Association between Serum Homocysteine Levels in Preeclampsia and its Severity among Women in Lagos, South-West Nigeria

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Abstract

Background: Clinical evidence had suggested that hyperhomocysteinaemia features in hypertensive disorders of pregnancy. However, there is still conflicting evidence on the extent at which elevated maternal homocysteine contributes to this deadly complication of pregnancy.

Objectives: This study investigated the impact of elevated maternal homocysteine levels in early pregnancy on preeclampsia and its severity among Nigerian women in Lagos.

Methods: This was a prospective cohort study conducted at the Lagos University Teaching Hospital. Participants were enrolled in the first trimester of pregnancy following which their sociodemographic data were obtained by interview. Venous blood samples were collected for measurement of homocysteine concentration using the ELISA method. Data on the occurrence of preeclampsia was obtained from the delivery records. Binary logistic regression model was used to study the effects of the major baseline characteristics on the development of preeclampsia.

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Results: The final analysis included 167 patients; hyperhomocysteinaemia was recorded in 24 (24.6%) patients. Women with hyperhomocysteinaemia had no statistically significant risk of developing preeclampsia or severe preeclampsia, compared with women with a normal homocysteine concentration.

Conclusions: The prevalence of hyperhomocysteinaemia in the study was relatively low. The absence of a significant association between maternal hyperhomocysteinaemia and preeclampsia reported in this study could create room for the conduct of a more robust, adequately powered longitudinal research needed to answer some of the major reservations that remain from the present study.

INTRODUCTION

In most developed countries, pregnancies are planned, complications are few and outcomes are generally favourable for both mother and infant. Adverse pregnancy outcomes are far more frequent in the developing world [1]. Vascular-related pregnancy complications are a major cause of these adverse maternal and fetal outcomes. The origin is thought to be related to early placentation, a process that involves trophoblast invasion and angiogenesis, but that is also dependent on vascular and endothelial function [2]. Preeclampsia, a pregnancy specific syndrome, is a major cause of maternal and perinatal morbidity and mortality with a worldwide incidence of 5–8% of all pregnancies [3,4]. Preeclampsia is characterized by the onset of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive pregnant woman [3,5]. Despite a fairly high incidence, the underlying aetiology of preeclampsia is still incomplete [3,4]. Clinical evidence had shown that hyperhomocysteinaemia features in hypertensive disorders of pregnancy [6,7]. Homocysteine, a Sulphur-containing amino acid, is involved in processes such as lipid peroxidation and oxidative stress [3,8]. Hyperhomocysteinaemia is a risk factor for endothelial dysfunction and vascular disease such as atherosclerosis [8]. In normal pregnancy there is conversion of spiral arteries from its highly tortuous thick-walled vessels to flaccid sinusoidal conduits of low resistance, however, the muscular coats of spiral arteries are retained in preeclampsia [9]. In preeclampsia there is elevated homocysteine injuries and abnormal vascular endothelium. In addition, vascular endothelium in pregnant women may be more sensitive to homocysteine injury [10]. Clinical alarm is always late to detect preeclampsia, thus laboratory assay of markers of endothelial injury may play an important role in such scenario. There is still conflicting evidence on the extent at which elevated maternal homocysteine is a risk factor for pregnancy complication such as preeclampsia, so prospective, sufficiently powered studies from early pregnancy onwards are required to establish this relationship. We therefore conducted this prospective cohort study that aimed to examining the impact of elevated maternal homocysteine concentrations in early pregnancy on vascular-related pregnancy complication of great clinical importance, such as preeclampsia. This will subsequently allow the establishment of a clinical alarm

system that may be useful as a marker to identify at-risk pregnant women in order to mitigate this undesirable potentially life-threatening pregnancy complication.

SUBJECTS, MATERIALS AND METHODS

The was a prospective cohort study conducted at the Antenatal clinics and Labour ward complex of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos. Participants healthy pregnant women attending the antenatal clinics of the hospital between July and December 2015. Ethical approval was obtained from the hospital's Health Research Ethics committee prior to the commencement of the study (ADM/DCST/HREC/018) and the ethical principles according to the Helsinki declaration were considered throughout the course of the study.

The sample size (N) for the study was determined using the formula [11]:

$$\frac{2SD\left(Z_{\alpha/2} + Z_{\beta}\right)^2}{d^2}$$

Using data from a published study by Mascarenhas et al [12], the Standard Deviation (SD) = 6.59 μmol/L, the unit normal deviate corresponding to the desired Type I error rate of 5% at 95% confidence interval ($Z_{\alpha/2}$) = 1.96, the desired type II error rate of 5% (Z_{β}) = 0.98 and between-group mean difference in serum homocysteine levels = 0.87 μmol/L. Making provision for attrition rate of 20%, the minimum sample size required was 163.6. However, for ease of data collection, collation and analysis, 200 women were enrolled at the point of entry for the study. Eligible participants at enrolment were consenting women aged 18 to 45 years with singleton pregnancy at gestational ages less than 14 weeks. Women with history of diabetes or hypertension, HIV, current or previous history of smoking and other described substance use, those with conflicting information regarding their gestational age at enrolment and those with incomplete or lost data at delivery were excluded from the final analyses.

Participants were enrolled by consecutive sampling method after which an informed written consent was obtained from each participant upon explanation of the nature and purpose of the study. Demographic and socioeconomic data were obtained from each participant by interview at entry to care using a pretested structured questionnaire. Venous blood samples (3–4mLs) were collected in universal specimen bottles and transported from the antenatal clinics to the hospital main laboratory within 2 hours of collection. The blood samples were processed by centrifugation at 3000rpm to obtain the sera which were then stored at −20°C until the final analysis. Total maternal homocysteine levels in serum were analyzed by the enzyme linked immunosorbent assay (ELISA) method using reagents from the manufacturer (Bioassay Technology Laboratory[R], Rockville, MD, USA). Elevated maternal serum homocysteine in pregnancies was defined as maternal serum homocysteine level greater than 15μmol/L [13]. The coefficient of variation within and between assays of <5% was be used. Data on confirmatory outcome variable (development of preeclampsia) was obtained by abstracting the antenatal and delivery records. Gestational duration was based upon gestation deduced from participants' last normal menstrual period confirmed or modified by

ultrasound. Preeclampsia was diagnosed in patients with hypertension defined as an absolute blood pressure greater than 140/90 mmHg after 20 weeks' gestation and proteinuria defined as greater than 0.3 g of urinary protein excretion per 24-h urine collection on at least two occasions 4 h apart [14]. Preeclampsia was further classified as mild and severe. It is severe, if systolic blood pressure increased to at least 160 mmHg, diastolic blood pressure increased to at least 110 mmHg, and proteinuria was >5 g/day [14].

Descriptive statistics for all data were computed using SPSS version 22.0 for windows manufactured by IBM, Armonk, NY, USA. Quantitative data were tested for normality with the Kolmogorov-Smirnov test. Associations between continuous variables were tested using the independent sample t test (normal distribution) or the Mann-Whitney U test (skewed data), whereas categorical variables were compared using the χ^2 test or the Fisher exact test, as appropriate. This was followed by multivariate analysis using binary logistic regression models to study the effects of the major baseline characteristics on the development of preeclampsia. $P<0.05$ was considered statistically significant.

RESULTS

Initially, 200 participants with singleton pregnancies at less than 14 weeks' gestation were enrolled. However, eight women withdrew their consent during the course of the study for personal or cultural reasons, fifteen women were lost to follow-up, seven had incomplete or lost data and three women experienced mid-trimester pregnancy loss. Therefore, the final analysis included 167 women; 41 (24.6%) patients had a homocysteine concentration above the reference range (>15μmol/L) and 126 (75.4%) with a normal homocysteine concentration.

Women with a high homocysteine concentration and those with a normal homocysteine level did not differ significantly in terms of age (P=0.684), level of education (P=0.866) and parity (P=0.647) (Table 1). Of the 167 participants, 18 (10.8%) had preeclampsia while severe preeclampsia was recorded in 7 (38.9%) of mothers with preeclampsia. The rate of preeclampsia was three times higher among women with maternal hyperhomocysteinaemia than those with a normal homocysteine concentration (P=0.011). However, no statistically significant association was detected between elevated serum homocysteine concentration and the severity of preeclampsia (RR 1.22, 95% CI 0.88–6.27, P=0.294). After controlling for age, parity, and level of education, maternal hyperhomocysteinaemia was also not independently associated with the occurrence of preeclampsia (adjusted RR 3.11, 95% CI 0.77–9.06, P=0.084).

DISCUSSION

In the present study, the prevalence of maternal hyperhomocysteinaemia in early pregnancy was 24.6%. This is similar to the rate of 22.2% found by Bergen et al [15] but much lower than the rate of 50.0% reported by Visternicean in Moldova [16]. This variation may be due to the geographical/racial differences and varying cutoff value for elevated serum homocysteine level chosen by Visternicean (12 μmol/L) [16], whereas the higher cutoff

value for the current study (15μmol/L) was based on the reference range for normal homocysteine levels used in a previous study by Hema [13].

The rate of preeclampsia recorded in this study was 10.8% and this was only slightly higher than the worldwide incidence of 5–8% [3, 4] but still within the range of 1.8% to 16.7% reported in most developing countries [17]. However, the proportion of these cases with severe preeclampsia (38.9%) was much lower than the 90.0% reported by our team in a similar setting in Lagos [18]. This may reflect the characteristics of participants in the current study who were healthy, low-risk and predominantly low parous pregnant women with a significant reduction in the risk of developing preeclampsia and severe preeclampsia. Just like our study, Zeeman et al [19] reported no detectable association between maternal hyperhomocysteinaemia and preeclampsia. Other previous studies, however, found statistically significant associations between elevated maternal serum levels of homocysteine and the occurrence of preeclampsia [15,20–25]. At variance to our study, some other studies have reported significantly higher maternal homocysteine concentration in women with severe preeclampsia compared to those with mild preeclampsia [21,22]. The current study was hospital-based, limiting the generalizability of the findings to the entire population of pregnant women in Lagos. It was also difficult to determine, through recall, the amount of folic acid tablets consumed by the mothers prior to their sample collection and homocysteine assay, therefore this may be a confounding factor, especially due to the fact that folic acid is routinely used by most in their first trimester of pregnancies even before their antenatal care bookings. A major strength of this study is that it is the only known study among black African women that prospectively examined the effects of a high homocysteine concentration on the development of preeclampsia among low-risk pregnant women while carefully adjusting for confounding factors. The study thus provides valuable information for future adequately powered longitudinal studies among pregnant women in Nigeria.

CONCLUSION

This study showed a relatively low prevalence of early pregnancy maternal hyperhomocysteinaemia among women in Lagos. The absence of a significant association between maternal hyperhomocysteinaemia and preeclampsia reported in this study could create room for the conduct of a more robust, adequately powered longitudinal research needed to answer some of the major reservations that remain from the present study.

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ABBREVIATIONS

ELISA   Enzyme Linked Immunosorbent Assay

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Table 1:

Distribution of participants' baseline characteristics and maternal serum homocysteine levels (n=167).[a]

Characteristic	Homocysteine levels		P-value
	Normal, n (%)	High, n (%)	
Age (years)			
<30	40 (71.4)	16 (28.6)	
30–34	57 (77.0)	17 (23.0)	0.684
>34	29 (78.4)	8 (21.6)	
Mean age ± SD	28.7 ± 4.3	30.1 ± 5.7	
Level of Education			
Primary	3 (100.0)	0 (0.0)	
Secondary	36 (76.6)	11 (23.4)	0.866
Tertiary	87 (74.4)	30 (25.6)	
Parity			
0–1	95 (82.6)	20 (17.4)	
2–4	28 (71.8)	11 (28.2)	0.647[b]
>4	3 (100.0)	0 (0.0)	
Median parity (IQR)	2.0 (0.0–4.0)	2.0 (0.0–4.0)	
Total	**126 (75.4)**	**41 (24.6)**	

Abbreviations: SD, standard deviation; IQR, interquartile range.

[a]Values are given as mean ± SD, median (IQR), or number (percentage), unless indicated otherwise.

[b]Fisher exact test.

Table 2:

Bivariate analysis of maternal homocysteine concentrations and development of preeclampsia (=167).[a]

Characteristic	Total (n=167)	Homocysteine levels		RR (95% CI)	P-value
		Normal (n=126)	High (n=41)		
Preeclampsia					
Yes	18 (10.8)	9 (7.1)	9 (22.0)	3.14 (1.97–8.43)	0.011
No	149 (89.2)	117 (92.9)	32 (78.0)	1.00 (ref.)	
Severity of preeclampsia[b]					
Severe	7 (38.9)	4 (36.4)	3 (42.9)	1.22 (0.88–6.27)	0.294[c]
Mild	11 (61.1)	7 (63.6)	4 (57.1)	1.00 (ref.)	

Abbreviations: CI, confidence interval; RR, risk ratio.

[a]Values are given as number (percentage) unless indicated otherwise.

[b]n=18.

[c]Fisher exact test

Table 3:

Bivariate and multivariate analyses of the relationships between baseline characteristics and development of preeclampsia.

Characteristics	Preeclampsia			
	Bivariate analyses		Multivariate analyses	
	RR (95%CI)	*P-value*	aRR (95%CI)	*P-value*
Age in years				
<30	1.00 (ref)	Ref	1.00 (ref)	Ref
30–34	0.78 (0.13–1.62)	0.701	0.84 (0.41–5.10)	0.111
>34	6.19 (2.27–13.01)	0.023	5.62 (2.44–14.79)	0.005
Parity				
0–1	1.00 (ref)	Ref	1.00 (ref)	Ref
2–4	2.33 (0.76–7.71)	0.651	1.47 (0.99–9.03)	0.885
>4	4.44 (1.08–10.22)	0.037	5.16 (3.55–8.74)	0.042
Level of Education				
Primary	1.00 (ref)	Ref	NA	
Secondary	1.05 (0.35–4.49)	0.998	NA	
Tertiary	3.78 (1.22–15.16)	0.055	NA	
Homocysteine levels				
Normal	1.00 (ref)	Ref	1.00 (ref)	Ref
High	4.57 (1.52–11.33)	0.048	3.11 (0.77–9.06)	0.084

Abbreviations: RR, crude risk ratio; CI, confidence interval; aRR, adjusted risk ratio.