ASSOCIATION OF PLASMA HOMOCYSTEINE AND ISCHAEMIC STROKE IN A NIGERIAN POPULATION

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ABSTRACT

Objective: Epidemiologic evidence suggests that raised plasma homocysteine is an independent risk factor for ischaemic stroke. However, other studies found no association between plasma homocysteine and stroke. Our objective was to determine the relationship between plasma homocysteine concentrations and ischaemic stroke in the Nigerian population where there is no existing published data.

Design: Case-control study.

Setting: University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. Fifty patients with ischaemic stroke and 50 control subjects, aged and sex-matched, were studied in relation to plasma homocysteine and other vascular risk factors.

Main Outcome Measures: Comparison of mean plasma homocysteine between stroke cases and control subjects and Odds Ratios for stroke in patients with hyperhomocysteinemia.

Results: Mean plasma homocysteine was significantly higher in stroke cases than in control subjects (mean ± SD: 20.8±10.2µmol/L vs. 13.1±4.5µmol/L; P<0.001). Other factors associated with ischaemic stroke were obesity, hypertension and elevated serum cholesterol. Using logistic regression analysis, there was an adjusted Odds Ratio of 1.9 (95% CI, 1.16–3.08) for ischaemic stroke for every 5µmol/L increase in plasma homocysteine concentrations.

Conclusions: Raised plasma homocysteine was significantly associated with ischaemic stroke and treating hyperhomocysteinemia may be an effective way of decreasing the incidence of stroke.

KEY WORDS: Stroke, Plasma homocysteine, Nigeria.

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INTRODUCTION

Stroke is a major cause of death and disability worldwide. Each year, about 4.4 million people die of stroke globally, of whom almost three million are from developing countries.[1] Traditional risk factors for stroke include advanced age, hypertension, diabetes mellitus, heart disease, elevated serum cholesterol, obesity, cigarette smoking, lower social class and drug use and abuse.[2-4] However, recent epidemiological evidence from several populations indicates that a moderately elevated plasma level of the amino acid homocysteine constitutes an additional risk factor for ischaemic stroke, coronary heart disease and deep venous thrombosis.[5-8]

The metabolism of homocysteine is dependent on folic acid, pyridoxal phosphate

(vitamin B6), cyanocobalamin (vitamin B12) and to a lesser extent, riboflavin (vitamin B2). Several studies have documented an inverse association between the plasma levels of these vitamins and total plasma homocysteine concentrations[8-9] while others have shown that raised plasma homocysteine levels can be normalized after treatment with these vitamins.[10]

Thus, in patients with hyperhomocysteinemia, treatment with folic acid, pyridoxine and cyanocobalamin may be a logical and inexpensive way of decreasing the incidence of stroke. Yet in much of sub-Saharan Africa, no study has investigated the association of hyperhomocysteinemia with ischaemic stroke. Our study aimed to determine the relationship between plasma homocysteine concentrations and ischaemic stroke in an adult population in Northeastern Nigeria.

PATIENTS AND METHODS

This was a case-control study conducted at the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Borno State, Nigeria, from January 2002 through January 2003. The UMTH is a tertiary referral centre for six states in northeastern Nigeria. Fifty consecutive patients aged 25-80 years were included in the study. They had a clinical diagnosis of stroke, supported by CT scan evidence of cerebral infarction. Control subjects were 25 healthy volunteers and 25 patients presenting at the same hospital with diagnosis other than stroke, transient ischaemic attack or any serious neurological disease. Both control groups had similar age and sex distribution to the cases. Exclusion criteria included haemorrhagic stroke, pregnancy, use of oral contraceptive pills, anticonvulsants or lipid lowering drugs and medical conditions such as liver disease, thyroid disease, leukemia and psoriasis.

A standard questionnaire was administered to each study participant; this included records of the subject's sex and age and whether they smoked tobacco at the time of the study. Participants were also asked of a prior physician diagnosis of hypertension, diabetes mellitus and hyperlipidemia. Each subject had a thorough physical examination details of body mass index, blood pressure and resting electrocardiogram were recorded. All study subjects signed informed consent and the ethical committee of the UMTH approved the study.

Hypertension was defined as a prior physician diagnosis of hypertension or persistent blood pressure levels of 140/90 mmHg or greater. Diabetes mellitus was defined as a prior physician diagnosis or fasting blood glucose levels of 7.0mmol/L or higher, in previously undiagnosed patients. Obesity was defined as body mass index greater than 25 kg/M2 in either sex.

Fasting blood samples were obtained from the forearm of each subject. For homocysteine measurement, blood samples were centrifuged within one hour of collection and plasma obtained was stored in a liquid nitrogen tank at – 40 to – 50^0C. Frozen plasma samples were later transported to the University of Ilorin Teaching Hospital, where homocysteine was assayed by a fluorescence polarization immunoassay method using the Abbott IMx automated immuno-analyzer (Abbott Labs., Illinois, USA), with a homocysteine assay kit (Lot No.88190HP00) as described by Schipchandler et al.[11] Serum total cholesterol and plasma glucose concentrations were performed by enzymatic methods.

Statistical analysis was performed with SPSS for Windows version 11.5. Categorical variables such as hypertension and obesity were compared between stroke cases and controls using Fisher's exact test, whilst means of continuous variables such as plasma homocysteine and serum cholesterol were compared using Student's unpaired t test. Logistic regression analysis was used to calculate Odds Ratios, and to adjust for confounders. P values less than 0.05 were considered significant.

RESULTS

The mean age of stroke cases was not significantly different from the control group (mean ± SD; 58.5±10.8years vs. 58.9±11.2 years; p=0.21). Plasma homocysteine in stroke patients

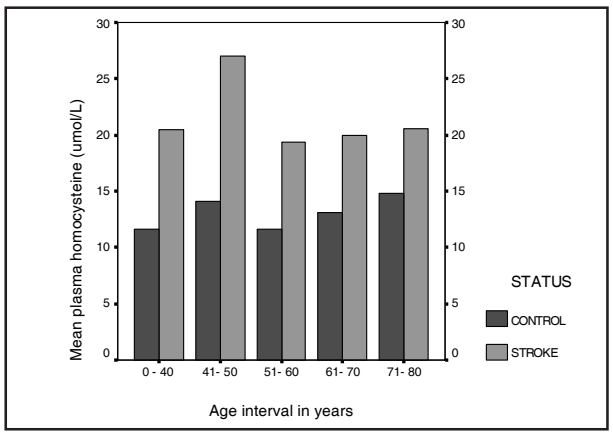

Fig-1: Bar chart showing plasma homocysteine distribution in stroke (grey) and control (dark) subjects.

was significantly higher than in control subjects (20.8±10.2µmol/L vs. 13.1 ± 4.5µmol/L; p< 0.001), with the difference being most marked in the fifth decade and least in the eighth decade (Figure-1). There were also statistically significant differences between cases and controls with respect to serum cholesterol and the prevalence of obesity and hypertension (Table-I). However, no difference was observed regarding the prevalence of diabetes mellitus or cigarette smoking.

To assess the risk of ischaemic stroke due to elevated plasma homocysteine, we performed a logistic regression analysis where stroke was the dependent variable, with plasma homocysteine, hypertension, obesity and serum cholesterol as the covariates. Results showed that for every 5µmol/L increase in plasma homocysteine, there was a corresponding Odds Ratio of 2.3 (95% CI, 1.2 – 3.4) for ischaemic stroke. After controlling for the confounding effects of the other risk factors, the adjusted Odds Ratio showed that every 5µmol/L increase in plasma homocysteine was associated with an Odds Ratio of 1.9 (95% CI, 1.16 – 3.08) for ischaemic stroke.

DISCUSSION

To our knowledge, this was the first study to document an association between elevated plasma homocysteine and ischaemic stroke in a Nigerian population. The only previous study on plasma homocysteine assessed cardiovascular risk factors and dietary habits of healthy persons among the nomadic Fulani of northern Nigeria.[9] In that study, mean plasma homocysteine was higher in males compared to females of the same age (14.7±6.2µmol/L vs. 10.8±4.3µmol/L) and an inverse association was observed between plasma homocysteine concentrations and serum levels of vitamin B12 and folate.

Our study has confirmed an association between raised plasma homocysteine and increased incidence of ischaemic stroke, similar to results obtained elsewhere.[5,6] While we did not find any difference in mean plasma homocysteine between males and females in the control group, male stroke patients in our study had significantly higher levels of plasma homocysteine compared to females (23.4 ±11.5µmol/L vs. 17.9 ± 7.6µmol/L; p<0.001).

The mean plasma homocysteine of 20.8µmol/L recorded among stroke patients in our study was higher than those of most other studies, perhaps reflecting very low intake of folate and vitamin B12 among Nigerians, an observation made in an earlier study.[9] A wide range of homocysteine values has been reported among stroke patients, ranging from 11.8µmol/L in South Korea to 14.6µmol/L in the U.K. and 16.4µmol/L in Israel.[5,6,12] In addition to nutritional factors, genetic variability is another important determinant of plasma homocysteine levels. Thus, variability in the prevalence of genetic mutations of the enzyme methylene

Table-I: Clinical and laboratory findings in stroke cases and control subjects

	Stroke (n = 50)	*Control subjects (n = 50)*	*P value*
Age (mean ± S.D.)	58.5 ± 10.8	58.9 ± 11.2	0.21
Smoking	2 (4%)	3 (6%)	1.0
Obesity	21 (42%)	5 (10%)	< 0.001
Hypertension	38 (76%)	13 (26%)	< 0.001
Diabetes mellitus	8 (16%)	4 (8%)	0.36
Serum cholesterol	5.16 ± 1.15	4.35 ± 1.04	0.02
Plasma homocysteine:			
All	20.8 ± 10.2	13.1 ± 4.5	< 0.001
Males	23.4 ± 11.5	13.2 ± 4.7	----
Females	17.9 ± 7.6	13.0 ± 4.3	----

tetrahydrofolate reductase and varied practices between countries regarding fortification of dietary flour with folic acid could explain differences in plasma homocysteine levels in different populations.

Optimal plasma homocysteine is defined as levels below 11.7µmol/L and values above 12.0µmol/L have been consistently associated with vascular disease.[13] By this definition, hyperhomocysteinemia is prevalent in the Nigerian population, not only among the stroke patients and control subjects in our study, but also among healthy males living in the community, as can be seen from the study by Glew et al.[9]

At present, the most effective way of decreasing the global burden of stroke remains preventive measures aimed at risk factor modification.[2] Brattstrom et al showed that daily administration of folic acid with pyridoxine hydrochloride reduced mean plasma homocysteine by 52% in patients with premature stroke and peripheral arterial disease.[10] Whether treating hyperhomocysteinemia will ultimately reduce the incidence of stroke can only be confirmed by prospective trials. Nonetheless, it will be logical to include plasma homocysteine assays in the diagnostic work-up of all stroke patients and those found to have hyperhomocysteinemia should receive therapy with vitamin B12, folic acid and pyridoxine.

Limitations of the Study: We have adjusted for the main causes of stroke that are present in our data set, so it is unlikely that our results can be explained by confounding. However, since some of our controls are volunteers, it is possible that they are not representative of those in the population from which the cases of stroke are drawn, in which case, their homocysteine levels may differ for reasons other than the absence of stroke.

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