INTRODUCTION

Atherosclerotic vascular disease is a multifactorial disease that requires the interaction of various factors to promote atherogenesis [1, 2].

Since the 1990’s, plasma homocysteine (Hcy) attained recognition as an “emerging” risk factor for atherosclerotic disease [3, 4, 5]. Consistently, elevated Hcy was found in vaso-occlusive diseases, such as stroke,
coronary artery disease, and acute myocardial infarction [6, 7, 8]. Causes of elevated tHcy include genetic and acquired aberrations in the metabolism of homocysteine, methionine, as well as dietary intake of proteins and vitamins [3, 4, 5]. Hyperhomocysteinaemia, defined as 1.5-5 times normal level, [6, 7] appears to act independent of other factors, in the development of atherosclerosis [5, 9]. The mechanisms of atherogenicity of Hcy include direct toxicity to the endothelium, [3, 4, 5], endothelial desquamation, increased synthesis and proliferation of vascular smooth muscle, and oxidation of low density cholesterol [5]. Haemostatic abnormalities have also been identified, such as decreasing the thrombomodulin cellular surface expression and inhibition of protein C activation [3, 5]. It is still unclear if elevated Hcy is the cause of the observed enhanced atherogenicity or a non-causal risk marker [5, 8].

Majority of the Hcy evidence has emerged from the industrialised populations [3, 4, 5], where the commonest vascular occlusive sequelae is coronary artery disease [1 – 6]. There is a dearth of Hcy studies in sub-Saharan Africa [10], where stroke is the commonest CVD, with hypertension and diabetes as frequent risk factors [10 – 12]. Despite the global explosion of studies of this “new” risk factor Hcy [3, 4, 13, 14, 15], only few studies have emanated from Nigeria [10, 11, 16]. Several cut-off values have been advocated, varying from 10 -24 μmol/L [3, 5]. Normal range of tHcy is accepted as 5-15 μmol/L the fasting state [5]. Hyperhomocystinaemia has been classified as moderate, 15-30μmol/L, intermediate >30-100 μmol/L, and severe >100 μmol/L [5].

Homocysteine (Hcy)-a highly reactive sulphur-containing amino acid is an intermediary product of methionine metabolism [3, 4, 5]. “Total plasma homocysteine” (tHcy) comprises of four moieties of the reduced, the protein-bound, the oxidised form of homocysteine and the mixed disulfides of homocysteine-cysteine [3, 5 – 8]. Hyperhomocysteinaemia can result from a deficiency of co-factors, such as folate or the B vitamins or abnormalities of the cystathione synthase pathways [3, 4, 5, 16].

This study aimed to compare tHcy levels amongst patients with stroke, hypertension and diabetes, respectively. In addition, relationships were sought between tHcy and lipid fractions in the different patient groups. We postulated the existence of a graded relationship in the degree of hyperhomocysteinaemia; being lowest in the healthy controls, intermediate in the “at risk” group of hypertension and diabetes and the highest levels in the stroke group.

**MATERIALS AND METHODS**

**Study Location and Sample Selection**

Study locations were the Departments of Medicine and Clinical Pathology of the Lagos University Teaching Hospital (LUTH) - a 700 bed-hospital. LUTH is one of the two Teaching Hospitals serving Lagos State-a major cosmopolitan centre in South-West Nigeria. Approval was obtained from the Ethics committee of LUTH, and all participants gave informed consent.

All participants were required to have normal liver function, defined as a plasma level of > 3.5 g/dL, and normal renal function (plasma creatinine ≤130 μmol/L). Exclusion criteria were current use of tobacco, excessive caffeine use, excessive alcohol use; and medications such as sulfadoxine-pyrimethamine, vitamin supplements.

Controls were 60 adult healthy subjects (HS), who were selected based on absence of history of hypertension, diabetes, and stroke. Other inclusion criteria were systolic blood pressure of <139 mm Hg, diastolic blood pressure of <89 mm Hg; normal fasting blood glucose; normal hepatic and renal function. Exclusion criteria were thyroid disease, current medication with sulfadoxine-pyrimethamine, chronic vitamin, and anticonvulsant preparations, pregnancy [7, 13].

Patients were appropriate consecutive patients purposefully selected from the out-patient clinics or admitted patients. There were 30 patients in each patient group of hypertension, diabetes and stroke. Patients were on appropriate therapy, as deemed necessary by their attending consultants.

Inclusion in the hypertension group was based on prior physician diagnosis, current use of anti-hypertensive agents, and fasting blood glucose < 5.6 mmol/L. The Keith Wagner hypertensive eye changes were assessed by AO; as an index of severity of hypertension. (The Keith Wagener classification of hypertensive changes grades the appearance and size of retinal “arteries” in relationship to retinal “veins, as well as presence of retinal haemorrhage, exudates and papilloedema).

Diabetes (DM) group selection was on the basis of a prior physician diagnosis, and/or use of anti-diabetic medications. Normotensive diabetic patients were selected (based on blood pressure of < 140/90 mm Hg). Severity of chronic hyperglycaemia was assessed by the level of blood haemoglobin (HbA1c).
Inclusion in the stroke group required current outpatient attendance in the Stroke clinic; current in-patient admission, with evidence that neurological deficit lasted more than 48 hours; duration of stroke syndrome > 28 days; and computed tomography scans showing brain infarction.

Total participants were 150 controls and patients, who were all clinically evaluated for appropriate medical history, and requisite physical examination to support their different categories as healthy subjects or patients.

**Procedure**

After an overnight fast, 10 mls of blood was collected without tourniquet occlusion (from antecubital veins), and divided into two 5-ml aliquots into heparinised and plain specimen bottles. Plasma was separated from red cells by immediate centrifugation at 3,000 rpm, for 5 minutes, with later analyses as detailed in reference [17]. Plasma total homocysteine (tHcy) was estimated by the enzyme immunoassay (EIA) microwell technique of the Diazyme Laboratories, 3350 General Atoms ct, San Diego CA, which employed a genetically engineered homocysteine binding protein (HBPI) as the capturing agent. IO – a clinical pathologist analysed all samples in batches, under the same prevailing condition of storage, and blinding to clinical group of participants.

Other biochemical tests that were obtained for all participants using standard methods were fasting blood glucose, fasting total cholesterol, HDL cholesterol, and triglycerides [18]. Glycosylated haemoglobin A1c was performed only for the diabetes group.

**Statistics**

Data analyses were performed with Excel worksheet and or Minitab Student 14 statistical package. The mean and standard deviation were calculated for each variable by group and percentages were calculated, as appropriate. Correlation of continuous variables with tHcy was established with Pearson correlation. Stepwise regression analyses were also used to examine associations between tHcy as dependent variable with demographic, disease-specific severity indices, and lipid profile parameters.

Partition value for hyperhomocystinaemia was 15 µmol/L [3, 5]. A p value of <0.05 was considered significant.

**RESULTS**

Group profile: Patient groups were comprised of 30 patients each in the stroke, diabetes, and hypertension groups, while controls were 60 healthy age- and sex-matched subjects (50% males and mean age of 53.0 ± 9.5 years). The average age of each patient group is shown in table 1, and is comparable to that of the controls. Of the hypertensive group, mean systolic, diastolic blood pressures and pulse pressure were 152 ± 24.7 mm Hg, BP 99.8 ± 13.9 mm Hg, and 49.8 ± 20.24 mm Hg respectively. Their mean Keith-Wagener class was 2.45 ± 0.86. For the diabetic group, their chronic glycaemic profile was 8.8± 1.5% using glycosylated haemoglobin (HBA1c). All the stroke patients had hemiparesis, and the average duration of the stroke syndrome was 3.4 years. The plasma tHcy of the control group was 8.29 ± 2.4 µmol/L.

The proportion of participants with hyperhomocystinaemia defined as ≥15 µmol/L is shown in table 1. Hyperhomocystinaemia was observed in 5% of controls, and up to 50% of all three patient groups.

Table 2 displays the biochemical parameters of the study group (lipid profile and plasma tHcy). The results show significant elevations of tHcy in stroke (37.27±14.5 µmol/L), hypertension (25.24 ±13.2 µmol/L) and diabetes (21.76 ± 10.2µmol/L), when compared to controls (8.29 ± 2.4µmol/L). In addition, the different patient groups had higher mean levels of plasma total cholesterol, and lower HDL-cholesterol than control subjects. However, triglyceride was comparable in all the groups as shown in Table 2.

**Associations with total homocysteine (tHcy)**

With clinical parameters, using univariate analysis tHcy correlated positively significantly with age in all subject groups, and glycosylated haemoglobin in the diabetes group. For age, the correlation factor respectively for healthy subjects, stroke, hypertension and diabetes was r = 0.56, p = 0.01; 0.44, p = 0.038; r = 0.42, p = 0.038; and 0.59, p = 0.01. In diabetes and hypertension, there was no significant relationship of tHcy with duration of disease (hypertension r = 0.036, p = 0.87; and diabetes r = 0.045, p = 0.8). For severity of disease, however, there was significant correlation with glycosylated haemoglobin in diabetes (r = 0.59, p = 0.006); but none with hypertensive Keith Wagner grade (r=0.04, p = 0.8). In Table 3 is displayed the Pearson correlation “r” for tHcy and plasma lipids, showing significant association of tHcy with TG in stroke (r = 0.46, p = 0.041; and positive but non-significant association in hypertension group, regarding HDL (r = 0.36, p = 0.11) and total cholesterol (r = 0.3, p = 0.19).
parameters

TC mmol/L 5.9 ± 2.1 6.0 ± 1.7 6.4 ± 3.1
HDL mmol/L 1.2 ± 0.1 1.2 ± 0.3 1.2 ± 0.4
TG mmol/L 1.9 ± 0.5 1.8 ± 0.7 1.6 ± 0.4

Mean age of healthy controls 53.0 ± 9.5 years; yrs = years; HHcy = hyperhomocysteinaemia; total homocysteine of ≥15 µmol/L.; male: female ratio of all groups 1:1; 5% of controls demonstrated HHcy

Table 3: This table shows Univariate Pearson Correlation of Plasma t-HCY and Plasma Lipid Parameters Amongst Patient Groups.

<table>
<thead>
<tr>
<th>Condition</th>
<th>TC</th>
<th>HDL</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Pearson r</td>
<td>0.30</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>Pearson r</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Stroke</td>
<td>Pearson r</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.24</td>
<td>0.6</td>
</tr>
</tbody>
</table>

“r” = Pearson r correlation; t-HCY = total homocysteine; TC=total cholesterol; HDL=high density cholesterol; TG = triglyceride

Stepwise multiple regression analysis was performed using tHcy as predictor and dependent variables were age, duration of disease, hypertensive eye changes, glycosylated haemoglobin, and lipid profile parameters. Again age had significant association with tHcy in all group, as follows: stroke (t = 2.24, p = 0.038); hypertension (t = 2.34, p = 0.032), diabetes (t = 2.29, p = 0.035, and controls t = 3.57, p = 0.002). In the hypertension and healthy subjects respectively, tHcy had negative association with marginal significance with HDL-cholesterol (t = -2.02, p = 0.059), (t = -2.05, p = 0.056). Same marginal relationship was noted for tHcy and glycosylated haemoglobin in the diabetes group (t = 2.11, p = 0.053). No other significant relationship was noted in any groups.

DISCUSSION

Amongst Africans with diverse cardiovascular disease, this study demonstrates the occurrence and establishes the severity of hyperhomocysteinaemia in vaso-occlusive stroke patients; compared to those with hypertension, diabetes, and controls. The level of hyperhomocysteinaemia in diabetes and hypertension were comparable, though significantly lower than that seen in stroke. Relationship of tHcy was noted with several other traditional cardiovascular risk factors. Hyperhomocysteinaemia is usually defined as tHcy ≥ 15 µmol/L, with levels of 15-100 µmol are described as moderate/intermediate severity [5]. In the present study, half or more of all the studied patient groups had elevated tHcy; and the levels were in the moderate/intermediate range. The results add to the body of evidence of the relationship of hyperhomocysteinaemia and stroke—an established occlusive vascular disease, and two other common cardiovascular risk factors in Nigerians [11,12]. Our principal finding was the elevation of fasting tHcy in 5% of controls, and up to 50% in all three patient groups. In addition, mean ± SD total Hcy differed significantly between the controls and all three patient groups respectively. The proportion with hyperhomocysteinaemia in the present study, is inconsistent with previous studies. Other studies have noted such degree of hyperhomocysteinaemia in only about one third of their vascular or stroke patients [3-6,7-9,15]. Such elevated tHcy levels is associated with high risk and mortality in different patient scenarios [9,13], and may be contributory to high mortality previously noted in stroke patients in our centre [12].

tHcy in Patient and Control Groups

The data obtained for stroke patients support previous African studies [8,10]; which showed an elevated tHcy in stroke patients, compared to healthy subjects. However, our study further examined the levels of tHcy in hypertension and diabetes, two common risk factors of stroke in South West Nigeria [11,12]. The mean tHcy values in the hypertension and diabetes were both comparable; but significantly higher than that of healthy

ISSN 2249-0574 World J Life Sci. and Medical Research 2011;11(6):129
Ajuluchukwu et al., 2011. Hyperhomocysteinaemia in Africans with CVD

Table 1: This table shows the Characteristics of the Patient Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>53.6 ± 10.2</td>
<td>54.0 ± 8.5</td>
<td>54.4 ± 7.5</td>
</tr>
<tr>
<td>Duration (yrs)</td>
<td>6.19 ± 5.75</td>
<td>6.0 ± 4.7</td>
<td>3.4 ± 1.5</td>
</tr>
<tr>
<td>HHcy (%)</td>
<td>56.0</td>
<td>50.0</td>
<td>70.0</td>
</tr>
</tbody>
</table>

Table 2: This table shows the Plasma Total Homocysteine and Lipids in Diverse Conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls N=60</th>
<th>Hypertension N=30</th>
<th>DM N=30</th>
<th>Stroke N=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>tHcy µL</td>
<td>8.3 ± 2.4</td>
<td>22.9 ± 11.2</td>
<td>22.6 ± 21.3</td>
<td>39.3 ± 14.5</td>
</tr>
<tr>
<td>TC mmol/L</td>
<td>4.8 ± 2.1</td>
<td>5.9 ± 1.1</td>
<td>5.7 ± 1.3</td>
<td>5.5 ± 0.9</td>
</tr>
<tr>
<td>HDL mmol/L</td>
<td>1.4 ± 0.6</td>
<td>1.2 ± 0.3</td>
<td>1.0 ± 0.5</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>TG mmol/L</td>
<td>1.4 ± 0.5</td>
<td>1.9 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

Legend: DM = T2 diabetes; HCY = homocysteine; TC = total cholesterol; HDL-C = high density cholesterol; TG = triglyceride
subjects; though significantly lower than values seen in stroke patients. Thus, the tHcy values of the “at risk” group were intermediate between those of the healthy subjects and stroke-the established occlusive disease. The present data further showed that in the stroke group, there was a moderate association of tHcy with triglyceride; though no association with total and high density cholesterol. This finding is consistent with other studies [7]. In this study, the mean tHcy values of the stroke patients (37.27 ±14.5 µmol/L) was much higher than values obtained in similar studies [3,7-10]. Previous studies in stroke patients, have reported average tHcy values of 10.9 µmol/L in South Africa [8], 24.0 µmol in Ireland [9] and 20.8µmol/L in Maiduguri [10]. It is noteworthy that the mean tHcy of the controls in the present study (8.29 ± 2.4 µmol/L) was comparable to other reported studies [8, 10]. Previous reported average values for healthy controls were in µmol/L were 8.73 for South Africa [8], 13.1 for Maiduguri [10], 16.3 by Ubbink et al [18] and 24.0 by Boushey et al [3]. In the present study, the samples of controls and patients were batched and analysed concurrently and blindly. Thus, any measurement factors would equally affect both controls and patient samples [6]. Therefore, the higher tHcy values of noted in the stroke patients in the present study has merit.

**Reasons for high tHcy**

Previous studies have shown that higher tHcy levels are frequent with genetic aberrations, especially in the presence of the C677R mutant gene and cystathionine synthase [3, 4, 5, 9, 19]. The status of these genetic aberrations and their polymorphism are currently unknown in Nigeria. Higher tHcy levels are also a consequence of poor intake of dietary factors necessary for metabolism of Hcy, such as vitamins B6, B12, folic acid, and certain proteins [3,4,5,19]. Such dietary aberrations have been alluded to in Nigerians from the Northern parts of the country [10,16]. In South Africa, a comparison of Black and White police recruits clearly demonstrated a more efficient Hcy metabolism, despite lower blood vitamin levels in young Black police recruits, compared to Whites [18]. The authors suggested that this finding may be a factor in the lower prevalence of coronary artery disease in Black Africans [2,18]. Other authors have documented ethnic and racial factors in cardiovascular disease with incomplete explanation [1,2,8,14]. Thus, race, ethnicity or geographical factors may explain or contribute to some of our findings.

**Relationships of tHcy in “at risk” Groups**

As in other studies, the current data showed that age was positively associated with tHcy in all the groups [5, 13, 16]. Parameters and duration of hypertension lacked association with tHcy. Even severity of hypertension, as determined by retinal eye changes demonstrated no association with tHcy. This is consistent with other studies that demonstrated no relationship of tHcy with hypertension [13,14]. Nevertheless, in the Framingham cohort study, tHcy was associated only with the use of antihypertensive medications, but not with systolic and diastolic blood pressure [13]. For diabetes, severity of diabetes, as determined by glycosylated haemoglobin was significantly associated with tHcy (r=0.5, p=0.001). To our knowledge, no investigators have studied tHcy with severity of diabetes or glycosylated haemoglobin. Other investigators have commonly used diabetes as a nominal group [7,9,14]; but those investigators failed to establish any relationship with tHcy and other cardiovascular risk factors. There is however consensus from other studies that elevated tHcy increases risk of cardiovascular disease in a graded fashion in different patient groups [3, 6-9, 14]. Thus the high levels noted in the hypertension, diabetes, and stroke patients place them on high risk.

Investigators have previously proposed that interactions of hyperhomocysteinaemia and lipid risk factors may contribute to atherosclerosis [9,15]. However, previous studies have demonstrated inconsistent relationship of lipids and tHcy [9, 14, 15]. Interestingly, in the present hypertension group, mild - moderate correlation, with marginal significance, was noted between tHcy and total cholesterol, and high density cholesterol. This relationship if confirmed in larger studies, may further identify hypertensive patients at higher risk, who may thus require and benefit from more aggressive hypertension care. Hypertension in people of African descent shows more rapid progression and outcome [11,12]. Studies of non-traditional cardiovascular markers, such as plasma tHcy may throw more light on the racial or geographic differences in hypertension. The current results also support the suggestion of an ominous interaction amongst multiple risk factors [1]. It has been suggested that the lack of association of tHcy to lipid parameters; especially total cholesterol, may partly explain the
occurrence of vascular occlusive disease in patients in normal cholesterol patients [3,21].

**Dietary and Genetic Influence tHcy Levels (3-5, 14,19)**

Some mutations of the cystathionine synthase gene and MTFR gene have been identified in the populations with hyperhomocystinaemia, ranging from 30% -70% in America, and Canada [3,5,7 9, 20]. No genetic studies of tHcy are currently available in Nigeria; but there is evidence of very low intake of intake of the requisite vitamins, in Northern parts of Nigeria [10,16], with resultant high tHcy levels. The present study concerns patients of a tertiary health facility in Lagos-a cosmopolitan and commercial nerve centre in the Southwest of Nigeria. It is note-worthy that plasma tHcy concentrations of hypertension and diabetes patients were even higher than the levels of stroke and myocardial infarction of western countries [9,15]. The reason and clinical implication of this finding is currently unclear; but may support the finding of more aggressive course and complication rate in hypertension and diabetes in Blacks, including Nigerians [11, 12]. On the other hand, the clinical implication of the higher tHcy levels found in the present study is unclear, as there are suggestions of “resistance” of African Blacks to coronary artery disease [2, 19].

**Limitations**

Our patient numbers were small, but several studies with valid conclusions have used similar patient numbers, such as total participant numbers or subgroups of 30 – 50 participants [7, 9, 10, 18]. Delport et al [8] showed that stroke patients with normal blood creatinine, and normal renal status had levels of tHcy comparable to controls. Their conclusion was that “while tHcy may increase risk of stroke, it is unlikely to be a primary initiating factor”. In the present study, normal renal status was determined for controls; but not in the patient groups (stroke, hypertension and diabetes). The contribution of a possibly impaired kidney function, in the patient subgroups, to a higher elevation of tHcy cannot be ruled out [5, 13].

**CONCLUSION**

The current data showed that tHcy of healthy controls was significantly lower than those of cardiovascular abnormalities. Proportion with hyperhomocystenaemia in our three groups of patients were much higher than similar studies. tHcy demonstrated a positive relationship with age in all groups; with HbAic in diabetes and with different lipid fractions in stroke and hypertension. It is foreseen that this additional “risk burden” on patients with already-defined cardiovascular disease may portend a poorer prognosis. It is still uncertain if tHcy is a risk marker or modifiable risk factor.

**RECOMMENDATIONS**

Our findings of a higher proportion and a more severe tHcy elevation in the selected diseases, require further elucidation (a) in other locales, (b) to determine any contributory interactions of dietary, genetic or geographical nature. A primordial preventive strategy of encouraging adequate dietary folate and B-vitamins intake may help in the reduction of plasma homocysteine [21, 22].

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ACKNOWLEDGEMENT / SOURCE(S) OF SUPPORT

We thank Professors Mustafa Danesi and Efe Ohwovoriole for useful comments in the preparation of the paper.

CONFLICT OF INTEREST

No conflict of interests was declared by authors.