PLASMA HOMOCYSTEINE AS A RISK FACTOR FOR STROKES IN GHANAIAN ADULTS.

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SUMMARY

Background: Stroke is an increasing problem in Ghana and the West African sub-region. New and modifiable risk factors for stroke have gained prominence in the last decade but have not been adequately researched in West Africa.

Methods: This was a case-control study with plasma Homocysteine (Hcy) as an independent risk factor for stroke. 80 consecutive stroke patients with 80 age-sex matched controls were venesected in the fasting state for assay of Hcy and other biochemical parameters.

Results: Mean Hcy level in stroke cases of 40.7 $\pm$ 9.5µmol/l was significantly higher than 16.8 $\pm$ 10.6µmol/l in controls [$p<0.0001$]. There was a significant association of hyperHcy with stroke [χ^2; $p<0.0001$]. OR of stroke calculated for quartiles of Hcy, demonstrated an increase in OR of 1.37 (25^{th} percentile) to an OR of 3.80 (75^{th} percentile).

Conclusions: Hcy was elevated in patients with stroke and should be considered as a modifiable risk factor for stroke in Ghanaian adults.

Keywords: Stroke, Homocysteine, Hyperhomocysteinemia, Infarction, Haemorrhage, Ghana.

INTRODUCTION

Cerebrovascular accidents are a major cause of neurological admission and account for major morbidity and mortality among hospital admissions in the West African sub-region.[1,2,3,4,5,6] Not much attention has been focused in Ghana or in the sub-region on modifiable risk factors, apart from hypertension, which is the dominant factor in the development of stroke in up to 77.3 % of cases.[1,2,7]

One of these hitherto unrecognized and untreated, but common risk factors for atherosclerotic ischaemic stroke may be an elevated plasma level of homocysteine(Hcy) which captured the stroke community's attention in the last decade as a definite modifiable risk factor for ischaemic stroke [8,9,10,11] and recently for haemorrhagic stroke[12]. Studies have been done on Hcy levels in Africa with regards to stroke. The last publications cited were work done in South Africa[13,14] in Egypt[15] and recently in Nigeria.[16]

Abundant epidemiologic evidence from at least 11 prospective cohort and nested case-control studies, and from more than 60 cross-sectional and retrospective case-control studies, have demonstrated that elevated Hcy levels are an independent risk factor for atherosclerosis in the coronary, cerebral, and peripheral vasculature.[8,10,11,17] This association is independent of other known risk factors, fairly and strongly consistent across many studies, dose related and meets the criteria for causality: consistency, strength, temporality, and biological plausibility.[10,17]

Despite the strong association between elevation of plasma Hcy and vascular risk, there is controversy regarding the causality of the association. However, with increasing understanding of the biology of hyperHcy, a causal relationship is increasingly plausible.[17,18]

Even though hyperhomcysteinemia is regarded as a risk factor for stroke, its pathogenetic role has not yet been established in black patients. At present, data on the distribution of total homocysteine (tHcy) levels in blacks are lacking; and limited information is available describing the correlates of elevated tHcy.[19,20]

METHODS

The study was a hospital based case-control study of adults aged eighteen years and above at the Korle Bu Teaching Hospital (KBTH) in Accra, Ghana. Consecutive patients (cases) admitted with a stroke using the WHO definition to the Surgical Medical Emergency Unit and the four medical wards of the KBTH were recruited over 6 months. Control patients, were selected from the same hospital setting and matched for age ($\pm$ 2 years), sex and race within study period.

Exclusion criteria

Cases with greater than one previous stroke, or recurrent strokes were not included in this study. Medical and surgical cases with a past or current history of a stroke or other vascular disease, diabetes, hypertension,

liver disease, renal failure, excessive alcohol use, anaemia, folic acid use, metabolic disease and evidence of cancer based mainly on clinical or investigative evidence were excluded as controls.

Initial clinical evaluation was done on arrival by the investigators, followed by a Computerized Tomographic Scan of the head, in all cases within 3 to 72 hours of admission. After informed consent was obtained from the patient or relatives (if the patient was unconscious) to partake in this study, a questionnaire was administered.

Laboratory Evaluation

Following the initial clinical evaluation, anthropometric measurements (weight, height and waist circumference) and written informed consent, both cases and controls were prepared for an overnight fast. Sampling for Hcy was timed to coincide with 48-72hrs post-stroke for the cases. Venepuncture was done from the antecubital veins in a recumbent position in all subjects between 7-8 am, and 10mls of blood collected into appropriate bottles for;

1. Determination of biochemical markers of alcohol intake: - Gamma Gluatmyl transferase levels were measured by standard semi automated methods
2. Fasting blood glucose (using a fluoride bottle on ice) and analysed by the Erba Smartlab (Transasia, Mumbai, India) automated machine using the Randox glucose reagent and an incubation period of 10 minutes.
3. Serum creatinine level measured by standard semi automated methods
4. Plasma Homocysteine(Hcy) - 5mls whole blood was collected into heparinized tubes [15,21] and kept on ice (<4^{o}C) after collection and centrifuged within 1hr (maximum 3hrs) after sampling. The plasma supernatant was aliquoted into new 2ml plastic vials and stored at -20^{o}C (in the freezer of the Public Health Reference Laboratory until analysed quantitatively using the Axis Homocysteine Enzyme Immunoassay (101) [IBL catalogue no. AX 513 01] Hamburg, Germany. Calibrators of known concentrations ranging from 2-50µmol/l (provided in the assay kit) were used as laboratory controls. Additionally, every fifth sample was run twice for reproducibility.

Statistics

The sample size was calculated for a hypothesis test of the Odds ratio (Level of significance 5%; Power 80%; Alternative hypothesis 2-sided), assuming an estimated prevalence of 20% and Odds ratio of 2.50 giving a sample size of 79.The data from the questionnaire were entered into a Microsoft Excel (2000) sheet and exported into SPSS version 10/11.5 for data analysis.

The Chi squared/Fisher's exact test was used to test the association between categorical variables. The independent T test was used to compare means of case-control data groups. The ANOVA test was used in the comparison of several groups. Pearsons correlation test was used to assess correlation between continuous variables.The Mantel-Haenszel estimate of the Odds ratio (which gives confidence intervals) was used for the case-control data for Hcy. This estimate takes into account possible confounding variables during analysis.

Continuous variables influencing the tHcy concentration were assessed by stepwise multiple regression analysis that included - age, serum creatinine, GGT, systolic and diastolic blood pressures in the model, to determine statistical significance for linear trend and the regression co-efficient ß. The level of significance was set at $p<0.05$, and in the tables shown in bold text.

Limitations

The primary limitation in this hospital-based case control design for Hcy is that levels are measured after the stroke, making it difficult to determine whether elevations in Hcy were a precursor or a consequence of the stroke.

The normal levels of Hcy in the healthy population are not known in Ghana or in the West-African sub region and this may impact on the interpretation of the results, even though the 75th percentile of controls was used as the cut off point as used in other studies.[13]

A second limitation of the study is that serum vitamin B12, B6 and Folate levels were not measured. Thus whether there was an association of vitamin status and stroke could not be determined and if so, whether the association was entirely mediated by Hcy.

RESULTS

Based on CT scanning done where feasible within 72hrs of the event, 61.3% of strokes were due to infarcts and 38.7% secondary to haemorrhage. Forty seven (58.7%) were males and 33(41.3%) females, with a male to female ratio of 1.4:1. The mean age of cases was 57.3 $\pm$ 13.7 years (range 28-81 yrs), and mean age of controls was 56.9 $\pm$ 13.8 years (range 28-83 yrs). For the subtype of stroke cases, mean age for ischaemic stroke was 60.1$\pm$11.6 years and mean age for haemorrhagic stroke was 52.9 $\pm$ 15.8 years.

56.3% of strokes were admitted within the first 24 hours, 18.8% within 48 hours, 6.3% within 72 hours and 18.8% > 72 hours. Glasgow coma scales (GCS) at presentation for the stroke cases were as follows: 48.7% (mild 11-13), 33.8% (moderate 8-10) and 17.5% (severe 3-7).

Homocysteine (Hcy)

The mean Hcy level measured in stroke cases of 40.7±9.5μmol/l was significantly higher than 16.8±10.6μmol/l measured in controls [p<0.0001] (see Table 1). Haemorrhagic strokes had slightly higher mean Hcy levels 41.5±8.7μmol/l than ischaemic strokes 40.2±10.0μmol/l (Table 2), but the difference did not reach statistical significance. The distribution of HyperHcy (classified in this study as >24.25μmol/l corresponding to the 75th quartile of control Hcy) in cases and controls is shown in Table 3. There was a significant association of hyperHcy with stroke [χ^2 ; p<0.0001], and gender [Pearson χ^2 ; p=0.031]. However there was no significant association between hyperHcy and the type of stroke [χ^2; p>0.05].

Table 4 shows the Odds ratios of stroke calculated for quartiles of total Hcy, with a steady increase in OR from the 25th percentile of 1.37 (95% CI 1.205 – 1.579) through to an OR of 3.80 (95% CI 2.591 – 5.573) for the 75th percentile with a statistically significant Mantel-Haenszel test for homogeneity of the OR [p<0.001].

Biochemical and haematological measurements

The mean ± SD and the range of all the biochemical parameters measured for both cases and controls are shown in Table 1. There was a statistically significant difference between the means of GGT, Creatinine, fasting blood glucose (FBS) and Haemoglobin [Independent T test with 2-tailed significance p<0.05].

The distribution of the biochemical parameters of Hcy, GGT, Creatinine, FBS, Haemoglobin and Mean cell volume (MCV) by haemorrhagic and ischaemic stroke subtypes is shown in Table 2. There was no statistically significant difference in any of the parameters for the type of stroke. MCV was not measured for the controls. There was a significant association seen between alcohol consumption and Hyperhcy [Fishers exact test χ^2 16.814 df(3) p=0.001].

Table 1 Biochemical Indices for cases and controls

	Cases		**Controls**		**P value****
	Mean ± SD	Range	Mean ± SD	Range	
Homocysteine (μmol/l)	40.7 ± 9.5	10.0 - 60.5	16.8 ± 10.6	0.0 – 51.5	0.0001
GGT (U/l)	61.8 ± 59.6	3.0 - 285.0	41.4 ± 46.4	4.0 - 237	0.017
Creatinine (μmol/l)	118.5 ± 61.5	53 – 446	101 ± 29.1	44 - 198	0.048
FBS (mmol/l)	6.2 ± 2.4	3.3 – 13.9	5.3 ± 1.9	3.10 – 7.1	0.022
Hb (g/dl)	12.6 ± 2.3 $^{\Psi}$	7.0 – 17.8	11.4 ± 2.2 $^{\Phi}$	6.7 – 16.3	0.002

** Independent T test, 2- tailed significance
$^{\Psi}$ N= 68 cases, $^{\Phi}$ N= 71 controls

Table 2 Biochemical indices amongst stroke subtypes

	Ischaemic stroke N=49		**Haemorrhagic stroke N=31**		**P value $^{\Phi}$**
	Mean ± SD	Range	Mean ± SD	Range	
Homocysteine (μmol/l)	40.2 ± 10.0	10.0 - 60.5	41.5 ± 8.7	16.8 - 55.6	NS
GGT (U/l)	62.6 ± 48.7	5.0 - 226.0	60.6 ± 74.5	3.0 - 285	NS
Creatinine (μmol/l)	114.5 ± 53.2	60 – 390	124.7 ± 73.0	53 - 446	NS
MCV $^{\beta}$	79.7 ± 9.1	62 – 104	77.1 ± 8.0	63.5 ± 91.6	NS

Φ-Mann-Whitney U Test, 2- tailed significance
$^{\beta}$- MCV (N=28; N=16) for Ischaemia and Haemorrhage respectively

Hcy levels positively correlated with the: age, strongly with Systolic blood pressure(SBP), Diastolic blood pressure (DBP), waist and hip measurements and with the Wasit to Hip Ratio(WHR). This implies that as the above parameters increase, the Hcy levels also increase, suggesting a link between obesity and Hcy levels. Hcy levels positively correlated with serum creatinine, haemoglobin and GGT levels.

Simultaneous multiple regression analysis with homocysteine levels as the dependent variable and age, GGT, serum creatinine, systolic BP and diastolic BP as independent variables (and excluding outliers outside 3 standard deviations) showed the proportion of variance accounted for by the regression to be 37.2% (adjusted R square), and the regression ANOVA [F=14.414 df(6) p=0.0005]. Only age and systolic blood pressure showed a significant linear relationship with Hcy.

Table 3 HyperHcy* in cases and controls

Hcy	Cases (%)	Controls (%)	Total
Normal	4(5%)	60(75%)	64
HyperHcy*	76(95%)	20(25%)	96
Total	80	80	160

χ^2 Pearson value 81.7 df(1) p<0.0001 for association of HyperHcy with stroke
HyperHcy* = >24.25 μmol/l corresponding to 75th quartile of control Hcy

Table 4 Odds ratios (OR) of stroke per quartiles of Hcy

Percentile	OR	95% CI Lower - Upper	P**
25th	1.379	1.205 - 1.579	0.001
50th	1.950	1.562 - 2.435	0.001
75th	3.80	2.591 - 5.573	0.001

P** Mantel-Haenszel statistic for homogeneity of the Odds ratio

DISCUSSION

Homocysteine (Hcy)

Most of the studies done on Hcy levels have been in relation to Caucasians and levels of Hcy measured in blacks have not been in relation to stroke.[20,22,23,24,25] However the mean Hcy levels seen in our study, 40.7μmol/l for cases and 16.8μmol/l for controls, were similar in trend to values reported for stroke in the meta-analysis by Boushey[26] in that: Hcy in strokes were significantly elevated compared to controls with mean values in the meta-analysis ranging from 10.1 - 40.5 μmol/l for cases and 8.6 - 31.7 μmol/l for controls. Similar findings were found for a case-control in Egypt 31.86μmol/l for cases vs. 12.6μmol/l for controls. [15]

Levels which could be considered as representative of a control population in sub-Saharan Africa include 15.9 ± 5.0μmol/l in adolescent girls in northern Nigeria [27] and a range of Hcy 2.8 - 37.6μmol/l seen in pregnant women in Harare[24], and 16.3μmol/l and 3.96-15.10μmol/l[13,22] in South African blacks and 12.6μmol/l in Egypt[15].The mean Hcy 40.2 μmol/l for infarcts {N=49} and 41.5 μmol/l for haemorrhage {N=31} concurs with the Brattstrom[28] and Lindgren[29] studies and recently by Li who included the largest number of haemorrhage {N=503}, found elevated Hcy to be frequently present in strokes with no significant difference in tHcy between infarcts and haemorrhage[12], but differs from the recent study {N=103} for haemorrhage by Boysen[30], that demonstrated a significant difference in tHcy level between ischaemic and haemorrhagic stroke.

This finding of difference between Hcy in ischaemic and haemorrhagic stroke in the Boysen study[30] indicated that the elevated level of Hcy in ischaemic stroke is not just a reaction to the acute illness but reflects the vascular differences between the two diseases – atherothrombotic for infarcts. Many inferences can be made from these observations: is elevated tHcy a risk factor or a risk marker for vascular disease? The findings of no difference between the stroke groups and supported by the large multicentre study in China [12] may give credence to the views of critics of the role of Hcy in stroke that: elevated tHcy may be a consequence rather than the possible cause of the acute event [31,32] but Brattstrom postulated that in conjunction with other vascular risk factors, longstanding moderate hyperhomocysteinaemia may well contribute to cerebral small vessel arteriosclerosis leading to haemorrhage and large vessel atherosclerosis and thrombosis leading to infarction.[28]

The debate still continues and the underlying cause for this elevation in tHcy remains to be clarified.[12, 17] The odds ratio of stroke per quartile increase in Hcy showed a graded, consistent and statistically significant increase with the risk of getting a stroke with Hcy in the 75th percentile {OR 3.80} at least 3 times the risk in the 25th percentile {OR 1.37}. This concurs with the

multivariate OR of 3.6 seen in the only study in African stroke patients[13], and a summary OR of 1.9-2.5 in the meta-analysis by Boushey [26] and OR 1.2-7.10 by Sacco [33], and an adjusted OR of 0.99- 4.7 in other meta-analyses.[11]

This and other evidence have brought into the fore the public health importance of hyperhomocysteinemia and the current efforts at modifying this with vitamin (mainly folate) supplementation. [18, 33,34] A positive correlation was seen between Hcy and age. This has been confirmed in epidemiological studies and has been attributed to the decline of the key Hcy metabolising enzyme cystathionine synthase with age.[35]A significant positive correlation was seen between Hcy and SBP/DBP a finding observed in several other studies. [35,36]

There are a number of pathophysiological mechanisms that could explain this relationship between elevated Hcy and vascular stiffness via Hcy-induced nitric oxide mediated relaxation of the vessel, smooth muscle cell proliferation and stimulation of elastinolytic processes in the arterial wall. The current view is that one of the mechanisms of Hcy-induced atherogenesis could be through elevations in blood pressure.[35,36] Knowledge of what the Hcy levels in hypertensives before and after a stroke are will help resolve whether Hcy is elevated pre, peri or post-stroke. Data on this however is currently unavailable[37] though results of prospective studies are awaited.

The correlation of serum creatinine demonstrated in this study, is a well-documented finding in the literature and has been attributed to the direct association of creatine/creatinine production with Hcy production and the major role the kidney plays in Hcy metabolism hence the mild-moderate elevations of Hcy commonly observed in end-stage renal disease.[9,28,38]The correlation of haemoglobin (which was higher in the stroke cases than controls, a finding also seen in the Danesi study[1]) with Hcy levels was not interpreted any further, in the absence of folate and vitamin B12 & B6 levels, which were outside the scope of this study. The fasting blood glucose did not correlate significantly with the Hcy. This could be evidence of the relative independence of Hcy as a risk factor of cardiovascular disease.

Finally, a significant, strong and positive correlation was seen for all the anthropometrical measurements with Hcy levels - indication of the possible association or interaction between obesity and Hcy, also mirrored in recent studies.[39,40]

The combined effects of these two risk factors suggest that Hcy could play a role in the higher risk of cardiovascular disease in obesity.[41] There is no consensus on the mechanism of interaction between obesity and Hcy, but nutritional factors, low plasma antioxidants and the insulin resistance syndrome in obese patients have been proposed as likely explanations for this link.[39,41,42] Whilst awaiting the results of these trials, it is prudent that with the evidence already accumulated over the years, and measures of fortifying flour with folic acid and B vitamins already instituted in the USA [18,33] and Japan [34] to address this modifiable risk factor need to be implemented in the west-African sub-region with the potential to lessen the burden of stroke on already overstretched health budgets and resources.

CONCLUSION

The findings in this study have demonstrated that:
Hcy is elevated in patients with stroke and should be considered as a risk factor of cardiovascular disease in Ghanaian adults. The issue of causality or risk however needs to be evaluated further in a prospective study. The high rates of stroke may be related to a combination of risk factors in addition to the traditional risk factors of hypertension and diabetes - obesity, moderate-excessive alcohol consumption and hyperhomocysteinemia. These risk factors can be prevented or treated both before and after the stroke.

However in a climate where under-nutrition and infectious diseases (including HIV/AIDS) are the major public health problems using the largest portion of the health budget, stroke is also a public health problem and must be considered as such. Addressing these modifiable risk factors in affordable, effective and culturally sensitive programmes remains a major challenge

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