Relationship between plasma homocysteine and blood pressure in hypertensive Northern-Nigerians

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Abstract

Aim: The study sought to determine whether there is any relationship between plasma homocysteine and blood pressure levels in Nigerians with essential hypertension.

Method: It was a cross-sectional analytical study done on 120 randomly selected hypertensive patients and 120 normal healthy controls seen at the large Conference hall of the Ahmadu Bello University (ABU) Medical Centre, Zaria as well as the ABU Teaching Hospital, Zaria, Northern-Nigeria. Pearson's Correlation and Binary Logistic Regression analysis determined the relationship between homocysteine and hypertension.

Results: Hyperhomocysteinaemia found in the hypertensive patients (22.8 ± 6.6 μmol/L) differed significantly ($p<0.001$) from controls (10.9 ± 2.8 μmol/L) with significant ($p<0.001$), blood pressure difference between both groups. Homocysteine significantly positively correlated with systolic ($r = 0.51$, $p<0.001$) and diastolic ($r = 0.47$, $p<0.001$) blood pressures in hypertensive subjects. The relation of plasma hcy to hypertension was statistically significant for SBP; OR: 1.08 (95% CI, 1.05-1.11) and DBP; OR: 1.08 (95% CI, 1.03-1.13) in the unadjusted model. When adjusted for confounding variables, hcy was significantly related to SBP; OR: 1.1 (95% CI, 1.04-1.18) but not DBP ($p=0.25$; OR: 1.06 (95 % CI, 0.96-1.18). The mean plasma folate level was high (115.2 ± 48.0 ng/mL) in the hypertensive subjects. The hyperhomocysteinaemic subjects showed a 2.8 times Odds of developing hypertension.

Conclusion: This study showed higher mean plasma homocysteine levels in hypertensives than controls not accounted for by sub-optimal folate levels. Hyperhomocysteinaemia showed a positive relationship to systolic hypertension after adjusting for confounders.

Keywords: Plasma homocysteine, hypertension, healthy controls, folic acid, blood pressure, Northern-Nigerians.

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Background

Hypertension is a major risk factor for arteriosclerosis which results in cardiovascular diseases such as stroke and myocardial infarction[1,2]. It was responsible for a third of all deaths worldwide[3] and it is projected that by the year 2025, 1.56 billion adults will be living with hypertension worldwide[4]. Hypertension was rare in Africans in the beginning of the twentieth century. Presently, it has been shown that in some African settings, more than 40% of adults are living with hypertension[5]. Furthermore, evidence shows that hypertensive related complications, especially stroke and heart failure, are becoming more rampant in sub-Saharan Africa[5].

Likewise, in Nigeria, hypertension prevalence as far back as the nineties was 11%, as documented by the nation-wide non-communicable disease (NCD) survey, with definition of hypertension in that survey being BP > 160/90 mmHg[6,7]. Adeloye et al. reported a review and meta-analysis of pooled prevalence of hypertension in Nigeria in the range of 22.5% to 28% with higher urban prevalence of 30.6% and rural prevalence of 26.4%[8]. These trends have strongly been linked with lifestyle changes of Africans which include an increase in tobacco use, excessive alcohol consumption, obesity, lack of exer-

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cise, adoption of "Western" lifestyle and intake of diets rich in salt, refined sugar, unhealthy fats and oils and of low fibre content[5].

The 7th report of the Joint National Committee on the Detection, Evaluation and Treatment of Hypertension advocates that hypertension is a controllable and preventable disease as modification of lifestyle such as smoking and alcohol cessation, exercise and dietary approaches to stop hypertension (DASH diet) have been shown as effective methods in its prevention and treatment[9].

However, these modifiable risk factors of hypertension as well as the non-modifiable (increasing age, black race, family history of hypertension or male sex/postmenopausal factors), can only partly explain why certain patients are at risk of hypertensive complications like stroke or heart attack[10]. Hence, there are newer vascular risk factors such as hyperhomocysteinaemia which has gained prominent focus in the recent past amongst other risk factors such as soluble endoglin, circulating soluble forms-like tyrosine kinase 1 (sFlt1), endostatins, endothelin-1, vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). These risk factors have been shown to predispose to pre-eclampsia and hypertension[11-13].

Homocysteine is a sulphur containing amino acid derived from breakdown product of methionine which is obtained from dietary protein with elevated levels regarded as > 15 μmol/L[14]. It exists in four forms in plasma: the majority (80-90%) being protein-bound thiol; disulfide and mixed disulfide (each consisting of 5-10%) and the free thiol which makes up 1%. The combination of all four forms of homocysteine is referred to as "total homocysteine" (tHcy)[15,16]. It is classified as normal (5-15 μmol/L), mild (15-30 μmol/L), moderate (31-100 μmol/L) and severe (>100 μmol/L) hyperhomocysteinaemia[14-16].

The vascular risk associated with hyperhomocysteinaemia has been shown to be stronger in hypertensive patients[16]. Homocysteine requires enzymes like methylene tetrahydrofolate reductase (MTHFR) and cysthathionine-β-synthase as well as vitamin co-factors such as folate (vitamin B9), cyanocobalamin (vitamin B12), pyridoxine (vitamin B6) or betaine in its metabolic breakdown pathways[14-17]. Therefore, when there is a genetic impairment or mutation in any of these enzymes or the presence of nutritional deficiencies of any of these vitamin co-factors, this may result in hyperhomocysteinaemia[17]. Consequently, there will be widespread endothelial damage manifesting clinically with raised blood pressure[17].

Other molecular mechanisms in which high homocysteine results in hypertension include a rise in asymmetric dimethyl arginine levels which causes direct endothelial injury[18,19]. Hyperhomocysteinaemia also leads to reduced activity of potent anti-oxidants like thioredoxin and peroxiredoxin which results in enhanced activation of NADPH oxidase and consequent increased reactive oxygen species and oxidative stress[18,19]. Furthermore, high levels of homocysteine can result in altered DNA and gene expression with resultant smooth muscle cell proliferation; decreased levels of vasodilatory molecules like nitric oxide; accelerated pro-coagulant activity of factor V, XII, von willebrand and tissue factors, resulting in platelet aggregation and thrombi formation[19,20]. Hyperhomocysteinaemia also inhibits the expression of anti-thrombin molecules[19,20]. All these molecular mechanisms consequently lead to accelerated atherosclerosis and hypertension[19]. However, high homocysteine levels leading to enhanced atherogenicity or being a non-causal risk marker has generated controversy[15].

The sub-Saharan Africa has insufficient data on homocysteine studies[15] with few studies emanating from Nigeria in hypertensive subjects[15,21-23]. There are also conflicting findings on the relationship between plasma homocysteine and blood pressure in hypertensives with or without cardiovascular disease. While some studies have shown that hypertension is not linked to hyperhomocysteinaemia[24,25] others have shown that there is a relationship[15,16,26,27].

The relationship of hyperhomocysteinaemia with essential hypertension in non-Caucasians is relatively understudied[15,27] especially bearing in mind the ethnic, geographic and genetic differences worldwide. Therefore, this study was aimed at determining whether there is a difference in homocysteine levels between hypertensive subjects and normal healthy controls and whether there is a significant relationship between hyperhomocysteinaemia and high blood pressures in Nigerian hypertensives living in Zaria.

Methods

Study location and research design

The design was a cross-sectional comparative analytical study, which was carried out among 120 randomly select-

ed hypertensive subjects who presented at the venue of the large conference hall of the Ahmadu Bello University (ABU) Medical Centre, Zaria. Some subjects were also recruited from the Out-patient Department (OPD) of the Ahmadu Bello University Teaching Hospital, Zaria in Northern-Nigeria. Healthy volunteers consisting of 120 subjects were randomly selected from willing patient escorts and volunteers of same hospitals.

Inclusion criteria for hypertensive patients were, adult subjects with willingness to participate, prior physician diagnosis of hypertension (BP ≥140/90 mmHg), current use of antihypertensive medications and non-diabetes (historically with Fasting blood glucose <7 mmol/L). Those for healthy controls were non-hypertensive (BP <140/90 mmHg), non-diabetes (FBG <7 mmol/L), willingness to participate without clinical evidence of renal, hepatic or cardiorespiratory disease. Exclusion criteria included patients with renal failure (serum creatinine >3 g/dl or GFR <60 ml/min) as determined by Cockcroft-Gault equation[28]; current tobacco use; excessive alcohol use; excessive caffeine use; chronic folic acid, vitamin B_{12} and vitamin B6 supplementation; history of heart failure, stroke, transient ischaemic attack, heart attack, sickle cell disease or pregnancy; as well as use of drugs known to interfere with homocysteine metabolism such as: methotrexate, anticonvulsants, nitrous oxide, sulfadoxine-pyrimethamine, penicillamine, vitamin supplements and contraceptives[14,15].

Ethical clearance was obtained from the Health Research Ethical Committee (HREC), Ministry of Health and Human Services, Kaduna State, Nigeria (Ref: MOH/ADM/744/VOL.1/369) and all participants gave written informed consent.

Screening evaluation and data collection

A total of 180 hypertensive subjects were screened from the venue of the large conference hall of the ABU Medical Centre, Zaria as well as the OPD of ABUTH, Zaria in Northern Nigeria from January, 2016 to June, 2016. Of these, 120 hypertensive subjects met eligibility criteria and were enrolled. There were 120 normal healthy controls that were randomly selected from willing patient escorts and volunteers of the same hospitals. Data collection was by well-structured interviewer-administered questionnaire by the author and 4 trained assisting medical doctors from all eligible subjects. The most important data obtained during the screening included: biodata (address, age, sex, tribe, and religion); establishment of hypertension and duration of hypertension; drug treatment of hypertension; detailed 24 hour dietary recall; family and social history. Physical examination, anthropometric measurements {weight, height & body mass index calculated as weight (kg)/ height^2 (m^2)} and blood pressures, were determined in both patients and controls. Blood pressures were measured using the Accoson Mercury Sphygmomanometer, twice in the left arm of seated subjects previously rested for 5 minutes and by standard protocol and the mean of the two readings was used[29]. Hypertension was defined from self-reported history, current use of anti-hypertensive therapy and or SBP ≥140mmHg or DBP ≥ 90 mmHg[29].

Blood Sample Collection

Blood samplies for plasma homocysteine and folate levels were obtained from the antecubital vein of either arm, after an overnight fast and without tourniquet application[30]. The blood was divided into two 5 ml aliquots and placed into labelled potassium EDTA-containing plastic lavender vacutainer tubes and plain specimen bottles respectively, in which 0.6 TIU/ml or 500 Kallikrein inactivator U/ml (a drop) of aprotinin (trasylol®) had been added[30]. The test tubes were taken to the Immunology laboratory of ABUTH, Zaria within 4 hours of collection in ice cubes, where they were centrifuged at 1800 rpm for 20 minutes. The plasma was separated within one to two hours and divided into aliquots in cryovials and stored at -70 ºC in the anti-retroviral laboratory of ABUTH, Zaria until assay. Baseline investigations such as serum electrolyte, urea and creatinine and fasting blood glucose, were also assayed in the Chemical pathology laboratory of the ABUTH, Zaria using the Chemray 120 automated clinical chemistry auto-analyser.

Measurement of plasma homocysteine and folate

The Human direct homocysteine enzyme linked immunosorbent assay kit (ELISA-Elabscience Biotechnology Co., Ltd., WuHan, P.R.C. with Lot No: AK0016JULI5066 and Catalog No: E-EL-HO156), was used for in-vitro quantitative determination of human homocysteine in plasma according to the manufacturer's manual and based on the Elisa principle[30]. The microtitre plate in this kit was pre-coated with an antibody specific to homocysteine.

Standards and samples were then added to the appropriate microtitre plate wells with a biotin-conjugated polyclonal antibody preparation specific for homocysteine. Avidin conjugated to horseradish peroxidase (A-HRP) which catalyses the substrate solution, was also added to each microplate well and incubated. Then a tetramethylbenzidine (TMB) substrate solution was added to each well. Only those wells that contained homocysteine peptide, biotin-conjugated antibody and enzyme-conjugated avidin exhibited a change in colour. The enzyme substrate reaction was terminated by adding a sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450nm ± 2nm. The concentration of homocysteine in the samples was determined by comparing the optical desity (O.D) of samples to the standard curve. The folic acid ELISA kit-Elabscience Biotechnology Co. Ltd., WuHan, P.R.C. with Lot No: AK0016JULI5067 and Catalog No: E-EL-0009 was used for the in-vitro quantitative determination of plasma folate levels in accordance with the manufacturer's manual[30,31]. Laboratory analysis was by the laboratory scientist in 2 batches under the same prevailing condition of storage and in the presence of the lead author.

Data analysis

Data was validated and analysed by SPSS version 22-software (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as numbers and percentages with difference determined using X^2 Comparison of Means was by the Independent Student's t-test. Pearson's correlation analysis was used to determine the relationship between plasma homocysteine and systolic and diastolic blood pressures. Binary Logistic Regression analysis was applied to examine the interaction of age, sex, body mass index (BMI), fasting plasma glucose (FPG), vegetables/fruits in daily diet, packed cell volume (PCV), family history and duration of hypertension with homocysteine and blood pressure. Relative Risk Assessment with Pearson's Chi-square analysis was used to determine the Odds Ratio of hypertension being associated with hyperhomocysteinaemia. Upper limit for hyperhomocysteinaemia was 15μmol/L[15]. Level of significance was assumed to be $p<0.05$ at 95% confidence interval.

Results

Group Profile

There were a total of 120 hypertensive subjects consisting of 83 (69.2%) females and 37 (30.8%) males and 120 normal healthy controls consisting of 65 (54.2%) females and 55 (45.8%) males. The females predominated over the males in both groups without any significant ($p=0.42$) difference (Table 1). The mean age of the hypertensive subjects was 49.7 ± 9.8 years which did not differ significantly ($p=0.06$) from the normal healthy controls (47.3 ± 10.1 years). The middle age group was more represented in both groups with no statistically significant difference ($p=0.73$) (Table 1). There were significantly ($p<0.001$) more hypertensive subjects, 61 (50.8%) than controls, who had a positive family history of hypertension (Table 1). There was no statistically significant ($p=0.61$) difference in daily dietary intake of fruits and vegetables in the normal healthy controls than in the hypertensive group (Table 1).

Table 1: Base-line socio-demographic characteristics of the study population

Variables	Subjects		
	Hypertensive Subjects (n=120)	Healthy Controls (n=120)	P-Value
Sex			
Male	37 (30.8 %)	55 (45.8%)	0.42
Female	83 (69.2 %)	65 (54.2%)	
Age	49.7 ± 9.7	47.3 ± 10.1	0.06
Young (< 45 years)	40 (33.3 %)	45 (37.5 %)	0.73
Middle age (45-65 years)	72 (60.0 %)	69 (57.5 %)	
Elderly (> 65 years)	8 (6.7 %)	6 (5.0 %)	
Level of education			
None	40 (33.3 %)	23 (19.2%)	0.14
Primary Education	28(23.3 %)	29 (24.2 %)	
Secondary Education	18 (15.0 %)	23 (19.2 %)	
Tertiary Education	20 (16.7 %)	29 (24.2 %)	
Post Graduate	14 (11.7 %)	16 (13.3 %)	
Tribe			
Hausa	75 (62.5 %)	78 (65 %)	
Yoruba	23 (19.2 %)	16 (13.3 %)	0.38
Igbo	8 (6.7 %)	14 (11.7 %)	
Others	14 (11.7 %)	12 (10.0 %)	
Fruits/Vegetables in Daily Diet			
Yes	69 (57.5 %)	72 (60 %)	0.69
No	51 (42.5 %)	48 (40 %)	
Family History of Hypertension			
Yes	61 (50.8 %)	24 (20.0 %)	< 0.01*
No	59 (49.2 %)	96 (80.0 %)	

Difference between the two groups by Chi-Square analysis. * Level of significance at *p<0.01*.

Clinical and Laboratory Parameters: The Mean systolic blood pressure of the hypertensive subjects (147.4 ± 20.0 mmHg), differed significantly (p<0.001), from that of the normal healthy controls (119.8 ± 10.0 mmHg). There was also a significant (p<0.001) difference between the Mean diastolic blood pressure of the hypertensive patients (92.7 ± 14.4 mmHg) and that of the controls (76.3 ± 7.9 mmHg) (Table 2).

There were significantly (p<0.001), higher Mean homocysteine levels in the hypertensive subjects (Mean ± SD, 22.8 ± 6.6 μmol/L), than in the normal healthy controls (Mean ± SD, 10.9 ± 2.8 μmol/L). There were higher plasma homocysteine levels in males than in female hypertensive (26.0 ± 9.2 μmol/L versus 21.4 ± 4.3 μmol/L) group. Likewise, there was a similar trend in the control group though not statistically significant (p=0.55) (Table 2).

Table 2: Clinical and Laboratory Parameters of the Study Population

VARIABLES	HYPERTENSIVE PATIENTS (n = 120)	NORMAL CONTROLS (n = 120)	P-VALUE
Body Mass Index (Kg/m^2)	28.2 ± 4.8	25.1 ± 4.2	< 0.001***
Underweight (<18 Kg/m^2)	2 (1.7 %)	2 (1.7 %)	
Normal (18-24.9 Kg/m^2)	28 (23.3 %)	57 (47.5 %)	< 0.01**
Overweight (25-29.9 Kg/m^2)	42 (35.0 %)	40 (33.3 %)	
Obese (>30 Kg/m^2)	48 (40.0 %)	21 (17.5 %)	
Weight (kg)	73.2 ± 12.7	69.6 ± 13.0	0.03*
Systolic Blood Pressure (mmHg)	147.4 ± 20.0	119.8 ± 10.0	< 0.001***
Diastolic Blood Pressure (mmHg)	92.7 ± 14.4	76.3 ± 7.9	< 0.001***
Plasma Homocysteine (µmol/L)	22.8 ± 6.6	10.9 ± 2.8	< 0.001***
Male (µmol/L)	26.0 ± 9.2^{ǂ}	11.0 ± 2.8†	< 0.001^{ǂ}
Female (µmol/L)	21.4 ± 4.3^{ǂ}	10.7 ± 2.9†	0.55†
Plasma Folate (ng/mL)	115.2 ± 48.0	117.7 ± 44.6	0.68
Male (ng/mL)	116.7 ± 45.6¶	112.0 ± 45.4§	0.75¶
Female (ng/mL)	113.9 ± 50.3¶	122.4 ± 43.6§	0.20§
Packed Cell Volume (%)	40.0 ± 4.6	41.8 ± 4.6	0.002***
Fasting Blood Glucose (mmol/L)	5.7 ± 1.0	5.4 ± 0.9	0.02*
GFR (ml/min/1.73m^2)	100.4 ± 17.3	106.0 ± 17.2	0.01**
Urea (mmol/L)	4.5 ± 1.2	4.3 ± 1.1	0.31
Creatinine (µmol/L)	72.9 ± 13.1	69.3 ± 14.7	0.05*

Difference between two groups by Independent Student's t - test. * Level of significance at $p \leq 0.05$. **Level of significance at $p \leq 0.01$. *** Level of significance at $p \leq 0.001$.^{ǂ} Difference in plasma Hcy levels between male and female hypertensive groups. † Difference in plasma Hcy levels between male and female control group. ¶ Difference in plasma folate levels between male and female hypertensive groups.§Difference in plasma folate levels between male and female control groups. Hcy: Homocysteine; GFR: Glomerular Filtration Rate.

The Mean plasma folate trended towards higher levels (117.7 ± 44.6 ng/mL) in the normal healthy controls than the hypertensive group (115.2 ± 48.0 ng/mL) though not statistically significant (p=0.68). There was no significant (p=0.75 and p=0.20) difference in plasma folate levels between males and females in the patient group as well as control group respectively (Table 2). Both groups trended towards obesity though more significantly (p<0.001), higher in patients than in controls (Table 2).

Furthermore, there were 118 (98.4%) out of 120 hypertensive subjects with hyperhomocysteinaemia. Out of these, 104 (86.7%) had mild hyperhomocysteinaemia (hcy, 15 - 30 µmol/L), while 14 (11.7%) had moderate hyperhomocysteinaemia (30 - 100 µmol/L) and only 2 (1.7%) hypertensive subjects had normal homocysteine (5 - 15 µmol/L) levels. (Table 3). There were 107 (89.2%) normal healthy controls with normal homocysteine levels, while 13 (10.8%) had mild hyperhomocysteinaemia (Table 3). There were no control subjects with moderate to severe hyperhomocysteinaemia.

Table 3: Classification of homocysteine and distribution between normal healthy controls and hypertensive subjects

Hcy Classification (µmol/L)	Normal Controls (n = 120)	Hypertensive Subjects (n = 120)	Total	P-Value
< 15 (Normal)	107 (89.2 %)	2 (1.7 %)	109 (45.4 %)	< 0.001*
15-30 (Mild)	13 (10.8 %)	104 (86.7%)	117 (48.8 %)	
31-100 (Moderate)	0 (0.0 %)	14 (11.7 %)	14 (5.8 %)	
>100 (Severe)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	
Total	**120 (100 %)**	**120 (100 %)**	**240 (100 %)**	

Difference between classes by Relative Risk Assessment with Pearson's Chi-Square analysis.
*Level of significance at *p<0.001*. Hcy: Homocysteine

Association of Homocysteine with Blood Pressure: There was a significant (p<0.001), positive correlation of plasma homocysteine levels with systolic (r = 0.71) and diastolic (r = 0.66) blood pressures in all subjects (Table 4). Homocysteine positively correlated with systolic (r = 0.51, p<0.001) and diastolic (r = 0.47, p<0.001) blood pressures in hypertensive subjects using the Pearson's Correlation analysis (Table 4). There was also a significant correlation of homocysteine with DBP (r = 0.2, p=0.03) in the normal healthy controls but no correlation with systolic (r = 0.09, p=0.35) blood pressure (Table 4).

Table 4: Correlation between plasma homocysteine and blood pressure levels in all subjects at baseline and in hypertensive subjects and healthy controls

VARIABLE	All Subjects Homocysteine Levels r (n = 240)	P	Hypertensive Subjects Homocysteine Levels r (n = 120)	P	Healthy Controls Homocysteine Levels r (n = 120)	P
SBP	0.71	< 0.001†	0.51	< 0.001†	0.09	0.35
DBP	0.66	< 0.001†	0.47	< 0.001†	0.20	0.03*

r: Pearson's correlation coefficient. * Level of significance at *p<0.05*. †Level of significance at *p<0.001*. SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; n = Number of subjects.

Furthermore, without adjusting for confounding variables, homocysteine showed a significant (p < 0.001, OR 1.08 (95% CI, 1.05 - 1.11) positive relationship to SBP and DBP (p=0.002, OR 1.08 (95 % CI, 1.03 - 1.13), in the combined hypertensive-control model (Table 5). Following adjustment for age, sex, weight, BMI, GFR, creatinine, urea, duration of hypertension, vegetables and fruits in daily diet, plasma folate, level of education, fasting blood glucose and PCV, homocysteine showed a significant (p=0.002, OR: 1.1 (95% CI 1.04 - 1.18) positive relationship to systolic blood pressure but not diastolic (p=0.25, OR: 1.06 (95 % CI, 0.96 - 1.18) blood pressure (Table 5).

Table 5: Relationship between Homocysteine and Blood Pressure

VARIABLES	Odds Ratio (OR)	95% Confidence Interval	P-Value
Unadjusted Model			
Systolic Blood Pressure	1.08	1.05 - 1.11	< 0.001*
Diastolic Blood Pressure	1.08	1.03 - 1.13	0.002*
ǂ **Adjusted Model**			
Systolic Blood Pressure	1.10	1.04 - 1.18	0.002*
Diastolic Blood Pressure	1.06	0.96 - 1.18	0.25

Binary Logistic Regression Analysis. *Level of significance at *p<0.01*. ǂAdjusted for age, sex, Body mass index (BMI), glomerular filtration rate (GFR), urea, creatinine, weight, height, duration of hypertension, vegetables/fruits in daily diet, plasma folate, packed cell volume (PCV), fasting blood glucose (FBG) and level of education.

Odds Ratio/Risk of Development of Hypertension

The hypertensive risk associated with hyperhomocysteinaemia (hcy > 15 μmol/L) as determined by the Odds ratio was 2.8 (95 % CI, 2.3 - 3.4). The Crude Odds ratio was 0.010 (95 % CI, 0.001 - 0.07, p<0.001) (Table 6).

Table 6: Hypertensive risk associated with hyperhomocysteinaemia by Odds ratio

Systolic Blood Pressure (n = 240)				
Plasma Homocysteine	**Odds Ratio**	**95% Confidence Interval**		**P-Value**
Combined (Patient/Control)	0.01	0.001	0.07	< 0.001*
Homocysteine (< 15 μmol/L)	0.03	0.007	0.12	
Homocysteine (> 15 μmol/L)	2.80	2.30	3.40	

Relative Risk Assessment and Pearson Chi-square analysis. * Level of significance at *p<0.001*.
n = total number of subjects

Discussion

The present study showed that hypertensive subjects resident in Northern-Nigeria had significantly (p<0.001) higher mean homocysteine levels than normal healthy Nigerians similar to previous reports[15,21]. Homocysteine also demonstrated a significant (p<0.001) positive correlation to SBP and DBP in all subjects as well as in the hypertensive subjects when compared to the controls. Furthermore, homocysteine showed a positive relationship to systolic (p<0.001) and diastolic (p=0.002) blood pressures in the unadjusted model and following adjustment for covariates, showed a positive relationship to only SBP (p=0.002) in the hypertensive subjects. This signifies that homocysteine might be a risk factor for hypertension. The high homocysteine levels in the hypertensive subjects ranged from mild to moderate levels with most of the patients having mild hyperhomocysteine levels and no subjects with severe hyperhomocysteinaemia. On the contrary, the normal healthy controls had just a few subjects with mildly raised homocysteine levels (>15 μmol/L) whereas the majority fell in the normal range.

The hyperhomocysteinaemia observed in the hypertensive subjects is consistent with reports from other stud-

ies outside Nigeria[14,16,27,31-34], which showed similar high levels. Likewise, similar reports were obtained in Nigeria on hypertensive subjects with or without cardiovascular disease[15,21,35]. This data however, contrasts reports from the meta-analysis of 25 randomised controlled trials done in the United States of America (USA) and European countries which reported median base-line homocysteine levels of 10.5 μmol/L (8.5-13.6 μmol/L)[36] which is significantly lower than that of this study.

The disparity between the current study and that of the USA and European countries may be attributed to racial and ethnic group differences. There are conflicting findings with regards to racial/ethnic differences in homocysteine levels. Report has shown that black Americans have higher prevalence of hyperhomocysteinaemia when compared to whites in both male and female counterparts[37]. Furthermore, while some study demonstrated higher homocysteine levels in young adult African-Americans[32], some others reported lower homocysteine levels in black South-Africans[38,39] and East London Bangladeshis[40] when compared to whites. It may be postulated that genetic mutation in the genes encoding the enzymes required in the homocysteine metabolism may explain the observed racial differences as established outside Nigeria[30,41]. Studies have shown higher ethnic differences in the genetic polymorphism of MTHFR gene with higher prevalence of TT genotype in Caucasians and an almost deficient level in African-Americans[41,42]. This genetic mutation can result in reduced enzyme activity and consequent increased plasma homocysteine levels in the presence of low folate status[41,42]. There is however, paucity of data on genetic studies in Nigeria as facilities are lacking and this was not determined in this study, hence the contribution of genetic factors to raised homocysteine in this study requires further investigation.

Furthermore, the difference between the high homocysteine levels in the hypertensive subjects in this study and that of reports by the meta-analysis of randomised controlled trials done in the USA and European countries may not be accounted for by suboptimal folate status as the folate levels obtained in the present study were well above the reference range for healthy adults in the United States[30]. The reason for the high plasma folate levels may be attributed to the high intake of fruits and vegetables in daily diet in the hypertensive subjects which did not differ significantly ($p=0.69$) from that of the normal healthy controls especially as dietary source of folate has been reported as the most important source of vitamins required for homocysteine metabolism[33]. Studies from USA, South Africa and Denmark suggested that about 40% of their population were not consuming enough folate to maintain low plasma homocysteine levels[41], but this study showed a high mean folate levels in the hypertensive patients which did not differ significantly ($p=0.68$) from the controls despite high homocysteine levels. Socio-environmental and geographical differences can further explain this disparity. The Northern part of Nigeria is largely a farming region where green leafy vegetables, lettuce, carrots, spinach, tomatoes and okra are grown and distributed to the Eastern part of the country for commercial purposes[43,44]. Consequently, the dietary pattern of inhabitants of the Northern part of the country largely involves intake of these fresh leafy vegetables and fruits which contain folate and other B-vitamins. These they also add to their staple diet as these are readily available and cheaper[43,44]. Additionally, deficiencies in other B vitamins like B_{12} and B_6 which can result in hyperhomocysteinemia will require further investigation in subsequent studies as they were not assayed in the present study; however some study done in Nigeria had shown that B_{12} deficiency was rare in Nigerians[30, 45,46].

There was no significant age difference between the patient and control groups with both groups being more represented by the middle age. This confirms earlier reports of hypertension occurring at a younger age in contrasts to the older age group in Western countries[8,15]. Increasing age has been documented to be associated with higher homocysteine levels[18]. Hyperhomocysteinaemia induces oxidative stress leading to the generation of an oxidant called peroxynitrite while reducing the bioavailability of a potent vasodilator called nitric oxide. Peroxynitrite results in nucleic acid oxidation, lipid peroxidation, protein oxidation and inactivation of enzymes, consequently leading to necrosis and apoptosis of cells[18-20]. This activation of abnormal proteins, programmed cell death, further increased oxidative stress via other mechanisms as well as enhanced shortening of telomere length, all lead to age-related degenerative diseases inclusive of atherosclerosis underlying the pathogenesis of hypertension[18-20, 47]. Renal dysfunction has also been documented to be associated with hyperhomocysteinaemia and high blood pressure levels though with incompletely elucidated mech-

anisms[38] and creatine/creatinine production has been shown to be directly associated with S-adenosyl-homocysteine/homocysteine production[19,48,49]. Hypertensive subjects in this study showed slightly lower GFR levels than normal healthy controls, however the mean GFR levels were within normal physiologic range in both patients and controls. Patients with renal failure were excluded abinitio, hence renal dysfunction as a cause of hyperhomocysteinamia in this study may not explain the high homocysteine levels as well as high blood pressures in the hypertensive subjects.

Furthermore, the hypertensive subjects had slightly higher FBG levels when compared to healthy controls however the mean FBG levels of both patients and controls were within normal physiologic range more so as diabetes subjects were excluded abinitio. Studies have shown association between diabetes and hyperhomocysteinaemia and consequently high blood pressure levels[15,19]. Obesity on the other hand may explain the higher homocysteine levels in the hypertensive patients when compared to healthy controls as obesity has been linked with higher homocysteine levels[18]. The differences in some baseline and laboratory parameters between the hypertensive patients and the healthy controls may be attributed to the presence of hypertension. In comparison to healthy controls, those with hypertension were more likely to be obese, have lower GFR and packed cell volume as well as higher FBG or pre-diabetes[50].

Males had higher homocysteine levels than females in both groups similar to previous reports[14-17,36,41] though not significantly different in the control group only. The sex difference may be attributed to differences in muscle mass as creatine synthesis is higher in men than women and plasma creatinine has been shown to positively correlate with homocysteine levels in normal subjects[36,51].
Studies have shown positive association between homocysteine and blood pressure both systolic and or diastolic[15,16,26,41,52] whereas, others showed no link[24,25]. The Hordaland Homocysteine study carried out on a large number of Norwegian subjects reported an association between plasma hcy and both systolic and diastolic blood pressures which was stronger in the 4th decade[52]. The Framingham Heart study, a 4 year longitudinal study involving 2, 104 predominantly female subjects in their 5th decade, showed a statistically significant association between elevated hcy and increased hypertension incidence and blood pressure progression in the unadjusted analyses[24]. However, following adjustment for age and sex, no significant association was found[24]. Likewise, the 3rd National Health and Nutrition Examination Survey (NHANES) showed that subjects in the highest quartile of plasma hcy compared to those in the lowest quartile had a 2 to 3 fold increased prevalence of hypertension. It was further demonstrated in that study that a 1 standard deviation increase in hcy levels was associated with a rise in SBP/DBP of 0.7/1.2 mmHg in females and 0.5/0.7 mmHg in males respectively[53].
This study confirms findings from previous studies as it showed a significant ($p<0.001$) positive correlation of homocysteine to systolic and diastolic blood pressures in hypertensive subjects meaning that higher homocysteine levels were associated with higher blood pressure levels in the hypertensive group. Several reasons may explain this association as hyperhomocysteinaemia causes direct endothelial injury via several pathological mechanisms[18-20,50]. These include increased asymmetric dimethylarginine levels; increased reactive oxygen species and transcription factor activation viz: nuclear factor kappa-β which consequently induces inflammatory chemokines, cytokines, leucocyte adhesion molecules and interleukins[18-20,50]. In addition to other mechanisms mentioned above, there is resultant endothelial injury and further stimulation of the coagulation cascade, all leading to atherosclerosis and consequently hypertension[18].

Importantly, the Binary Logistic Regression analysis further showed that homocysteine was positively related to systolic ($p<0.001$) and diastolic blood pressures ($p=0.002$) in the combined unadjusted model of hypertensive patients and controls. Additionally, the hyperhomocysteinaemic subjects had almost 3 times risk of developing high blood pressure when compared to the normal healthy controls similar to study by Lu et al[50]. This further confirms that hyperhomocysteinaemia is associated with hypertension. However, following adjustment for confounding variables such as age, sex, weight, BMI, GFR, creatinine, urea, duration of hypertension, vegetables and fruits in daily diet, plasma folate, level of education, FBG and PCV; homocysteine showed a significant ($p=0.002$) positive relationship to systolic blood pressure only and not diastolic ($p=0.25$) blood pressure. Possible mechanisms include the effect of hcy on arterial stiffness/ar-

terial resistance and pulse pressure as well as its reduced vasodilatory effect and consequently increased cardiac output[50]. However the pulse pressure or gold standard aortic pulse wave velocity was not assessed in this study hence cannot objectively explain this.

On a further note, the findings prior to and following adjustment are similar to some previous reports[50,52]. The Hordaland study involved young individuals similar to the present study however differed from this by its very large sample size[52]. That done in China on hypertensive patients and non-hypertensive controls showed significant association of hcy to BP in both unadjusted and age, sex, BMI, diabetes, alcohol and smoking adjusted model. The difference however was the mean age of hypertensives in that study falling within the 6th decade unlike the younger age here and the fact that diabetes subjects, smokers and alcoholics were not excluded abinitio. Also, the adjustment for confounding variabes was done in relation to blood pressure as a whole without looking at systolic and diastolic BP's separately[50]. Contrary findings have also been documented in the Framingham Heart Study which was a prospective longitudinal study that included a larger number of non-hypertensive older adults[24].

Cross-sectional studies may not detect the causal relationship between hyperhomocysteinaemia and raised blood pressure[24]. Furthermore, the relationship of hcy to systolic and DBP in hypertensive subjects using Pearson's correlation coefficient was moderate, therefore it can be proposed that homocysteine may be a risk factor for development of hypertension in Nigerians but might not be the primary initiator. Moreover, findings from our previous work showed that homocysteine-lowering therapy with folic acid reduced hcy levels significantly but had no significant decrease in blood pressure levels similar to some global reports[30,54] and also contrary to some other[55]. Therefore, a high plasma hcy associated with hypertension may not necessarily translate to beneficial cardiovascular outcomes following treatment of hcy with folic acid though still a controversial debate topic[50]. It becomes imperative that other non-traditional vascular risk factors should be looked out for. Also, lifestyle modification inclusive of increased consumption of high fibre, fruits & vegetables, exercise and avoidance of smoking /alcohol should be emphasized, as part of the cardiovascular disease preventive strategy[30,50].

Traditional vascular risk factors associated with hypertension like hyperlipidaemia were not determined in this study. Other cardiovascular risk factors like excessive smoking and alcohol shown to be associated with hyperhomocysteinaemia and consequently raised blood pressure were not found in the hypertensive subjects in this study.

Conclusion

This study showed higher mean plasma homocysteine levels in hypertensive patients than controls not accounted for by sub-optimal folate levels. Hyperhomocysteinaemia showed a positive relationship to systolic hypertension after adjusting for confounders.

Recommendations

Longitudinal larger population based studies should be carried out in Nigeria across all geopolitical zones to determine whether there is a causal relationship between hcy and blood pressure progression. Other non-traditional vascular risk factors of hypertension should be evaluated in Nigerian hypertensive subjects in further studies (on-going presently).

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Author contribution

All authors contributed to conceptualization, data collection, data analysis, drafting or revising the article, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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No conflict of interest

Abbreviations
Hcy, Homocysteine; BP, Blood Pressure; MTHF, MethylTetraHydroFolate; FA/VB9, Folic acid/ Vitamin B9; r, Correlation; OR: Odds Ratio; CI: Confidence Interval.

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