umol/L).[27]The relatively higher cut off for HHCY is perhaps reflecting very low intake of folate and vitamin B12 among northern Nigerians, an observation made in an earlier study.[32] Glew et al, has also suggested that the relatively high level of HCY in the people of Gombe region in northern Nigeria may be as a result of a high prevalence among the population of a mutation in the gene encoding 5,10-methylene tetrahydrofolate reductase (MTHFR).[29]Mutations affecting the activity or expression of MTHFR have been shown to be associated with hyperhomocysteinaemia.[33] Several laboratories have varying cut off for HHCY that ranged from 15 to 20 umol/L especially in adults who do not supplement vitamins or eat folate fortified food.[34] Hence, the 90th percentile cut off value used in this index study still falls within the range quoted for the caucasian populations.

Plasma HCY has been found to have a linear relationship with severity of HF based on the NYHA classification in some earlier studies done among caucasians.[22,30] This study, after post hoc analysis, only revealed a significant increase between HCY level in those that presented in NHYA class 1 when compared with other classes. This observed non linear relationship may be explained by the fact that most of the patients would have sought alternative care from non orthodox practitioners, with several medications used, before presenting at the specialist center. This could have affected their plasma level of HCY unlike class 1 individuals who most likely presented earlier. Moreso, Glew et al, from his findings reported no significant rise in HCY level in patients with coronary artery disease risks within a similar population.[29]This finding is however in contrast to what was reported by Herrmann et al,[30]amongs HF patients in Germany. They reported a stepwise increase in mean total plasma HCY levels with increasing NYHA class, from 10.6umol/L in class I to 17.4umol/L in class IV (p=0.002). The difference could be as a result of late presentation and also the relatively younger age of our HF patients. Hence, plasma HCY levels do not directly correlate with the clinical severity of HF in the study population.

This study revealed no significant relationship between increasing HCY level and the LVEF in the subjects (P =0.160). However there is a correlation between the LVMI and mean HCY level (P= 0.019). Data from the Framingham Study also revealed significant associations of HCY with left ventricular mass and left ventricular wall thickness. The Framingham‘s data also showed no relation between HCY and the echocardiograpically assessed left ventricular function.[35] This was also reported by Wocial et al in subjects with essential hypertension.[36] This is however in contrast to what was reported by Vasan et al[20] and Hermann et al[30] where HCY level was found to have inversely correlated with LVEF. More so, clinical data relating plasma homocysteine to LV function have yielded inconsistent results. Negative, positive, and no association of plasma homocysteine with LV ejection fraction have been reported in referral samples of patients with coronary artery disease.[37] Several mechanisms have been adduced for the observed relationship between LVMI and plasma HCY levels which include; the critical role of homocysteine as a source of increased oxidative stress, a factor known to promote myocardial dysfunction. HHCY induced cardiac fibrosis and increased activation of matrix metalloproteinases, which in turn promote left ventricular remodeling, a known precursor of elevated LVMI.[22] Homocysteine also has growth-promoting and collagen production-stimulating effects on vascular smooth muscle cells and inhibitory effects on endothelial cell growth.

5. CONCLUSION

The study found that homocysteine levels are not significantly elevated in Nigerians with heart failure when compared to the healthy general population. The hyperhomocysteinaemia cut off in the study population is higher than the Caucasian reference value. Plasma HCY levels do not increase with heart failure severity (Using LVEF and NYHA). However, Plasma HCY levels increase with increasing left ventricular mass index (LVMI) which suggests its role in cardiac remodeling.

CONSENT

Informed consent was obtained from all participants in the study.

ETHICAL APPROVAL

The design of the study was approved by the Research and Ethics Committee of the Federal Teaching Hospital, Gombe.

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