Original Paper

Homocysteine Levels in Nigerian Women with Pre-eclampsis/Eclampsia

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ABSTRACT

Hyperhomocysteinaemia has been linked in various studies worldwide to the occurrence of cardiovascular disorders and endothelial cell injury. In Nigeria with one of the highest maternal/neonatal mortality and morbidity, it is significant to explore means of predicting pregnancy-induced hypertension. This study was carried out at the Lagos University Teaching Hospital which is a major referral center in the South-western part of Nigeria. It was set to determine the mean plasma homocysteine levels and corpuscular haemoglobin in pre-eclamptic/eclamptic Nigerian women. A total of 150 subjects consisting of 100 primigravidae and 25 diagnosed cases of pre-eclampsia/eclampsia and 25 non-pregnant females were enrolled in the study. 60 primigravidae were lost to follow up or had incomplete data. The mean value of homocysteine in the control group, and eclamptic group was 7.5±3.4 µmol/L and 16.1±6.5 µmol/L respectively. There is positive and significant correlation between plasma homocysteine in the eclamptic group and the mean MCV (r=0.97, p=0.01), and between plasma homocysteine, systolic and diastolic blood pressure of the eclamptic group (r=0.944, p=0.0001; r=0.98, p=0.0001)**.** The study further supports earlier findings of increased homocysteine levels in the occurrence of pre-eclampsia/eclampsia.

Keywords: **Hyperhomocysteinaemia, Pre-eclampsia/eclampsia, Primigravidae, Cardiovascular disorder**

INTRODUCTION

Hypertensive disorders in pregnancy (pre-eclampsia/eclampsia) are among the leading causes of maternal mortality worldwide accounting for approximately 17% of all maternal deaths (Keinth, 1999). Overall perinatal mortality in pre-eclampsia is about 35/1000 total births, but may reach 160/1000 births in severe cases. Pre-eclampsia complicates 3-5% of first pregnancy and 1-2% of subsequent pregnancies with about 5-10% of cases being severe (Keinth, 1999). Hyperhomocysteinaemia has been linked in various studies worldwide to the occurrence of cardiovascular disorders, and endothelial cell injury (Holmes *et al.*, 2005).

Epidemiological studies worldwide have also demonstrated strong correlation between homocysteine levels in the blood and cardiovascular diseases, and wide spectrum of thromboembolic disorders (Mas Valerie, 2003). The theoretical background for this study of the relationship between hyperhomocysteinaemia and pre-eclampsia/eclampsia, is based on the understanding that when the enzyme reactions involved in the two metabolic pathways of homocysteine metabolism are impaired, or there are nutritional deficiencies of vitamins such as folate, vitamin B12, and vitamin B6, these may manifest clinically as widespread endothelial damage with laboratory evidence of increasing mean corpuscular volume (MCV).

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However, current research efforts are being directed at the 677C→T polymorphism in the MTHFR gene, with some studies documenting higher values of total plasma homocysteine among subjects with TT genotype compared with those with the CC genotypic variant (up to 16% in some studies). These genetic variants, together with dietary and other geographical factors could be responsible for ethnic and racial variations in plasma homocysteine levels worldwide (Rajkovic *et al.*, 2004).

This study was carried out in Lagos, which is known to be the most populated city in Nigeria and the most representative of the multi-ethnic background of the nation. However, only few data exists (from Northern Nigeria) on this subject matter (Cotter *et al.*, 2001). The challenges of management, and difficulty in predicting possible pregnancy outcome in the face of dwindling resources, where folate, vitamin B12 assay and genetic studies are not immediately feasible. This study therefore attempts to document baseline values and possible predictive values for occurrence of pre-eclampsia/eclampsia using plasma homocysteine levels and mean corpuscular volume in pregnant females.

MATERIALS AND METHODS
Study Centre, Population and Design
This study was carried out among 100 primigravidae attending the ante-natal clinic of the Lagos University Teaching Hospital, Idi-Araba between January to October 2006. The hospital is located in the South West region of Nigeria and serves as a referral center serving the most populated and ethnically diverse state in Nigeria. The participants were all those presenting at the clinic in the month of January 2006, who voluntarily agreed to participate in the study and were registered for the first time at less than fourteen weeks of gestation. Twenty-five (25) clinically diagnosed cases of eclampsia were also recruited for the study which was the total number of cases admitted in the study center between January and October 2006. With 25 non-pregnant females randomly selected as control, all primigravidae with history of hypertension, diabetes mellitus, renal disease, deranged urinalysis report, use of alcoholic beverage and smoking were excluded to limit study bias. All participants were to strictly maintain the drug regimen prescribed for routine antenatal use only.

Ethical approval for the study was obtained from the hospital ethical committee.

Collection of Samples and Analysis
On collection of 4.5ml of venous blood in EDTA bottle, plasma levels of homocysteine were determined at the time of registration, at 26 weeks and at 34 weeks of gestation respectively for all participants, and on all the twenty-five (25) cases of eclampsia using the enzyme linked immuno-assay technique (Diazyme Laboratories, San-Diego, USA), which has a high level of precision, with a total coefficient of variation of 4.5 - 7.4% and a turnover of 82 samples for 2.5 hours. Quality control sample was run with each batch of test. Estimation of Mean cell volume (MCV) was carried out on 4.5ml of venous blood in EDTA bottle using the electronic coulter counter (ADVIA-TM 60). Supine blood pressure readings were taking and urinalysis done on each participant using the dry chemistry method.

Data Analysis
The data was analysed using the statistical software SPSS version 11 (SPSS Inc, Chicago). The data on qualitative variable were indicated by frequency and percentage, qualitative variables by range and mean, and association. Verified by Chi-Square with statistically significant value placed at $P < 0.05$.

RESULTS
A total of 150 subjects were enrolled in the study, this consisted of 100 primigravidae and 25 diagnosed cases of eclampsia and 25 non-pregnant females (Table 1). Sixty (60) primigravidae were either lost to follow up or had incomplete data. Mean ages of respondents were 25±5.8 years for control group, 27±5.8years for primigravidae and 28 ± 4.6 years for the pre-eclamptic/eclamptic study group. Mean value of homocysteine in the control group was 7.5±3.4 µmol/L. Mean values of plasma homocysteine declined progressively throughout pregnancy among the primigravidae (7.1±2.9µmol/L; 6.0±1.7 µmol/L and 5.4±1.7 µmol/L for 1st, 2nd and 3rd trimester respectively).

As shown in table 1, there was marked elevation of plasma homocysteine in the eclamptic subjects which ranged from 11.6µmol/L to 38.5µmol/L with a mean value of 16.1 ± 6.5µmol/L. The mean corpuscular volume for the eclamptic group (85±6.6fl) was significantly higher than those for

the non-pregnant control, and primigravidae throughout pregnancy (80±4.3fl, p=0.032 and 82±6.0fl, p=0.046 respectively).

Positive and significant correlation exists between the plasma homocysteine levels among the eclamptic group and MCV of respondents in that group (r=0.97, p=0.01).

			Primigravidae		
	Non-pregnant control	**Pre-eclamptic/ Eclamptic**	1st trimester	2nd trimester	3rd trimester
	n=25	n=26	n=39	n=39	n=39
Mean age of participant in years±SD	25±5.8	28±4.6		27±5.8	
Mean G. A (Weeks)	-	33	14	26	34
Mean Homocysteine level (µmol/L)	7.5 ± 3.4	16.1 ± 6.5	7.1 ± 2.9	6.0 ± 1.7	5.4 ± 1.7
Mean MCV (Fl)	80 ± 4.3	85 ± 6.6	81 ± 4.7	82 ± 6.1	83 ± 6.0

Table 1: Age, Plasma Homocysteine and Mean Cell Volume of Participants

Table 2: Correlations between Plasma Homocysteine and Blood Pressure of the Study Group.

PARAMETERS	*Control subjects*	*Pre-eclamptic/ Eclamptic*	*Primigravidae 1st trimester*	*Primigravidae 2nd trimester*	*Primigravidae 3rd trimester*
Homocysteine level (µmol/L)	**7.5±3.4**	**16.1±6.5**	**7.1±2.9**	**6.0±**	**5.4±1.7**
SYSTOLIC B.P (mmHg)	108 ± 10	177 ± 29	110 ± 8	108± 8	114 ± 7
Pearson correlation co-efficient(r)	-0.065	0.944	0.07	0.04	0.064
P-value	>0.05	<0.05	>0.05	>0.05	>0.05
DIASTOLIC B.P (mmHg)	66 ± 7	110 ± 17	66 ± 6	67 ± 9	69 ± 6
Pearson correlation co-efficient(r)	-0.07	0.98	0.09	-0.22	0.10
P-value	0.70	0.0001	0.56	0.17	0.53

There was a statistically significant association between rising homocysteine level in the eclamptic group and blood pressure levels (Table 2). The mean systolic and diastolic blood pressure within the group (177±29 mmHg; 110±17 mmHg respectively) correlates well with mean plasma homocysteine levels (r=0.944, p=0.0001; r = 0.98; p=0.0001). The mean plasma

homocysteine level in the 3rd trimester of normal pregnancy was significantly different from that of the eclampatic group at the third trimester of pregnancy (5.4 ± 1.7µmol/L and 16.1 ± 6.5µmol/L respectively, p=0.048 and 0.014). The relative risk for developing pre-eclampsia/eclampsia in this study was 7.74 (Table 3).
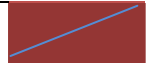
Table 3: Association of Hyperhomocysteine with Normal Pregnancy and Pre-Eclampsia/Eclampsia

Homocysteine Level(μmol/L)	Pre-eclampsia/ Eclampsia	Normal Primigravidae	Chi-Square (Yates Corrected)	P-Value	Relative Risk
>10.4	22	5			
<10.4	4	34	30.22	0.000	7.74
Total	26	39			

DISCUSSION

Numerous studies have been done on homocysteine and its association with toxemia of pregnancy. Makedos *et al.* (2007) in the study of 28 pre-eclamptic patients and 26 normal control subjects gave a recorded value of 11.11μmol/L and 6.40μmol/L for pre-eclamptic and normal control respectively. This is not much different from that obtained in this study (16.1μmol/L and 7.5μmol/L respectively). However, they found no significant difference in the plasma folate and vitamin B12 of both groups. The mean value of plasma tHcy of 16.1 ± 6.5μmol/L in this study, showed a significant elevation when compared with mean third trimester value of 5.4±1.7 μmol/L in the primigravidae (p=0.00), thus putting the relative risk of developing pre-eclampsia at 7.7.

A similar study by Cotter *et al.* (2001) however recorded a lower value of 9.8 μmol/L in some patients with pregnancy induced hypertension in Northern Nigeria. Megahad and Taher in a study of 25 subjects with eclampsia, reported a decline in the value of red cell folate among the respondents, but no significant difference in the levels of plasma vitamin B12 (Megahad and Taher, 2004).

Michelle *et al.* (2002) documented a progressive fall in plasma homocysteine level throughout pregnancy irrespective of use or non-use of folic acid in pregnancy. This may imply that subtle folate deficiency, or defect in folate metabolic pathway is a more important variable in the pathogenesis of hyperhomocysteinaemia. Current studies however are aimed at 677 (C→T) gene polymorphism to establish new aetiological relationship between rising plasma homocysteine level and folate metabolism. A study of 356 subjects in Zimbabwe found that one subject with pre-eclampsia had homozygous 677(C→T) gene even though this association was not statistically significant (Tanya *et al.*, 1999).

Similarly, Angius *et al.* (2007) attributed lower values of tHcy seen among Burkinabes to a more effective homocysteine metabolism.

Geographical and racial variations in plasma homocysteine have also been documented. Peter *et al.* (2004) documented a significantly lower plasma homocysteine among pregnant white females (5.5 μmol/L), compared to black pregnant females (7.6 μmol/L), and reported that though plasma homocysteine levels where elevated in subjects with pre-eclampsia, the black pre-eclamptic females still had a higher mean plasma homocysteine levels. This, they attributed to racial difference in diet and adherence to folic acid supplement. However, this finding is at variance with those of Moore *et al.* (2006) in a racial comparison study of 158 non-pregnant females and 12 pregnant Gambian women, with their female British counterpart. They recorded a mean plasma homocysteine level of 9.0 μmol/L in non-pregnant female Gambians and a lower value of 6.2 μmol/L in the pregnant females. These values approximated well with those of their British counterpart (9.4 μmol/L for non-pregnant white females). Findings in this study agree with those from the Gambian studies.

Agyemang and Bhopal (2005) recorded higher blood pressure and Body Mass Index (BMI) among women of African origin in the UK. However, homocysteine level correlated poorly with systolic and diastolic blood pressure of respondents in the control group and throughout pregnancy in normal pregnant respondents (r= -0.065/-0.70, p>0.05, r=0.064/0.10, p>0.05). In this study, there was a strong correlation between the homocysteine level and the systolic/diastolic blood pressure in the eclamptic group (r=0.944, p=0.0001; r = 0.98; p=0.0001). Of the 40 primigravidae that was followed up, one of the respondents showed a rising pattern of plasma homocysteine throughout the 1st to 3rd trimester of pregnancy (10.2 μmol/L, 11.4μmol/L and

17.0µmol/L respectively). The pre-registration value of blood pressure rose from 120/80mmHg at 14 weeks) to 140/90mmHg at 34 weeks with associated proteinuria. These imply that in health, homocysteine has no correlation with blood pressure, until homocysteine level rises to a point where it induces widespread endothelial damage, vasospasm and microangiopathic changes with subsequent elevation of blood pressure. However, despite earlier submission by Michelle *et al.* (2002) and Makedos *et al.* (2007) on the relative insignificant difference in folate and vitamin B12 levels between the pre-eclamptic and normal control study groups, the observed significant difference in the mean corpuscular volume seen in the eclamptic group (mean MCV value of 85±6.6/L) and those of the non-pregnant controls (80±4.3/L, p <0.05) might be attributable to subtle nutritional deficiencies in folate and vitamin B12 metabolism (Mueller *et al.*, 2001).

CONCLUSION

This study has been able to document the pattern of plasma homocysteine among pregnant Nigerian women in the Southwestern part of the country, and cases of pre-eclampsia/eclampsia. It has further provided possible predictive value for increasing mean corpuscular volume, plasma homocysteine level and rising blood pressure during pregnancy as a prelude to the development of pre-eclampsia/eclampsia.

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