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Original Paper

Relationship Between Plasma Homocysteine and Vitamin B_{12} Levels in Clinically Diagnosed Cases of Cardiovascular Accident in Lagos, Nigeria

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ABSTRACT

Vitamin B_{12} deficiency interferes with the normal pathway for homocysteine metabolism, even in the presence of adequate serum folate and pyridoxine. Homocysteinaemia, is an elevated level of homocysteine in plasma which has been established as a risk factor for the development of cardiovascular disorders. However, there has been conflicting report from various research works on the role of Vitamin B_{12} in the occurrence of major cardiovascular disorders. The study was set to determine the mean values of homocysteine, and correlate same with mean plasma levels of vitamin B_{12} in the subjects, and to estimate the prevalence of anaemia in the stroke patients, using Haemoglobin concentration (Hb) and mean corpuscular volume (MCV) as indices. One hundred (100) participants were recruited, comprising 40 clinically diagnosed stroke patients (hemorrhagic or thrombo-embolic), and 60 normal adults as control group. Plasma homocysteine levels were measured by HPLC and plasma vitamin B_{12} levels by Chemiluminescent Microparticle Immuno-assay method. Anticoagulated whole blood samples were evaluated for Hb, and MCV using automated Advia-60. Mean value of plasma homocysteine for the stroke patients (17.7±4.4umol/l) was significantly higher than values obtained in the control group at 9.5±2.4umol/l ($p<0.001$). Mean plasma vitamin B_{12} levels for both the stroke patients and controls were 249±14.5 *p*mol/l and 203.6±20.5 *p*mol/l respectively ($p=0.029$). The MCV for the study group (85fl) were significantly higher than those of the control group (82fl), $p=0.040$. However, the study showed no correlation between rising plasma homocysteine in stroke patient and vitamin B_{12} deficiency.

Keywords: Cerebrovascular accident, Homocysteine, Polymorphism, Stroke, Vitamin B_{12}

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INTRODUCTION

Stroke is the second commonest cause of death and the principal cause of adult disability in Nigeria (Nwosu *et al*., 1992). The current prevalence of stroke in Nigeria is 1·14 per 1000 while the 30-day case fatality rate is as high as 40% (Nwosu *et al*., 1992). Management of the disease is largely conservative therefore primary prevention is the key to reducing the increasing burden of stroke in Nigeria. Besides, socio-demographic factors, other biochemical factors such as: rising plasma homocysteine, folate deficiency, vitamin B_{12} deficiency, including genetic disorders of folate metabolism (677C→T polymorphism in MTHFR gene) are being implicated in the possible aetio-pathogenesis of cerebrovascular accident (Rajkovic *et al*., 2004).

Normal fasting plasma homocysteine level ranges between 5-15 µmol/l (Boushey *et al*., 1995). Intracellular homocysteine either enters the trans-sulfuration pathway or the remethylation cycle (Hoffbrand *et al*., 2001).

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Approximately 50% of homocysteine enters the trans-sulfuration pathway, where it is irreversibly combined with serine by the vitamin B6-dependent enzyme cysthathionine beta-synthase to form cysthathionine (Rosenblatt *et al.*, 1999) This is then metabolized to cysteine and ultimately to sulphate which is excreted in the urine (Tan, 2000). The methylation of Vitamin B_{12} to methyl B_{12} by tetrahydrofolate and subsequent methylation of homocysteine to methionine ensures a low plasma level of harmful homocysteine (Rosenblatt *et al*., 1999). Impairment of these mechanisms resulting from deficiencies of folate, vitamin B_{12}, or other enzymes involved in these pathways, ultimately lead to rising plasma homocysteine levels with subsequent vascular endothelial damage (Allen *et al*., 1993). Clinical manifestation in these patients may include: mental retardation (Barbe *et al*, 2009), skeletal abnormalities (Haddad *et al*., 1999), lens dislocation (Nwosu *et al*.,1992), early arterosclerosis (Lentz *et al*., 1996) and various thrombo-embolic phenomenon of which stroke is of major public health concern in our environment.

The effect of folate lack as possible risk factor for hyperhomocysteinaemia and ultimately cardiovascular disorders have been extensively documented. Therefore, this study seeks to establish possible relationship between plasma homocysteine and vitamin B_{12} in clinically diagnosed stroke patients in Nigeria, as limited local data exists on this subject among the population.

MATERIALS AND METHODS

Study Centre, Population and Design

The study was carried out between March and April 2007, at the Lagos University Teaching Hospital Idi-Araba and the Lagos State University Teaching Hospital, Ikeja both located in Lagos State, South West of Nigeria and represent the largest referral centers in the geographical region. A total of 100 subjects participated in this study after obtaining verbal consents of participants and ethical clearance from the hospital ethical committee. The study group comprised forty (40) clinically diagnosed cases of stroke for the period under study, who presented primarily with blood pressure readings above 140/90mm/Hg; hemi-paresis, and varying degrees of unconsciousness and had no other documented history of chronic systemic disorders. The remaining 60 participants who were clinically stable, age-sex matched and apparently healthy individuals were recruited by systematic random sampling from out-patient department into the control group.

Sample Collection and Processing

Two venous, non-fasting blood samples of 4.5 mls each were drawn from individual subjects into Na-EDTA and heparin specimen tubes and were immediately placed on ice in a cooler. Plasmas obtained after centrifugation at 3,000 rpm were stored at -20^0C prior to analysis. Total homocysteine assay (tHcy) was determined by HPLC using commercial homocysteine determination kit (Chromosysteins, Germany) in HP Agilent 1100 HPLC systems. Plasma vitamin B12 was determined by Chemiluminescent Microparticle Immunoassay (CMIA) methods using commercial kits (Abbott Laboratory Abott park, Chicago, IL) in Architech i 2000 Analyzer. Normal ranges in the laboratory are <10µmol/l for tHcy and 200-883pmol/l for vitamin B_{12}. Haemoglobin concentration (Hb) and mean cell volume (MCV) were determined using the automated coulter cell counter (Advia TM 60).

Statistical Analysis

Data obtained were analyzed using the SPSS version 11 (SPSS Inc. Chicago). Quantitative variables were expressed as mean ± SD; differences in mean were subjected to Student t-test and Fishers exact test where applicable and associations verified by Chi-square and Pearson correlation coefficient.

RESULT

Of the total number of stroke patients in the study, 70% (28 of 40) were males and 30% (12 of 40) were females. Mean age of the stroke patients was 62±12 years. The mean value of homocysteine among the stroke patients was 17.7±4.4umol/l, and this is significantly higher than that of the control at 9.5±2.4umol/l; p<0.001. The mean systolic blood pressure and diastolic blood pressure for both study group and control group as shown in table 1, were 160/102mmHg and 125/85mmHg respectively (p=0.000). The mean value of plasma vitamin B_{12} was higher in patients with stroke than in the normal subjects (249±14.5*p*mol/l and 203.6±20.5*p*mol/l respectively; p=0.029).

Table 1: **Mean values of Measured Parameters among the Respondents**

Measured parameters	Study Group	Control Group	t-test (p-value)
Hb (g/dl)	11.6±2.3	12.1±1.6	0.514
MCV(fl)	85±4.8	82±2.9	0.040
Homocysteine (umol/L)	17.7±4.4	9.5±2.4	0.000
Vitamin B_{12} (*p*mol/L)	249±14.5	203.6±20.5	0.029
Mean systolic blood pressure(mmHg)	160	125	0.000
Mean diastolic blood pressure(mmHg)	102	85	0.000

The mean haemoglobin concentration of the stroke patients was 11.6±2.3g/dl. This value was lower than the mean haemoglobin concentration for the control subjects at 12.1±1.6g/dl, but is not statistically significant (p=0.514). However, the mean cell volume (MCV) of the stroke patients (85 fl) was higher than those of control subjects (82 fl) p=0.040. High mean values of plasma homocysteine (>10µmol/l) associates well with the occurrence of stroke in the respondents, as 88% of subjects with stroke had elevated homocysteine levels above 10µmol/l as shown in table 2 (p<0.001;OR 14). Table 3 shows, poor association of stroke with plasma vitamin B_{12} level below 200*p*mol/l (p=0.568; OR 1.08).

Table 2: Relationship between High Homocysteine values and Occurrence of Stroke.

Groups	Plasma Homocysteine Level (>10µmol/l)	Plasma Homocysteine Level (<10µmol/l)	Total	Chi square	P- value
Study Group	35(87.5)	5(12.5)	40		
Control Group	20(33.3)	40(66.7)	60	28.45	0.000
TOTAL	55(100)	45(100)	100		

Odds ratio=14

Table 3: Association between Low Plasma Vitamin B_{12} and Occurrence of Stroke among the Respondents

Groups	Plasma Vitamin B_{12} level (<200ng/l)	Plasma Vitamin B_{12} level (>200ng/l)	Total	Fisher exact test P- value
Study Group	5(45.0)	35(55.0)	40	0.568
Control Group	7(25.0)	53(75.0)	60	
TOTAL	12(100)	88(100)	100	

Odds ratio=1.08

DISCUSSION

Of the total number of stroke patients who participated in the study, 70% (28 of 40) were males and 30% (12 of 40) were females. A previous study by Andrew *et al.* (1999) had shown no significant difference in sex prevalence of homocysteinaemia. Mean homocysteine level for both male and female participants in this study were not statistically different (18.2µmol/l and 17.1µmol/l respectively; p=0.217)]. The mean value for plasma homocysteine in the stroke patients (17.7±4.4umol/l) was significantly higher than that of the control group at 9.5±2.4µmol/l (p<0.05).

These values are similar to that reported by Nura *et al*. (2006) in a study of plasma homocysteine in Maiduguri (Northern Nigeria), where they recorded mean plasma homocysteine levels of 20.8umol/l and 13.1umol/l for ischaemic stroke and normal control group respectively. The association between high levels of plasma homocysteine and the incidence of stroke has been well documented. Andrew *et al*. (1999) in a study of stroke in some elderly persons concluded that total non-fasting plasma homocysteine levels are an independent risk factor for the incidence of stroke in elderly persons.

In Gombe, Northern Nigeria, Glew *et al*. (2004) showed no significant difference in the plasma homocysteine levels of stroke patients and control group. In our study however, 85% of the stroke patients had plasma homocysteine levels above 10umol/l, the mean plasma vitamin B_{12} level among the stroke patients (249±14.5pmol/l) showed no correlation with rising plasma homocysteine levels. This mean value of plasma vitamin B_{12} in the stroke patients is significantly higher than plasma vitamin B_{12} level in the control group at 203.6±20.5pmol/l (p=0.029). These findings therefore are at variance with other studies where lower values of plasma vitamin B_{12} were found (Barbe *et al*., 2009). However, Van Guelpan *et al*. (2005) concluded from a study of ischaemic and haemorrhagic stroke that neither dietary nor plasma vitamin B_{12} was associated with risk of either subtype of stroke. This seeming disparity might be traceable to subtle deficiencies in vitamin B_{12} utilization and disturbances in the metabolic pathway as possible aetiological factors in some of these patients with hyperhomocysteinaemia.

Our study therefore lends support to earlier position that vitamin B_{12} play no significant role in the occurrence of stroke. Mean corpuscular volume of the stroke patients (85±4.8 fl) was significantly higher than those of the control group with mean corpuscular volume of 82±2.9fl (p=0.040).This when considered with the relatively lower haemoglobin concentration among the stroke patients (11.6±2.3g/dl vs 12.1±1.6g/dl), might suggest a possible folate deficiency. Haltmayer *et al*. (2002) in a study involving 200 hospitalized patients at the Konvent hospital Barmherzige Brueder reported that cobalamin (Vitamin B_{12}) and folate deficiencies are related to both increased erythrocyte mean cellular volume (MCV) and raised serum total homocysteine (tHcy) values. The mean values of plasma vitamin B_{12}, correlated inversely with the plasma homocysteine levels only in the control group($r= -0.629$, $p<0.001$).This may imply that in health, homocysteine levels rises with decreasing levels of vitamin B_{12}. Although, this study made attempt to study the controversial relationship between vitamin B_{12} and observed changes in plasma homocysteine levels in stroke patients in Nigeria; we did not set out to determine plasma folate levels. Additionally, almost all previous studies were in agreement with the occurrence of low plasma folate in patients with stroke. Nevertheless, a larger, multi-centre study involving larger number of patients might be required to further establish these findings among stroke patients in Nigeria.

CONCLUSION

Though this study has been able to indicate an inverse relationship between vitamin B_{12} level and homocysteine in healthy individuals, its (vitamin B_{12}) role in the aetiology of stroke is largely speculative as indicated in similar studies, but may in addition to other prevailing factors be a potential risk factor for hyperhomocysteinaemia and subsequently cardiovascular disorders.

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