Original Article

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

Obiageli U. Onyemelukwe[1, 2] and Bilkisu B. Maiha[2]

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[1]Department of Medicine, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria
[2]Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria

Corresponding author: Obiageli U. Onyemelukwe **E-mail:** obiageliuo629@gmail.com
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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 (Ln_{10}Homocysteine) was correlated with systolic and diastolic blood pressures as well as age, body mass index, fasting blood glucose, glomerular filtration rate, hypertension duration and Ln_{10}folate 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. Ln_{10}Homocysteine 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 antihypertensive 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%.[3]

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

ergocalciferol-containing foods are other attributable risks.[5,6] 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 yoghourt, can reduce the incidence of hypertension and its attendant complications like stroke, heart failure, heart attacks and kidney failure.[3,7-8]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.[5,8]

Furthermore, there are non-traditional molecular vascular risks factors of hypertension of which hyperhomocysteinaemia have gained focus over recent past.[9] Homocysteine is an amino acid biosynthesized from methionine, an essential amino acid obtained from dietary protein such as meat, eggs, seafoods and dairy products.[10] It can be recycled into methionine or converted to cysteine in the presence of vitamin cofactors like folic acid (vitamin B_9) majorly, pyridoxal phosphate (vitamin B_6), cyanocobalamin (vitamin B_{12}) and riboflavin (vitamin B_2) to a lesser extent.[11-13] Enzymes such as methylene tetrahydrofolate reductase (MTHFR) and cystathionine-β-synthase are also required in the process.[11]

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.[11-12, 14-15] It has also been reported that vitamin supplementation can reduce homocysteine levels, however, its effect on cardiovascular morbidity and blood pressure is rather controversial.[11-14, 16-21] 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.[22]

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 homozygous deficiency of MTHFR enzyme resulting in homocystinuria.[14-15,23] 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.

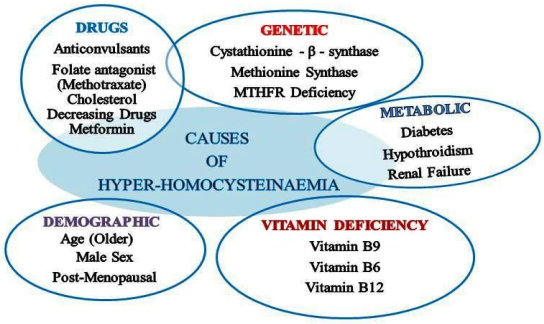

Figure 1: Major causes of hyperhomocysteinaemia. MTHFR: Methylene Tetra Hydro Folate Reductase. B_9 (Folate); B_6 (Pyridoxine); B_{12} (Cyanocobalamin).

Globally and in sub-Saharan Africa, studies have documented high prevalence of hyperhomocysteinaemia associated with ischaemic stroke;[12,23-24] myocardial infarction;[11,21,25] diverse cardiovascular diseases;[9,11-19,26-29] pre-eclampsia;[30-31]and in the general population.[32-33] However there is a paucity of data regarding homocysteine in adult Nigerian hypertensives with few studies emanating from Ajuluchukwu *et al.,*[9] Akande *et al*.,[34] Alkali *et al*.,[35] and El-Mabchour *et al*.[36] 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.[37] 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. [37]

Subjects were also recruited from the Medical Out-patient Department of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.[37] 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 < 60ml/min},[38] 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 B_{12} and B_6 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)/ height^2 (m^2)} 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.[8] 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 (trasylol®) 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 ºC 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-analyser.[20]

Measurement of Plasma Folate and Homocysteine

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.[20,39]

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 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 horseradish peroxidase (A-HRP) which catalyses the substrate solution was also added to each microplate well and incubated.[20,39] Then a tetramethylbenzidine (TMB) substrate solution was added to each well.

Only those wells that contained homocysteine peptide, enzyme-conjugated avidin and biotin-conjugated antibody exhibited a colour change.[39] The enzyme substrate reaction was terminated by adding a sulphuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450±2 nm. The homocysteine concentrations in the samples were determined by comparing the optical density (OD) of samples to the standard curve. The coefficient of variation was <10%. 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.[40]

Data Analysis

Data was validated on excel and analysed by SPSS version-22 software (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as numbers and percentages with difference determined via Chi-square (X^2). 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 homocysteine and folate were presented as Median and Interquartile range. Homocysteine was classified into normal (<15 µmol/L), moderate (15.1-30.9 µmol/L), intermediate (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 pressure (SBP) <120 mmHg as normal, 120.1-139 mmHg as pre-hypertension, 140-159 mmHg as Stage 1 hypertension 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 determine the Mean ± SD homocysteine levels at the different classes of blood pressure. Kruskal Wallis test with Pairwise comparison was used to determine the relationship 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-transformed homocysteine values were used to determine its relationship with blood pressures; plasma folate; glomerular filtration rate (GFR) as well as age, body mass index (BMI), duration of hypertension and fasting blood glucose (FBG) using the Pearson's Correlation analysis.

One-way ANOVA was also used to determine the plasma homocysteine and folate distribution according to age group classification and Kruskal Wallis test with Pairwise comparison was used to determine the difference in Mean ranks at the various levels.

Plasma homocysteine was recoded into different variable viz.: >15 µmol/L (hyperhomocysteinaemia) and <15 µmol/L and BMI was also grouped into >25 kg for overweight/obese and <25 kg for normal. Relative Risk assessment with Pearson's Chi-square was performed to determine any further association of BMI with plasma homocysteine.

The upper limit for hyperhomocysteinaemia was 15 µmol/L[9,26,38] and the level of significance was assumed to be $p \leq 0.05$ 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 with mean age of 49.7 ± 9.8 years. The Median plasma homocysteine level was 20.8 µmol/L while that of plasma folate was 111.8 µmol/L (Table 1). The Median homocysteine in males was 22.0 µmol/L which differed significantly (p=0.003) from females (20.8 µmol/L).

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 (n = 37)	FEMALES (n = 83)	P-Value	TOTAL (n = 120)	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) ǂ	22.0	20.8	0.003** ǂ	20.8	19.5	20.8	22.0
Folate (ng/mL) ǂ	111.0	112.6	0.81 ǂ	111.8	84.7	111.8	137.9
Glomerular Filtration Rate (mls/min)	104.6±18.9	98.5 ± 16.3	0.09	100.4 ± 17.3			
Packed Cell Volume (%)	43.7 ± 4.8	38.3 ± 3.3	< 0.001*	40.0 ± 4.6			
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. ǂNon-Parametric Data presented as Median + Interquartile range. ǂMann 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			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 %)	13 (10.8 %)	0 (0 %)	120 (100%)	
Males	0 (0 %)	28 (26.7 %)	9 (6.9 %)	0 (0 %)	37 (30.8 %)	
Females	2 (1.7 %)	77 (73.3 %)	4 (3.1%)	0 (0 %)	83 (69.2 %)	
Homocysteine Levels (µmol/L)	14.9 [a]	20.8 [b]	40.0 [c]	0.0		0.02*‡; <0.001**†
Males	0.0	21.6 [d]	40.0 [e]	0.0		<0.001**¶
Females	14.9 [f]	20.8 [g]	8.9 [h]	0.0		0.02*ǂ; 0.001**₸

Difference between Median Normal and Median Hyperhomocysteinaemia by Mann Whitney U test for Non-Parametric data. Difference between the Mean Rank of classes of hyperhomocysteinaemia by Kruskal Wallis test with Pairwise comparison. [a – c, d - e, f - h] Median in a row without a common superscript letter differ. ‡ Pairwise comparison between a and b significant. † Pairwise comparison between a and c as well as b and c significant. ¶ Pairwise comparison between d and e significant. ǂ Pairwise comparison between f and g significant. ₸ Pairwise comparison between f and h as well as g and h significant. * Level of significance at p ≤ 0.05.
** Level of significance at p ≤ 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 homocysteinemia (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 ($p \leq 0.05$) 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 (%) n = 120	Plasma Homocysteine (µmol/L)	P-Value	CLASSIFICATION Diastolic Blood Pressure (mmHg)	n (%) n = 120	Plasma Homocysteine (µmol/L)	P-Value
Normal (< 120)	11 (9.2%)	20.2 ± 1.0^{a}	$0.007^{*\dagger}$	**Normal (< 80)**	5 (29.2%)	20.6 ± 0.4^{b}	0.18
Prehypertension (120 – 139)	24 (20%)	20.8 ± 0.5^{ba}	0.63	**Prehypertension (80–89)**	2 (1.7 %)	19.4 ± 1.4^{b}	0.44
Stage 1 (140 – 159)	50 (41.7%)	21.5 ± 0.7^{ca}	0.93	**Stage 1 (90 – 99)**	41 (34.2%)	22.8 ± 1.0^{b}	0.17
Stage 2 (≥ 160)	35 (29.2%)	26.9 ± 1.5^{d}	$0.003^{**\ddagger}$	**Stage 2 (≥ 100)**	42 (35 %)	24.9 ± 1.3^{b}	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. $^{a-d\dagger}$ Mean Rank in a column without a common superscript letter differ. †Pairwise comparison between a and d significant. ‡Pairwise comparison between c and d significant. $^{a-a, b-b}$ Mean Rank in a column with a common superscript letter do not differ. *Level of significance at $p \leq 0.01$. **Level of significance at $p \leq 0.001$. Level of Non-significance at $p > 0.05$. SEM: Standard Error of Mean. n: Total sample size. JNC-7 Classification of hypertension; JNC-7: The 7th report of the Joint National Committee on the Prevention, Detection and Treatment of Hypertension. Stage 1: Hypertension; Stage 2: Severe Hypertension. ANOVA: Analysis of Variance.

Pearson's Correlation analysis was used to determine the relationship between log transformed homocysteine (L_{10}Homocysteine) concentrations and systolic/diastolic blood pressures. Findings showed a significant (p<0.001) positive relationship between systolic blood pressure and L_{10}Homocysteine (r=0.45) as well as diastolic blood pressure and L_{10}Homocysteine (r=0.40) in males and females respectively.

Log transformed homocysteine showed a significant *(p=0.01)* positive correlation with age. There was no significant (p>0.05) correlation of log transformed homocysteine with log transformed folate, glomerular filtration rate, body mass index, fasting blood glucose and duration of hypertension respectively (Table 4).

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

PARAMETERS	MALE (n=37) r	P-VALUE	FEMALE (n=83) r	P-VALUE	TOTAL (n=120) r	P-VALUE
Systolic Blood Pressure (mmHg)	0.48	0.003*	0.43	<0.001*	0.45	<0.001*
Diastolic Blood Pressure (mmHg)	0.52	0.001*	0.44	<0.001*	0.40	<0.001*
Log10 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*	0.17	0.12	0.33	<0.001*
Body Mass Index (kg/m^2)	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. *Level of significance at $p \leq 0.001$. 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 hyperhomocysteinaemia 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 FOLIATE (ng/mL)	P-VALUE
MALE	Very Young (<30)	0 (0%)	0.0 ± 0.0	0.00	0.00	0.00
	Young (30-44)	7 (18.9%)	22.3 ± 1.3[a]	0.14[†]	121.4 ± 13.8[b]	0.85[†]
	Middle Aged (45-65)	24 (64.9%)	24.8 ± 1.8[a]	0.74	112.7 ± 9.6[b]	0.57
	Elderly (>65)	6 (16.2%)	35.3 ± 4.7[a]	0.06	115.6 ± 15.5[b]	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[a]	0.008[*¶]	100.0 ± 9.2[c]	0.74[‡]
	Young (30-44)	31 (37.4%)	20.4 ± 0.7[b]	0.95	112.3 ± 9.5[c]	0.76
	Middle Aged (45-65)	47 (56.6%)	22.1 ± 0.7[c]	0.001[*Ł]	118.4 ± 7.5[c]	0.44
	Elderly (>65)	2 (2.4%)	21.4 ± 0.6[d]	0.86	108.5 ± 19.5[c]	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[e]	0.03[*#]	100.0 ± 9.2[d]	0.89[‡]
	Young	38 (31.7%)	20.8 ± 0.6f	0.08	114.2 ± 8.1[d]	0.67
	Middle Aged	71 (59.2%)	23.0 ± 0.8[g]	0.02[*†]	116.5 ± 5.9[d]	0.68
	Elderly	8 (6.7%)	31.8 ± 4.1[h]	0.22	113.8 ± 12.0[d]	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. ŁPairwise comparison between b and c significant. [# †] Pairwise Comparison between e and h as well as f and g significant.[a - a, b - b, c – c, d – d † ‡ ‡ ŧ] 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;[41] homocysteine's association with ischaemic stroke [25,35,42-44]and or myocardial infarction;[45] homocysteine in Nigerian pre-eclamptic/eclamptic;[31] homocysteine in 36 hypertensives with or without cardiovascular disease in comparison to controls[34] and homocysteine in diverse cardiovascular diseases in South-West Nigeria.[9] Consistent with some of those studies,[9,34-35,42-47] 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.[16,45]

Similarly, Malinow et al. study[46] showed 77% of hypertensives had hyperhomocysteinaemia compared to 40% of normal subjects, while Mendis et al.[47] 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%.[26]

In sub-Saharan Africa, the prevalence of hyperhomocysteinaemia has ranged from 85% in stroke subjects in Gombe town of Nigeria[44] as well as South-West Nigeria[31] respectively; 70% and 56% out of three patient groups inclusive of stroke and hypertension respectively in Lagos State of Nigeria;[9] 56.5% in coronary heart disease subjects in Togo[25] and 95.3% in coronary artery disease

subjects in an Indian study.[45] 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.[10-12,14,18,23] It also surpasses the study done by Ajuluchukwu et al.[9] in South-Western Nigeria but may be somewhat similar to some other study done in the Northern part of Nigeria.[44]

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[20] 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%.[48] 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.[9,24,34-35,42-44] Studies have documented more rapid progression of hypertension and poorer outcome in hypertensive subjects of African ancestry.[9,35,44]

Furthermore, there is no consensus definition for hyperhomocysteinaemia as values used amongst studies varied with different techniques used.[26] These ranged from 10 to 10.5 µmol/L;[19,26,41,45] 11.4 and 10.4 µmol/L in males and females respectively in the NHANES study;[16] 15 µmol/L in some other studies;[9,21,24,27,32] while some other used 18 µmol/L.[47] 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.[9,26,35,49]

However, the strikingly high prevalence rate in this study somewhat similar to that reported in Gombe town of Northern Nigeria[44] may be attributed to vitamin cofactor deficiencies which have been reported to exist in sub-Saharan Africa and are a major dietary determinant of hyperhomocysteinaemia.[24,32-33] However, folate levels assayed in this study were beyond the normal reference range for healthy adults (2-20 ng/mL). Log-transformed plasma folate also showed no inverse relationship with log transformed homocysteine levels hence sub-optimal folate concentrations cannot account for this high prevalence. Other B-vitamins were not assayed in this study as such cannot be accounted for as a possible cause.[20] 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 B_{12} status.[33] This may not extrapolate for an adult population as adolescent girls were studied[20] but previous study by Flemming et al.[20,40,50] showed that vitamin B_{12} deficiency was rare in Northern-Nigerians.[20,40] This was confirmed by a recent study done in Zaria comparing stroke patients with normal healthy subjects which showed that vitamin B_{12} deficiency is not the problem of healthy Nigerians as the levels were well within normal reference range.[43] 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.[9,14,15,18,26,34-35] 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.[12,51-52]

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.[14-15,18,20,24]

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.[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.[51-53]Additionally, the duration of hypertension showed no significant relations to plasma homocysteine consistent with some other report locally.[9]

Furthermore, genetic deficiencies or mutation in cystathionine-β-synthase, as well as Methylene Tetra-Hydrofolate Reductase (MTHFR) enzymes, have been documented to result in impaired homocysteine metabolism consequently leading to hyperhomocysteinaemia.[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.[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.[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^{o}C till analysis. Addition of aprotinin was to inhibit the proteases enzymes that degrade homocysteine so as to obtain the actual homocysteine concentration.[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.[54]

Consistent with previous findings, even in pre-eclampsia/eclampsia, plasma homocysteine was significantly ($p<0.001$) higher in subjects with higher severity of systolic blood pressure.[9,11,19,30,32] Log-transformed homocysteine concentration was positively related to both systolic and diastolic blood pressures similar to previous reports.[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,[11,16,26,28,46] some other studies found no such association.[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 μmol/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.[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.[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.[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 ($p>0.05$).[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.[13,57] There were instead, a greater proportion of the subjects with moderate hyperhomocysteinaemia. Studies have shown that severe hyperhomocysteinaemia (>100 μmol/L) is linked to a rare autosomal recessive disease called homocystinuria reported in Europe and the USA.[29,54] Homozygotes lack one of the major metabolic enzymes required for homocysteine degradation, consequently leading to premature cardiovascular disease with multi-systemic involvement.[29,53] The present study therefore, confirms previous reports of moderate to intermediate hyperhomocysteinaemia in Nigerian-Africans,[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.[18,29] Furthermore, it causes endothelial injury mediated by increased reactive oxygen species and asymmetric dimethyl arginine which are cell cytotoxic.[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 ($ONOO^{-}$), 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 endoglins, 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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REFERENCES

1. WHO. World Health Organization in collaboration with the World Heart Federation and World Stroke Organization. In: Puska P, Norrving B (Eds). Global Atlas on Cardiovascular Disease Prevention and Control, Geneva: USA; 2011. pp. 155.
2. Bakris G, Sorrentino M. Redefining Hypertension-assessing the new blood pressure guidelines. *New Eng J Med* 2018; 378(6): 497 - 499.
3. Van de Vijver S, Akinyi H, Oti S, Olajide A, Agyemang CM, Aboderin I, et al. Status report on hypertension in Africa-consultative review for the 6th session of the African union conference of ministers of health on non-communicable diseases. *Pan Afr Med J* 2013; 16: 38 - 55.
4. Adeloye D, Basquill C, Aderemi A, Thompson JY, Obi FA. An estimate of the prevalence of hypertension in Nigeria: a systematic review and meta-analysis. *J Hypertens* 2015; 33(2): 230 - 242.
5. Rajakumar K, Greenspan SL, Thomas SB, Holick MF. Solar ultraviolet radiation and vitamin D: a historical perspective. *Am J Pub Health* 2007; 97(10): 1746 - 1754.
6. Brijesh M, Saurav P. Prevalence of vitamin D deficiency in type 2 diabetes mellitus patients and its correlation with glycaemic control. *Int J Bioassays* 2014; 3(9): 3313 - 3317.
7. Go AS, Bauman M, King SM, Fonarow GC, Lawrence W, Williams KA, et al. An effective approach to high blood pressure control: A science advisory from the American Heart Association, the American College of Cardiology and the Centre for Disease Control and Prevention. *Hypertens* 2013; 63(4): 878 - 885.
8. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the Joint National Committee on prevention, detection,

evaluation, and treatment of high blood pressure. *Hypertens* 2003; 42(6): 1206 - 1252.
9. Ajuluchukwu J, Oluwatowoju IO, Adebayo K, Onakoya A. Plasma homocysteine in diverse cardiovascular diseases in urban Africans. *World Life Sci Med Res* 2011; 1(6): 126 - 132.
10. Ueland PM, Refsum H, Stabler SP, Malinow MR, Anderson A, Allen R. Total homocysteine in plasma and serum: methods and clinical applications. *Clin Chem* 1993; 39(9):1764 - 1779.
11. Nygård O, Vollset SE, Refsum H, Brattström L, Ueland PM. Total homocysteine and cardiovascular disease. *J Intern Med.* 1999; 246(5): 425 - 454.
12. Clarke R, Frost C, Sherliker P, Lewington S, Collins R. Dose-dependent effect of folic acid on blood concentrations of homocysteine: a meta-analysis of randomised trials, homocysteine lowering trialists collaboration. *Amer J Clin Nutr* 2005; 82(4): 806 - 812.
13. Fakhrzadeh H, Ghotbi S, Pourebrahim R, Heshmat R, Nouri M, Shafaee A, et al. Plasma homocysteine concentration and blood pressure in healthy Iranian adults: the Tehran Homocysteine Survey (2003-2004). *J Hum Hypertens* 2005; 19(11): 869 - 876.
14. Selhub J. Public health significance of hyperhomocysteinaemia. *Food Nutr Bullet* 2008; 29(2): S116 - S125.
15. Balakumar P, Singh PA, Subrahmanya SG, Singh M. Hyperhomocysteinemia and cardiovascular disorders: is there a correlation? *Trends in Med Res* 2007; 2(4): 160 - 166.
16. Lim U, Cassano PA. Homocysteine and blood pressure in the Third National Health and Nutrition Examination Survey, 1988-1994. *Amer J Epidem* 2002; 156(12): 1105 -1113.
17. Spence DJ, Bang H, Chambless EL, Stampfer JM. Vitamin intervention for stroke prevention trial: An efficacy analysis. *Stroke* 2005; 36(5): 2404 - 2409.
18. Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, et al. The Hordaland homocysteine study: Community-based study of homocysteine, its determinants, and associations with disease. *J Nutr* 2006; 136(6): 1731 - 1740.
19. Sundström J, Sullivan L, D"Agostino RB, Jacques PJ, Selhub J, Rosenberg IH, Vasan RS. Plasma homocysteine, hypertension incidence, and blood pressure tracking: the Framingham heart study. *Hypertens* 2003; 42(6): 1100 - 1105.
20. Onyemelukwe OU, Maiha BB. Hyperhomocysteinemia and folate levels in normal healthy Nigerians living in Zaria: Sub-analysis of ABU homocysteine cross-sectional survey. *Ann Afr Med* 2019; 18(3):143-52.
21. Ganeshan S, Karthikumar BA., Renjith AA, Alin B. Effect of folic acid on serum homocysteine levels in patients with cardiovascular diseases (CVD). *J Chem Pharm Res* 2014; 6(3): 1141 - 1148.
22. Govindaraju V, Neelam, Manjunath CN, Venkataramiah H, Raghu TR. Hyperhomocysteinemia: an emerging risk factor for cardiovascular disease. *Ind J Clin Biochem* 2003; 18(1): 8 - 14.
23. Brattstrom L, Israelsson B, Lindegarde F, Hultberg B. Higher total plasma homocysteine in vitamin B_{12} deficiency than in heterozygosity for homocystinuria due to beta-synthase deficiency. *Metab* 1988; 37(2): 175 - 178.
24. Akpalu AK, Nyame PK. Plasma homocysteine as a risk factor for strokes in Ghanaian adults. *Ghana Med J* 2009; 43(4): 157 - 163.
25. Yayehd K, Damorou F, Randrianarisoa F, Tcherou T, N`cho MP, N`da NW, et al. Correlation between homocysteinemia and coronary heart diseases in African patients. *Res J Cardiol* 2012; 5(1): 1-11.
26. Wang Y, Li X, Qin X, Cai Y, He M, Sun L, et al. Prevalence of hyperhomocysteinaemia and its major determinants in rural Chinese hypertensive patients aged 45-75 years. *Br J Nutr* 2013; 109(7): 1284 - 1293.
27. Damorou F, Tcherou T, Yayehd K, Pessinaba S, Diop IB. Homocysteine level and cardiovascular afflictions in the black african patients in Lome. *Res J Cardiol* 2010; 3(1): 1 - 8.
28. Scazzone C, Bono A, Tornese F, Arsena R, Schillaci R, Butera D, Cottone S. Correlation between low folate levels and hyperhomocysteinemia but not vitamin B_{12} in hypertensive patients. *Ann Clin Lab Sci* 2014; 44(3): 286 - 290.
29. Tripathi P. Molecular and biochemical aspects of homocysteine in cardiovascular diseases. *Intern Cardiovasc Forum J* 2016; 6: 13 - 17.
30. Peter T, Powers RW, Dctarry AR, Ness RD, Robert JM. Homocysteine and folic acid are inversely related in black women with pre-eclampsia. *J Hypertens* 2004; 43(6): 1279 - 1282.
31. Osunkalu VO, Akanmu AS, Adediran A, Abudu O. Homocysteine levels in Nigerian women with preeclampsia/eclampsia. *Sierra Leone J Biomed Res*, 2009; 1(1): 55 - 60.
32. Amouzou EK, Chabi NW, Adjalla CE, Rodriguez-Gueant RM, Feillet F. High prevalence of hyperhomocysteinemia related to folate deficiency and the 677C→T mutation of the gene encoding methylenetetrahydrofolate reductase in coastal West Africa. *Am J Clin Nutr* 2004; 79(4): 619 - 624.
33. Vanderjagt, DJ, Spelman K, Ambe J, Datta P, Blackwell W, Glew RH. Folate and vitamin B_{12} status of adolescent girls in Northern Nigeria. *J Nat Med Assoc* 2000; 92(7): 334 - 340.
34. Akande AA, Salisu OT, Omotoso AB and Kolo, PM. Plasma homocysteine level and other biochemical

risk factors in hypertensives with and without cardiovascular events. *J Hainan Med Coll* 2009; 15(911): 1387-1390.
35. Alkali NH, Watt H, Bwala SA and Gadzama A. Association of plasma homocysteine and ischaemic stroke in a Nigerian population. *Pak J Med Sci* 2011; 22(4): 405 - 408.
36. El-Mabchour A, Agueh V, Delisle H. Determinants and relationship of homocysteinemia with cardiometabolic risk factors. *Presse Med J* 2010; 39(11): 238 - 246.
37. Onyemelukwe OU, Maiha BB, Ayanwuyi LO, Dahiru T, Madugu NH, Aliyu M , *et al*. Selected Cardiometabolic Risk-Factor Clusters of Urban Hypertensive Adults in Response to 72-hour ABU Radio Frequency Modulated (F.M.) Call for Free-Medical Screening. *J Cardiovasc Disease Res* 2019;10(1):35-46.
38. Cockroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16(1): 31 - 41.
39. Frantzen F, Faaren Al, Alfheim I, Nordhei AK. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. *Clin Chem* 1998; 44(2): 311 - 316.
40. Onyemelukwe OU, Maiha BB, Ayanwuyi LO, Dahiru T. Randomised double-blind placebo-controlled study of folic acid adjunct for 8 weeks in hyperhomocysteinaemic hypertensive patients in Zaria, Nigeria. *J Drug Deliv Ther* 2018; 8(5): 338-348
41. Glew RH, Williams M, Conn CA, Cedena SM, Crossey M, Okolo SN, et al. Cardiovascular disease risk factors and diet of Fulani pastoralists of Northern Nigeria *Am J Clin Nutr* 2001; 74(6): 730 - 736.
42. Okubadejo NU, Oladipo OO, Adeyomoye AA, Awosanya GO, Danesi MA. Exploratory study of plasma total homocysteine and its relationship to short term outcome in acute ischaemic stroke in Nigerians. *BioMed Central Neurol* 2008; 8(1): 26 - 32.
43. Suleiman HM, Aliyu IS, Abubakar SA, Anaja P, El-Bashir JM, Adamu R, et al. Assessment of homocysteine, Vitamin B_{12}, and Zinc levels among patients with acute ischemic stroke in northwestern Nigeria. *Niger J Basic Clin Sci* 2017; 14 (2): 105 - 108
44. Glew RH, Okolie H, Crossey M, Suberu O, Trujillo M, Pereyra M, et al. Serum lipid profiles and homocysteine levels in adults with stroke or myocardial infarction in the town of Gombe in Northern Nigeria. *J Health Pop Nutr* 2004; 22(4): 341 - 347.
45. Mahalle N, Kulkarni MV, Garg MK, Naik S. Vitamin B_{12} deficiency and hyperhomocysteinaemia as correlates of cardiovascular risk factors in Indian subjects with coronary artery disease. *J Cardiol* 2013; 61(4): 289 - 294.
46. Malinow MR, Kang SS, Taylor LM, Wong PW, Coull B, Inahara T, et al. Prevalence of hyperhomocysteinaemia in patients with peripheral arterial occlusive disease. *Circulation* 1989; 79:1180 - 1188.
47. Mendis S, Athauda SDP, Nase M, Takahashi K. Association between hyperhomocystenaemia and hypertension in Sri Lankans. *J Intern Med Res* 1999:27: 38 - 44.
48. Ubbink JB. Vitamin status and hyperhomocysteinaemia in a healthy population. In: Graham I, Refsum H, Rosenberg IH, Ueland PM, Shuman JM (Eds.). Homocysteine metabolism: from basic science to clinical medicine. Developments in cardiovascular medicine. Springer, Boston, MA. 1997; 196:93-98.
49. Hashmi SFA, Dasti Ma, Jaffery MH, Sheh SZA. Serum homocysteine in hypertensive patients. *Prof Med J* 2013; 20(6): 932 - 937.
50. Flemming FA, Ogunfunmilade TA, Carmel R. Serum vitamin B_{12} levels and vitamin B_{12} binding proteins of serum and saliva of healthy Nigerians and Europeans. *Amer J Clin Nutr* 1978; 31(10): 1732 - 1738.
51. Anderson A, Brattstom L, Israelsson B, Isakson A, Hamfelt A, Hultberg B. Plasma homocysteine before and after methionine loading with regard to age, gender, and menopausal status. *Eur J Clin Invest* 1992; 22(5): 74 - 81.
52. Guo S, Pang H, Guo H, Zhang M, He J, Yan Y, et al. Ethnic Differences in the prevalence of high homocysteine levels among low-income rural Kazakh and Uyghur adults in far Western China and its implications for preventive public health. *Int J Environ Res Public Health*. 2015; 12: 5373 - 5385.
53. Strassburg A, Krems C, Lührmann PM, Hartmann B, Neuhäuser-Berthold M. Effect of age on plasma homocysteine concentrations in young and elderly subjects considering serum vitamin concentrations and different lifestyle factors. *Int J Vitam Nutr Res* 2004; 74(2):129-36.
54. Faeh D, Chiolero A, Paccaud F. Homocysteine as a risk factor for cardiovascular disease: should we (still) worry about it? *Swiss Med Wkly* 2006:136(47-48); 745 - 756.
55. Friskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine; automated determination and sample stability. *Clin Chem* 1993; 39(2); 263 - 271.
56. McMahon JA, Skeaff CM, Williams SM, Green TJ. Lowering B vitamins has no effect on blood pressure in older adults. *J Nutr* 2007; 137(5):1183 - 1187.
57. Sharifi F, Fakhrzadeh H, Mirarefin, M, Pourebrahim R, Nouri M, Forouzanfar MH, et al. The effects of high-dose folic acid on blood pressure of hypertensive adults with hyperhomocysteinemia: a

randomised double-blind placebo controlled clinical trial (Tehran Homocysteine Survey). *Iran J Diab Lipid Disord* 2010; 9:13 - 20.
58. Gupta R. Trends in hypertension epidemiology in India. *J Human Hypertens* 2004; 18(2): 73 - 78.
59. Van-Guldener C, Nanayakkara PW, Stehouwer CD. Homocysteine and blood pressure. *Curr Hypertens Res* 2003; 5(1): 26 - 31