Prevalence of hyperhomocysteinaemia, selected determinants and relation to hypertension severity in Northern-Nigerian hypertensives: the ABU homocysteine survey.

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SUMMARY

Background: This study aimed at evaluating the prevalence of hyperhomocysteinaemia in Northern-Nigerian hypertensives and its association with hypertension severity and some major determinants as data regarding these are lacking in sub-Saharan Africa.

Method: A Community-based cross-sectional study done on 120 randomly-selected hypertensive patients who responded to an ABU radio frequency modulated invitation for free health-screening at the Ahmadu Bello University (ABU) Medical Centre from January 2016 to June 2016. The percentage of participants with high homocysteine levels, their anthropometric parameters and blood pressures were determined. Plasma homocysteine (hcy) was classified as normal (5-15), moderate (>15-30), intermediate (31-100) and severe (>100) µmol/L. Kruskal-Wallis test was applied and log-transformed homocysteine (Ln10Homocysteine) was correlated with systolic and diastolic blood pressures as well as age, body mass index, fasting blood glucose, glomerular filtration rate, hypertension duration and Ln10folate in males and females using the Pearson’s Correlation analysis.

Results: There were 83(69.2%) females and 37(30.8%) males with Median homocysteine of 20.8 µmol/L and 22.0 µmol/L respectively (p=0.003). Hyperhomocysteinaemia was found in 118(98.3%) hypertensives while 2(1.7%) subjects had normo-homocysteinaemia. Moderate hyperhomocysteinaemia (Median, 20.8 µmol/L) was identified in 105(87.5%) and intermediate (Median, 40 µmol/L) in 13(10.8%) (p<0.001). No subject had severe hyperhomocysteinaemia. Homocysteine was higher (p=0.003) in subjects with Stage 2 systolic hypertension. Ln10Homocysteine was significantly (p<0.001) correlated with blood pressure (SBP: r=0.45; DBP: r=0.40) and age (r=0.33).

Conclusion: The prevalence of hyperhomocysteinaemia in North-Western Nigerian hypertensives is high as against normal healthy controls. Plasma homocysteine is higher with severe systolic hypertension and positively associated with age.

Keywords: Hypertension, Homocysteine, Blood pressure, Northern-Nigerians

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INTRODUCTION

Hypertension, which is a persistently sustained raised blood pressure ≥ 140/90 mmHg or current use of anti-hypertensive therapy has been re-defined to a lower threshold value of 130/80 mmHg, thereby increasing the percentage of United States adults with hypertension to 46% from 32% with the previous definition.² In Sub-Saharan Africa, hypertension was said to be rare in the first phase of the twentieth century, however current trends rates it at more than 40%.

Systematic review and meta-analysis by Adeloye et al. rates the overall prevalence of hypertension in Nigeria at 28.9% with higher urban than rural prevalence of 30.6% and 26.4% respectively.⁴ This rising trend in Africans is attributable to traditional risk factors of hypertension viz-a-viz: increased tobacco use; excessive alcohol intake; sedentary lifestyle consequently leading to obesity as well as adopted "Western" lifestyle and diets rich in salt, refined sugars, low fibre and unhealthy fats and oils.³ Likewise, vitamin D deficiency which may result from limited exposure to early morning sunlight, long-term wearing of covering clothing, use of sunscreen, aging, malabsorption as well as less consumption of

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ergocalciferol-containing foods are other attributable risks. These risk factors if modified via lifestyle changes such as exercise, avoidance of alcohol and smoking, intake of high fibre meals, fruits, vegetables and low dairy yoyurt, can reduce the incidence of hypertension and its attendant complications like stroke, heart failure, heart attacks and kidney failure. The non-modifiable traditional risk factors of hypertension includes: black race, family history of hypertension, increasing age, male sex/post-menopausal status, genetic predisposition and childhood under-nutrition.

Furthermore, there are non-traditional molecular vascular risks factors of hypertension of which hyperhomocysteinaemia have gained focus over recent past. Homocysteine is an amino acid obtained from dietary protein such as meat, eggs, seafoods and dairy products. It can be recycled into methionine or converted to cysteine in the presence of vitamin cofactors like folic acid (vitamin B₉) majorly, pyridoxal phosphate (vitamin B₆), cyanocobalamin (vitamin B₁₂) and riboflavin (vitamin B₂) to a lesser extent. Enzymes such as methylene tetrahydrofolate reductase (MTHFR) and cystathionine-β-synthase are also required in the process.

Studies have documented that deficiencies of vitamin cofactors and inherited enzyme deficiency or mutation predispose to hyperhomocysteinaemia which has been shown to be associated with endothelial injury and consequently raised blood pressure. It has also been reported that vitamin supplementation can reduce homocysteine levels, however, its effect on cardiovascular morbidity and blood pressure is rather controversial. Hyperhomocysteinaemia has been classified into moderate (>15-30 µmol/L), intermediate (>30-100 µmol/L) and severe (>100 µmol/L) forms with normal levels between 5-15 µmol/L.

The moderate to intermediate class has been linked to vitamin cofactor deficiencies especially folate, however, the more severe form which is rare is mostly associated with inherited deficiencies in cystathionine-β-synthase or homozgyous deficiency of MTHFR enzyme resulting in homocystinuria. Renal insufficiency, certain medications, smoking, alcohol, coffee intake, end stage diabetes, obesity, systemic lupus erythematosus amongst others can also result in hyperhomocysteinaemia as summarised in Figure 1.

Globally and in sub-Saharan Africa, studies have documented high prevalence of hyperhomocysteinaemia associated with ischaemic stroke, myocardial infarction, diverse cardiovascular diseases, preeclampsia and in the general population. However there is a paucity of data regarding homocysteine in adult Nigerian hypertensives with few studies emanating from Ajuluwuchwu et al., Akande et al., Alkali et al., and El-Mabchour et al. Previous analysis from the original study of same cohort of subjects had looked at the relationship between homocysteine levels in hypertensive subjects in comparison to age and sex-matched normal healthy controls (Unpublished data), hence this study focussed on homocysteine’s prevalence in hypertensive subjects with respect to sex differences, some determinants of homocysteine and its relation to hypertension severity based on the JNC-7 classification.

METHODS
Study Location and Research Design
It was a community based cross-sectional study carried out among 120 randomly selected hypertensive subjects presenting at the large hall of the Ahmadu Bello University (ABU) Medical Centre, Zaria following a 3-day ABU radio frequency modulated (F.M) announcement inviting hypertensive subjects in Zaria to come for free medical screening exercise, free drug delivery as well free health talk on hypertension management. Emphasis was also made on the need to come in a fasted state ensuring their last meal did not exceed 10 p.m of the previous day.

Subjects were also recruited from the Medical Out-patient Department of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. There were also 120 apparently healthy controls randomly selected from willing patient escorts and staff of the ABU Medical Centre as well as ABUTH, Zaria as reported previously (Unpublished data).
Inclusion criteria for hypertensive patients were adult subjects with the willingness to participate, prior physician diagnosis of hypertension (BP ≥ 140/90 mmHg), current use of antihypertensive medications and non-diabetics (historically and with fasting blood glucose < 7 mmol/L).

Exclusion criteria included patients with renal failure (serum creatinine > 3 mg/dL or > 264 µmol/L or glomerular filtration rate (GFR) as determined by Cockcroft-Gault equation < 60 ml/min),28 excessive alcohol use (Having > 1 wine glass of red wine per day for women and > 2 wine glasses of red wine per day for men in the past 1 year);26 current tobacco use (Having smoked at least one cigarette per day/18 packs in the past 1 year);27 chronic folic acid, vitamin B12 and B6 supplementation for greater than 3 months; history of heart failure, stroke, transient ischaemic attack, myocardial infarction, sickle cell disease or pregnancy; as well as use of drugs known to interfere with homocysteine metabolism such as: methotrexate, nitrous oxide, sulfadoxine-pyrimethamine, penicillamine, anticonvulsants and contraceptives.9,21

Ethical clearance was obtained from the Health Research Ethical Committee (HREC), Ministry of Health, Kaduna (ref: MOH/ADM/744/VOL.1/369) and all participants gave written informed consent. The Hausa version of the informed consent form was made available to subjects who had no formal education.

Screening Evaluation and Data Collection
A total of 180 hypertensive subjects were selected by simple random sampling from an original sample frame of 250 subjects screened from the large hall of the ABU Medical Centre, Zaria as well as the medical out-patient department of ABUTH, Zaria from January 2016 to June 2016. Of these, 120 hypertensive subjects met eligibility criteria and were enrolled. Subjects were recruited in two batches based on the requirement of being in a fasted state.37 A standard well-structured questionnaire was interviewer-administered to each eligible study participant by the author and 4 trained assisting senior medical doctors.

This included record of the subjects’ bio-data (address, age, sex, tribe, and religion); prior physician diagnosis of hypertension and duration of hypertension (subjects were asked if hypertensive and if “Yes,” for how long?); drug treatment of hypertension (subjects were asked if they had been on any anti-hypertensive and if “Yes,” for how long and whether compliant to therapy). Subjects were also asked when last they were off drugs and whether on any other drugs or supplements. A detailed 24 hour dietary recall; family history of hypertension, diabetes, hyperlipidaemia and sudden cardiac death were obtained. Subjects were asked of previous history of stroke, heart failure, heart attack or sickle cell disease and if “Yes,” the duration was determined.

Social history i.e. alcohol and smoking history were also enquired (Subjects were asked whether they smoked or took alcohol and if the response was “Yes,” the quantity of alcohol or cigarette/day, duration of intake and brand was documented. The pack-year (the number of sticks/number of packs × 10 years) for cigarette smoking was then determined. Physical examination, anthropometric measurements {weight, height & body mass index calculated as weight (kg)/height² (m²)} and blood pressures, were determined.

Blood pressures were measured using 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.4 Hypertension was defined from self-reported history, current use of anti-hypertensive therapy and or SBP ≥ 140 mmHg or DBP ≥ 90 mmHg and classified based on the 7th Joint National Committee on the detection, prevention and control of hypertension (JNC-7) classification.8

Blood Sample Collection
Blood samples for plasma homocysteine and folate levels were obtained from the antecubital vein of either arm, following an overnight fast and without tourniquet application.20 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 (trasylo16) had been added.20

The test tubes were taken to the Immunology laboratory of the 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 °C in the human immunodeficiency viral (HIV) laboratory of ABUTH, Zaria until assay. The serum electrolyte, urea and creatinine and fasting blood glucose of the subjects, were also assayed in the Chemical pathology laboratory of ABUTH, Zaria using the Chemray 120 automated clinical chemistry auto-analysers.20

Measurement of Plasma Folate and Homocysteine
The folic acid ELISA kit-Elabscience Biotechnology Co. Ltd., WuHan, P.R.C. with Lot No: AK0016JUL5067 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.20,39

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The Human direct homocysteine enzyme linked immunosorbent assay kit (ELISA-Elabscience Biotechnol-
y Co., Ltd., WuHan, P.R.C. with Lot No: AK0016JUL15066 and Catalog No: E-EL-HO156), was used for in-vitro quantita-
tive determination of human homocysteine in plasma based on the Elisa principle and according to the manufacturer’s manual.20,39

The microtiter plate in this kit was pre-coated with an antibody specific to homocysteine. Standards and samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for homocysteine. Avidin conjugated to hors-\n radish peroxidase (A-HRP) which catalyses the substrate solution was also added to each microplate well and in-
cubated.20,39 Then a tetramethylbenzidine (TMB) sub-
strate solution was added to each well. Only those wells that contained homocysteine peptide, enzyme-conjugated avidin and biotin-conjugated anti-
body exhibited a colour change.39 The enzyme substrate reaction was terminated by adding a sulphuric acid solu-
tion and the colour change was measured spectrophotome-
trically at a wavelength of 450±2 nm. The homocys-
teine concentrations in the samples were determined by comparing the optical density (OD) of samples to the standard curve. The coefficient of variation was <10%.

Data Analysis

Data was validated on excel and analysed by SPSS ver-
sion-22 software (SPSS Inc., Chicago, IL, USA). Cate-
gorical variables were presented as numbers and percent-
ages with difference determined via Chi-square (X²). The differences in age, body mass index (BMI), SBP, DBP, GFR, fasting blood glucose (FBG), packed cell volume (PCV) between males and females were determined by the Independent Student’s Sample t-test with unequal variances assumed.

Plasma homocysteine and folate levels were compared between males and females using the Mann-Whitney U test of significance for non-parametric data. Plasma ho-
mcysteine and folate were presented as Median and In-
terquartile range. Homocysteine was classified into nor-
mal (<15 µmol/L), moderate (15.1-30.9 µmol/L), inter-
mediate (31-100 µmol/L) and severe (>100 µmol/L) and Kruskal Wallis test with Pairwise comparison was used to compare homocysteine concentration at the different classes.

Blood pressure was transformed into systolic blood pres-
ure (SBP) <120 mmHg as normal, 120.1-139 mmHg as pre-hypertension, 140-159 mmHg as Stage 1 hyperten-
sion and ≥160 mmHg as Stage 2 systolic hypertension. Diastolic blood pressure (DBP) was grouped into <80 
mmHg as normal, 80.1-89 mmHg as pre-hypertension, 
90-99 mmHg as Stage 1 diastolic hypertension and ≥100 
mmHg as Stage 2 diastolic hypertension.

One-way analysis of Variance (ANOVA) was used to de-
temine the Mean ± SD homocysteine levels at the differ-
cent classes of blood pressure. Kruskal Wallis test with 
Pairwise comparison was used to determine the rela-
tionship between homocysteine and severity of systolic and
diastolic blood pressures respectively.

One sample Kolmogorov-Smirnov test was applied ab-
\initio to test for normality of distribution of data of which 
plasma homocysteine and plasma folate levels were 
skewed hence values were log-transformed. Log-trans-
formed homocysteine values were used to determine its
relationship with blood pressures; plasma folate; glom-
erular filtration rate (GFR) as well as age, body mass index
(BMI), duration of hypertension and fasting blood glu-
cose (FBG) using the Pearson’s Correlation analysis.

One-way ANOVA was also used to determine the plasma 

cysteine and folate distribution according to age

and the 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 consist-
ing of 83(69.2%) females and 37(30.8%) males with 
mean age of 49.7 ± 9.8 years. The Median plasma homo-
cysteine level was 20.8 µmol/L while that of plasma fo-
late was 111.8 µmol/L (Table 1). The Median homocys-
teine in males was 22.0 µmol/L which differed signifi-
cantly (p=0.003) from females (20.8 µmol/L).

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The clinical and laboratory parameters between male and female subjects were as shown in Table 1. The Mean ± SD age and PCV were significantly (p<0.001) higher in males than females. The BMI was however significantly (p = 0.003) higher in females than males.

Table 1 Clinical and laboratory distribution parameters of hypertensive subjects according to sex

| PARAMETERS                        | MALES   | FEMALES  | P-Value | TOTAL  | 1st Quartile | Median  | 3rd Quartile |
|-----------------------------------|---------|----------|---------|--------|--------------|---------|--------------|
| SEX                               | 30.8 %  | 69.2 %   | 100 %   |        |              |         |              |
| AGE (Years)                       | 54.2 ± 10.1 | 47.6 ± 9.1 | <0.001† | 49.7 ± 9.8 |             |         |              |
| Body Mass Index (Kg/m²)           | 26.6 ± 3.7 | 29.1 ± 4.9 | 0.003‡  | 28.2 ± 4.8 |             |         |              |
| Systolic Blood Pressure (mmHg)    | 150 ± 23.0 | 146.3 ± 18.6 | 0.38    | 147.4 ± 20.0 |             |         |              |
| Diastolic Blood Pressure (mmHg)   | 91 ± 14.6 | 93.6 ± 14.3 | 0.36    | 92.7 ± 14.4 |             |         |              |
| Homocysteine (µmol/L)             | 22.0 ± 14.6 | 20.8 ± 13.4 | <0.001‡ | 19.5 ± 13.4 | 20.8 ± 14.4 | 22.0 ± 14.4 |              |
| Fasting Blood Glucose (mmol/L)    | 5.8 ± 1.1 | 5.7 ± 0.9 | 0.46    | 5.7 ± 1.0 |              |         |              |

Data expressed as Mean ± SD. Difference between two groups by Independent Student’s Sample t-test with unequal variances assumed. *Non-Parametric Data presented as Median + Interquartile range. Mann Whitney U test for Non-Parametric Data. **Level of significance at p ≤ 0.001.†*Level of significance at p ≤ 0.01.

Table 2 Distribution and classification of plasma homocysteine levels among hypertensive subjects

| PARAMETERS                        | Normal Homocysteine (<15 µmol/L) | Hyperhomocysteine (>15 µmol/L) | TOTAL  | P-Value |
|-----------------------------------|----------------------------------|--------------------------------|--------|---------|
| Distribution of Homocysteine (%)  | 2 (1.7 %)                        | 118 (98.3 %)                   | 120 (100%) |        |
| Median Homocysteine (µmol/L)      | 14.9                             |                                | 20.8   | <0.001** |
| Classification of Homocysteinaemia (µmol/L) | Normal (<15)  | Moderate (15 - 30) | Intermediate (31 - 100) | Severe (>100) | TOTAL  | 2 (1.7 %) | 105 (87.5 %) | 9 (6.9 %) | 0 (0 %) | 120 (100%) | 40.0 c | 0.0 | 0.02*; <0.001*** |
| Medieval                                 | 14.9                             |                                | 20.8   | <0.001** |
| Classification of Homocysteinaemia (µmol/L) | Normal (<15)  | Moderate (15 - 30) | Intermediate (31 - 100) | Severe (>100) | TOTAL  | 2 (1.7 %) | 77 (73.3 %) | 4 (3.1 %) | 0 (0 %) | 83 (69.2 %) | 40.0 d | 0.0 | <0.001** |
| Homocysteine Levels (µmol/L)        | 14.9 e                           | 20.8 f                         | 40.0 e | 0.0 | 0.02*; <0.001*** |
| Males                               | 0.0                              | 21.6 e                         | 40.0 e | 0.0 | <0.001** |
| Females                             | 14.9 e                           | 20.8 f                         | 8.9 f | 0.0 | 0.02*; <0.001*** |

Hyperhomocysteinaemia was observed in 118 (98.3%) hypertensive subjects, of which 37 (30.8%) were males and 81 (67.5%) females, while 2 (1.7%) subjects had normal homocysteinaemia (Median, 14.9 µmol/L) (Table 2). There was a significantly (p<0.001) higher Median homocysteine level in the hyperhomocysteinaemic subjects when compared to those with normal homocysteine levels by Man Whitney U test (Table 2). Furthermore, out of the hyperhomocysteinaemic hypertensives, moderate hyperhomocysteinaemia (Median, 20.8 µmol/L) was identified in 105 (87.5%), intermediate (Median, 40 µmol/L) in 13 (10.8%) and none had severe hyperhomocysteinaemia (Kruskal Wallis; p<0.001) (Table 2).

There were 2 (26.7%) male hypertensives with moderate homocysteine levels while the females had 77 (73.3%) with the same. Whilst there were 9 (6.9%) males with intermediate homocysteine levels, 4 (3.1%) females had the same (Table 2). The pairwise comparison showed that the significant difference was between the Median homocysteine levels in hypertensive subjects with normal homocysteine and moderate homocysteine levels (p=0.02); normal homocysteine and intermediate homocysteine levels (p<0.001) as well as hypertensive subjects with moderate homocysteine and intermediate homocysteine levels (p<0.001) (Table 2).
The between-subjects effect showed a significant (p<0.05) difference in homocysteine concentration with higher severity of systolic blood pressure (SBP) using the Kruskal Wallis test. The actual difference on Pairwise comparison, was a significantly (p=0.007) higher homocysteine concentration in subjects with Stage 2 systolic hypertension (≥160 mmHg) when compared with those with normal SBP as well as a significantly (p=0.003) higher homocysteine levels in subjects with Stage 2 systolic hypertension when compared with Stage 1 systolic hypertension (140-159 mmHg).

Additionally, there were 50 (41.7%) of the subjects with Stage 1 systolic hypertension and 35 (29.2%) with Stage 2 systolic hypertension. The Mean ± SEM homocysteine level was 26.9 ± 1.5 μmol/L in subjects with Stage 2 systolic hypertension (Table 3).

On the contrary, with regards to diastolic blood pressure classification, the between-subjects effect showed no significant difference (p=0.18) in homocysteine concentrations with increasing severity of diastolic blood pressure using the Kruskal Wallis test with Pairwise comparison (Table 3). There were 41(34.2%) of the subjects with Stage 1 diastolic hypertension and 42 (35%) with Stage 2 diastolic hypertension (Table 3).

**Table 3** Relationship of plasma homocysteine concentrations to severity of hypertension in hypertensives

| CLASSIFICATION | Systolic Blood Pressure (mmHg) | n (%) | Plasma Homocysteine (µmol/L) | P-Value | CLASSIFICATION | Diastolic Blood Pressure (mmHg) | n (%) | Plasma Homocysteine (µmol/L) | P-Value |
|----------------|-------------------------------|-------|-------------------------------|---------|----------------|-------------------------------|-------|-------------------------------|---------|
| Normal (<120) | 11 (9.2%)                     | 20.2 ± 1.0<sup>a</sup> | 0.007<sup>b</sup> | Normal (<80) | 5 (29.2%) | 20.6 ± 0.4<sup>b</sup> | 0.18 |
| Prehypertension (120 – 139) | 24 (20%) | 20.8 ± 0.5<sup>ab</sup> | 0.63 | Prehypertension (80 – 90) | 2 (1.7%) | 19.4 ± 1.4<sup>b</sup> | 0.44 |
| Stage 1 (140 – 159) | 50 (41.7%) | 21.5 ± 0.7<sup>a</sup> | 0.93 | Stage 1 (90 – 99) | 41 (34.2%) | 22.8 ± 1.0<sup>b</sup> | 0.17 |
| Stage 2 (≥160) | 35 (29.2%) | 26.9 ± 1.5<sup>ab</sup> | 0.003<sup>b</sup> | Stage 2 (≥100) | 42 (35%) | 24.9 ± 1.3<sup>b</sup> | 0.89 |

Data presented as Mean ± SEM by One-Way ANOVA. Difference between systolic and diastolic blood pressure severity in relation to Hyperhomocysteinaemia by Kruskal Wallis test with Pairwise comparison. <sup>a</sup> Pairwise comparison between a and d significant. <sup>b</sup> Pairwise comparison between c and d significant. <sup>ab</sup> Mean Rank in a column with a common superscript letter differ. Pearson’s Correlation analysis was used to determine the relationship between log transformed homocysteine (L<sub>10</sub>Homocysteine) concentrations and systolic/diastolic blood pressures. Findings showed a significant (p<0.001) positive relationship between systolic blood pressure and L<sub>10</sub>Homocysteine (r=0.45) as well as diastolic blood pressure and L<sub>10</sub>Homocysteine (r=0.40) in males and females respectively.

**Table 4** Relationship between log-transformed plasma homocysteine concentration and other parameters in hypertensive subjects

| PARAMETERS | MALE (n=37) | FEMALE (n=83) | TOTAL (n=120) |
|------------|-------------|---------------|---------------|
| Systolic Blood Pressure (mmHg)     | 0.48 | 0.003<sup>*</sup> | 0.43 | <0.001<sup>*</sup> | 0.45 | <0.001<sup>*</sup> |
| Diastolic Blood Pressure (mmHg)    | 0.52 | 0.001<sup>*</sup> | 0.44 | <0.001<sup>*</sup> | 0.40 | <0.001<sup>*</sup> |
| Log<sub>10</sub> Plasma Folate (ng/mL) | -0.16 | 0.34 | 0.11 | 0.31 | 0.03 | 0.77 |
| Glomerular Filtration Rate (GFR, mls/min) | -0.13 | 0.45 | 0.06 | 0.58 | 0.02 | 0.80 |
| Fasting Blood Glucose (mmol/L)     | -0.22 | 0.20 | 0.13 | 0.23 | -0.02 | 0.86 |
| Age (Years)                        | 0.32 | 0.05<sup>*</sup> | 0.17 | 0.12 | 0.33 | <0.001<sup>*</sup> |
| Body Mass Index (kg/m<sup>2</sup>) | 0.30 | 0.07 | 0.21 | 0.06 | 0.11 | 0.24 |
| Duration of Hypertension           | 0.04 | 0.81 | -0.03 | 0.83 | 0.04 | 0.66 |

Pearson’s Correlation Analysis. <sup>*</sup> Level of significance at p ≤ 0.001. n = Total sample size. r = Correlation coefficient.

Furthermore, BMI showed no significant (p=0.53) relationship with homocysteine however the Odds of overweight/obese subjects having hyperhomocysteinæma was 1.4 with an OR of 1.4 (95% CI, 1.2 - 1.5).
Table 5 shows the age and sex distribution of plasma homocysteine and folate levels. There was a rising trend in homocysteine level from young age (<30 years & 30-45 years) to middle age (45-65 years) to elderly (>65 years) with a significant (p=0.03) difference in all subjects as well as female hypertensives (p=0.008) using the Kruskal-Wallis test. In the female hypertensive subjects, the actual difference found was the significantly (p=0.001) higher homocysteine levels in the middle age group than the younger age group.

The male hypertensive subjects showed a similar rise in homocysteine levels progressively with age however this was not statistically significant (p=0.14). (Table 5). The contrary however was the trend for plasma folate as there was no statistically significant (p>0.05) difference between the different age groups in both male and female subjects as well as in all subjects, even though, the folate levels appeared to be lower in the elderly group (>65 years) and very young (<30 years) in all subjects as well as female hypertensives (Table 5).

Table 5: Prevalence of hyperhomocysteinaemia and folate concentrations according to sex and age group

| SEX      | AGE GROUP (Years) | PROPORTION (n = 120) | HOMOCYSTEINE (µmol/L) | P-VALUE | PLASMA ATE (ng/mL) | FOLATE (µmol/L) | P-VALUE |
|----------|-------------------|-----------------------|-----------------------|---------|-------------------|-----------------|---------|
| MALE     | Very Young (<30)  | 0 (0%)                | 0.0 ± 0.0             | 0.00    | 0.00              | 0.00            | 0.00    |
|          | Young (30-44)     | 7 (18.9%)             | 22.3 ± 1.3*           | 0.14†   | 121.4 ± 13.8*     | 0.85†           |         |
|          | Middle Aged (45-65) | 24 (64.9%)           | 24.8 ± 1.8†           | 0.74    | 112.7 ± 9.6†      | 0.57            |         |
|          | Elderly (>65)     | 6 (16.2%)             | 35.3 ± 4.7*           | 0.06    | 115.6 ± 15.5†     | 0.84            |         |
| TOTAL    |                   | 37 (30.8%)            | 26.0 ± 1.5            | 114.8 ± 7.1 |                   |
| FEMALE   | Very Young (<30)  | 3 (3.6%)              | 19.8 ± 0.9*           | 0.008*  | 100.0 ± 9.2*      | 0.74*           |         |
|          | Young (30-44)     | 31 (37.4%)            | 20.4 ± 0.7*           | 0.95    | 112.3 ± 9.5*      | 0.76            |         |
|          | Middle Aged (45-65)| 47 (56.6%)            | 22.1 ± 0.7*           | 0.001*  | 118.4 ± 7.5*      | 0.44            |         |
|          | Elderly (>65)     | 2 (2.4%)              | 21.4 ± 0.6*           | 0.86    | 108.5 ± 19.5*     | 0.67            |         |
| TOTAL    |                   | 83 (69.2%)            | 21.4 ± 0.5            | 115.3 ± 5.5 |                   |
| ALL SUBJECTS | Very Young | 3 (2.5%)               | 19.8 ± 0.9*           | 0.03*   | 100.0 ± 9.2*      | 0.89*           |         |
|          | Young             | 38 (31.7%)            | 20.8 ± 0.6f           | 0.08    | 114.2 ± 8.1†      | 0.67            |         |
|          | Middle Aged       | 71 (59.2%)            | 23.0 ± 0.8†           | 0.02†   | 116.5 ± 5.9*      | 0.68            |         |
|          | Elderly           | 8 (6.7%)              | 31.8 ± 4.1*           | 0.22    | 113.8 ± 12.0*     | 0.81            |         |
| TOTAL    |                   | 120 (100%)            | 22.8 ± 0.6            | 115.2 ± 4.4 |                   |

Data presented as Mean ± SEM by One-way ANOVA. Kruskal-Wallis test with Pairwise Comparison. *Level of significance at p ≤ 0.01. Non-significant at p > 0.05. a – d, e – h† † Mean Rank in a column without a common superscript letter differ. †Pairwise comparison between b and c significant. # Pairwise Comparison between e and f as well as g and significant. ‡ Pairwise Comparison between c and d significant. † † † † † † Mean Rank in a row with a common superscript letter do not differ. HHcy: Hyperhomocysteinaemia.

**DISCUSSION**

This Nigerian study is peculiar as it was targeted at determining the prevalence of hyperhomocysteinaemia in a larger sample of adult subjects with essential hypertension whilst comparing the plasma homocysteine concentrations with the JNC-7 classification of hypertension severity as well as some selected determinants of homocysteine. The previous Nigerian studies assessed homocysteine levels and other cardiovascular risk factors in Fulani pastoralists of Northern-Nigeria in relation to their diets; homocysteine’s association with ischaemic stroke and or myocardial infarction; homocysteine in Nigerian pre-eclamptic/eclamptic; homocysteine in 36 hypertensives with or without cardiovascular disease in comparison to controls; homocysteine in diverse cardiovascular diseases in South-West Nigeria. Consistent with some of those studies, hyperhomocysteinaemia was prevalent albeit with differing prevalence levels. This study found that almost all the hypertensive subjects (98.3%) had hyperhomocysteinaemia (homocysteine >15 µmol/L) with high Median plasma homocysteine concentration. This is somewhat similar though higher than previous reports globally viz.: a large observational trial, the third National Health and Nutrition Examination Survey (NHANES III) done in the United States which showed high prevalence of hypertension in subjects with higher homocysteine levels compared to those with lower levels. Similarly, Malinow et al. study showed 77% of hypertensives had hyperhomocysteinaemia compared to 40% of normal subjects, while Mendis et al. documented a prevalence of 63% in hypertensive Sri Lankans compared to 33% in controls. Another population based study done in China showed a prevalence of 51.6%.

In sub-Saharan Africa, the prevalence of hyperhomocysteinaemia has ranged from 85% in stroke subjects in Gombe town of Nigeria as well as South-West Nigeria respectively; 70% and 56% out of three patient groups inclusive of stroke and hypertension respectively in Lagos State of Nigeria; 56.5% in coronary heart disease subjects in Togo and 95.3% in coronary artery disease...
subjects in an Indian study. The proportion of hypertensive subjects with hyperhomocysteinaemia in this study far exceeds that of other studies especially those of the United States and European countries. Such studies documented hyperhomocysteinaemia in only a third of their vascular or stroke subjects. It also surpasses the study done by Ajuluchukwu et al. in South-Western Nigeria but may be somewhat similar to some other study done in the Northern part of Nigeria. A sub-analysis of the homocysteine levels of 65 apparently normal healthy Nigerians from the original study of same cohort documented a 9.2% prevalence of hyperhomocysteinaemia (>15 µmol/L) in the general population and further analysis of a larger sample of normal healthy Nigerians from Zaria documented a 10.8% prevalence (Unpublished) using a partition limit of 15 µmol/L, similar to previous global reports in the range of 5-10%. This marked finding of hyperhomocysteinaemia in Northern-Nigerian hypertensive subjects, significantly (p<0.001) different from that of age and sex matched normal healthy controls (Unpublished data), is of public health significance and a cause for concern as such degree of hyperhomocysteinaemia may be associated with high risk for cardiovascular events like stroke. Studies have documented more rapid progression of hypertension and poorer outcome in hypertensive subjects of African ancestry.

Furthermore, there is no consensus definition for hyperhomocysteinaemia as values used amongst studies varied with different techniques used. These ranged from 10 to 10.5 µmol/L, 11.4 and 10.4 µmol/L in males and females respectively in the NHANES study; 15 µmol/L in some other studies; while some other used 18 µmol/L. The reason for the disparity in prevalence rates might be the different partition values of homocysteine used in the different studies. Other reasons may be due to differences in inclusion criteria, racial, geographic, genetic, and risk factor profiles as well as sampling techniques and methods used.

However, a study by Vander Jagt on adolescent girls in Northern-Nigeria showed that folate levels were within satisfactory limits despite high mean homocysteine levels and this was attributed to their marginal vitamin B12 status. This may not extrapolate for an adult population as adolescent girls were studied but previous study by Flemming et al. showed that vitamin B12 deficiency was rare in Northern-Nigerians. This was confirmed by a recent study done in Zaria comparing stroke patients with normal healthy subjects which showed that vitamin B12 deficiency is not the problem of healthy Nigerians as the levels were well within normal reference range. Further studies on hypertensive subjects should be done to determine if there are any such deficiencies in them which is currently on-going.

Consistent with previous reports globally and locally, the Median plasma homocysteine level was higher in males than in females. The males also had a higher proportion of subjects with intermediate hyperhomocysteinaemia and no subjects with mild hyperhomocysteinaemia as against the females who had very few subjects with intermediate as well as mild hyperhomocysteinaemia. This may indicate a higher severity of hyperhomocysteinaemia in males than females. The difference may be attributed to higher creatine synthesis in males than females accounting for sex differences in muscle mass and the female hormone oestrogen which has been shown to have a reduction effect on homocysteine levels.

Several factors that affect homocysteine levels such as alcohol and smoking were not assessed in these subjects because such patients were excluded ab-initio hence these cannot explain the high prevalence and concentrations of homocysteine in this study. Subjects with renal impairment were also excluded hence cannot be a confounding variable as the hypertensive subjects had normal mean GFR and log transformed homocysteine did not show positive correlation with GFR. Likewise, diabetes subjects were excluded from this study and FBG did not show significant correlation with plasma homocysteine levels. BMI also showed no significant association with log transformed homocysteine however the Odd of overweight/obese hypertensive subjects having hyperhomocysteinaemia was high. Previous studies have shown that elevated homocysteine levels is associated with alcohol, smoking, renal dysfunction, diabetes and obesity respectively.

Age on the other hand was shown to be positively related to log-transformed homocysteine levels. In both males and female subjects, there was a rising trend in plasma homocysteine levels with increasing age from young to middle age to highest values seen in the elderly; which
was more significant in the females especially between the young and middle age group. The rising levels with older age is similar to previous reports in which homocysteine was documented to be associated with increasing age and positively correlated to age.\textsuperscript{51-53} It is important to note that the plasma homocysteine levels were significantly higher in the middle age group in females with no difference between the young and very young age groups. This may be attributed to the protective effects of oestrogen in the young females as shown from previous reports.\textsuperscript{51-53} Additionally, the duration of hypertension showed no significant relations to plasma homocysteine consistent with some other report locally.\textsuperscript{8}

Furthermore, genetic deficiencies or mutation in cystathionine-\(\beta\)-synthase, as well as Methylene Tetra-Hydrofolate Reductase (MTHFR) enzymes, have been documented to result in impaired homocysteine metabolism consequently leading to hyperhomocysteinaemia.\textsuperscript{11,13,32} However, this cannot be accounted for by this study as it was not assayed due to its lack of availability in Nigeria and high costs even when available.\textsuperscript{9,20} Of important mention, is the fact that concentrations of homocysteine have been reported to be higher in serum than in plasma with a 10\% rise in postprandial states and samples stored at room temperature can result to an artefactual rise in extracellular homocysteine levels.\textsuperscript{29,54-55} However, for the purpose of this study, fasting plasma samples in which aprotinin was previously added were used and samples were stored at -70\(^\circ\)C till analysis. Addition of aprotinin was to inhibit the proteases enzymes that degrade homocysteine so as to obtain the actual homocysteine concentration.\textsuperscript{21} Moreover, specimens were placed on ice immediately following collection by venipuncture which was done without tourniquet application and plasma was separated within 2 and not more than 4 hours to prevent a time and temperature dependent release of homocysteine from blood cells.\textsuperscript{54}

Consistent with previous findings, even in pre-eclampsia/eclampsia, plasma homocysteine was significantly (\(<0.001\)) higher in subjects with higher severity of systolic blood pressure.\textsuperscript{9,11,19,30,32} Log-transformed homocysteine concentration was positively related to both systolic and diastolic blood pressures similar to previous reports.\textsuperscript{12,16,18,19,21,28,34} There are controversial findings on the association of homocysteine with blood pressure: while some studies showed that higher homocysteine concentration is associated with higher blood pressure levels,\textsuperscript{11,16,26,28,46} some other studies found no such association.\textsuperscript{19,55}

The third National Health and Nutrition Examination Survey (NHANES) from 1988-1994 done on 7,103 United States subjects showed that a 1 standard deviation (~5 \(\mu\)mol/L) increase in homocysteine levels was associated with an increase in systolic and diastolic blood pressures of 1.2 mmHg and 0.7 mmHg respectively in women which was stronger than in men.\textsuperscript{17}

Similarly, the Hordaland homocysteine survey, the largest population-based study on the relationship between plasma homocysteine and cardiovascular disease risk factors, showed that elevated plasma homocysteine was weakly associated with systolic and diastolic BP in subjects within the early fourth decade as against those between ages 65-74 years.\textsuperscript{18} Contrary reports, however, exist from the Framingham Heart Study of 2,104 participants, which investigated the relations of plasma homocysteine to hypertension incidence and blood pressure tracking in a community-based setting.\textsuperscript{19} Plasma homocysteine in that study was found to be positively associated with hypertension incidence and BP progression in the unadjusted model but when adjusted for age and sex, the association was no longer statistically significant (\(<0.05\)).\textsuperscript{19}

The present study done on a sample of 28-80 year old hypertensive adults showed no cases of severe hyperhomocysteinaemia similar to the Tehran homocysteine survey done in Iran.\textsuperscript{13,57} There were instead, a greater proportion of the subjects with moderate hyperhomocysteinaemia. Studies have shown that severe hyperhomocysteinaemia (>100 \(\mu\)mol/L) is linked to a rare autosomal recessive disease called homocystinuria reported in Europe and the USA.\textsuperscript{29,54} Homozygotes lack one of the major metabolic enzymes required for homocysteine degradation, consequently leading to premature cardiovascular disease with multi-systemic involvement.\textsuperscript{29,53} The present study therefore, confirms previous reports of moderate to intermediate hyperhomocysteinaemia in Nigerian-Africans,\textsuperscript{9,35-36,41-42,44} hence this may explain the absence of this rare vascular disease in Nigerians.

Mechanisms in which hyperhomocysteinaemia causes raised blood pressure have been shown from experimental evidence viz-a-viz: Hyperhomocysteinaemia has prothrombotic and pro-atherosclerotic effects leading to increased risk for atherosclerotic cardiovascular disease.\textsuperscript{18,29} Furthermore, it causes endothelial injury mediated by increased reactive oxygen species and asymmetric dimethyl arginine which are cell cytotoxic.\textsuperscript{12,14,21}

It results in an increase in oxidative stress and consequently, impaired nitric oxide mediated vasodilation which leads to increased generation of peroxynitrite (\(\text{ONOO}^\cdot\)), a strong oxidant that enhances nucleic acid oxidation, lipid peroxidation, protein oxidation and
inactivation of enzymes which cause necrosis and programmed cell death.14-19,29

During the early stages of atherosclerosis induced by hyperhomocysteinaemia, endothelial cells are stimulated to secrete chemokines, cytokines and adhesion molecules like monocyte chemo-attractant protein-1 (MCP-1) as well as interleukins, which contribute to inflammation and atherogenesis.14-15, 29 These processes lead to endothelial dysfunction with resulting platelet aggregation and thrombus formation. This occurs, consequent on procoagulant activity of factors V, XII, tissue and Von-Willebrand factor as well as inhibition of anti-thrombin expression by homocysteine.9,11,15,29

Homocysteine also causes altered DNA methylation and gene expression leading to vascular smooth muscle proliferation and atherosclerosis.9,14-15,29 It has also been documented to cause damage to elastin fibres with increased collagen production, in addition to its smooth muscle proliferation effect, consequently resulting in arterial stiffness.58 Increased arterial stiffness will lead to elevated systolic blood pressure and cardiac load with resultant increased pulse pressure.59

Several studies have shown that vitamin supplementation can reduce homocysteine levels but the findings on its blood pressure reduction effect are conflicting with some reporting no BP reduction effect,17,20,56 while others documented both systolic and diastolic BP reduction,12,16,21,28 yet some others had only systolic BP reduction effect.13,57 More so, the association of log-transformed homocysteine with blood pressure in this study was rather moderate, hence other molecular vascular risk factors of hypertension such as soluble endoglin, endothelin-1 and vascular endothelial growth factor-1 (VEGF-1) should be sort for in subsequent studies as possible causes of hypertension in Nigerian-Africans (presently on-going).3,20

Recommendation and Limitations
Lifestyle factors should, therefore, be advocated and encouraged at all tiers of government via organization of health talks and hypertension awareness programs so as to encourage the masses to adopt lifestyle modification as recommended by the World Health Organization in a bid to reduce cardiovascular risk.37

Population and longitudinal-based studies should be carried out across all geopolitical zones of the country to determine the cause-effect relationship between hyperhomocysteinaemia and raised blood pressure as this study is limited by its cross-sectional design.

Other B-vitamins were not assessed in this study as well as genetic assay to determine whether there is any genetic mutation in the MTHFR enzyme responsible for homocysteine metabolism, hence, further studies should be done to explore this in hypertensive Nigerians.20 It is further recommended to assess homocysteine levels as a routine investigation in hypertensive Nigerian-Africans.

CONCLUSION
This study shows that there is a high prevalence of hyperhomocysteinaemia in hypertensive patients of North-Western Nigeria as against normal healthy controls. Hyperhomocysteinaemia was positively related to age and severe systolic hypertension.

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