Association between Maternal Serum Homocysteine Concentrations in Early Pregnancy and Adverse Pregnancy Outcomes

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Abstract

Background: There is still conflicting evidence on the extent to which maternal hyperhomocysteinemia is a risk factor for pregnancy complications. Aims: The study aimed to investigate the impact of elevated maternal homocysteine concentrations on adverse pregnancy outcomes among Nigerian women in Lagos. Materials and Methods: This was a prospective cohort study conducted at the Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria. Participants were enrolled during the first trimester of pregnancy following which relevant data were obtained by the interview. Fasting blood samples were collected for the measurement of maternal homocysteine concentration using the enzyme-linked immunosorbent assay method. Pregnancy outcomes and complications were obtained by abstracting the antenatal, delivery, and newborn medical records. Preterm births, low-birth weight (LBW), and antepartum fetal death were used as confirmatory outcome variables in the final analysis. Descriptive statistics for all data were computed using SPSS version 22.0. The associations between the variables were tested and multivariate analyses were used to study the effects of the major baseline characteristics on the pregnancy outcome. P < 0.05 was considered statistically significant. Results: Hyperhomocysteinemia was recorded in 41 (24.6%) patients. 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). Women with hyperhomocysteinemia had an approximately twelve-fold higher risk of preterm birth (P = 0.001) and a ten-fold higher risk of delivering a term neonate with LBW (P = 0.004), but had no risk of antepartum fetal death (P = 0.118) compared to women with a normal homocysteine concentration. Conclusions: The prevalence of hyperhomocysteinemia among mothers in Lagos was relatively low. The associations between hyperhomocysteinemia and adverse pregnancy outcomes could have implications in future for the prevention of these adverse outcomes.

Keywords: Enzyme-linked immunosorbent assay, hyperhomocysteinemia, Lagos, low-birth weight, preterm birth

Résumé

Historique: Il existe encore des preuves contradictoires sur la mesure dans laquelle l’hyperhomocystéine maternel est un facteur de risque de complications de grossesse. Objectifs: L’étude visait à étudier l’impact des concentrations élevées d’homocystéine maternelle sur les résultats défavorables de la grossesse chez les Femmes nigérianes à Lagos. Matériel et Méthodes: Il s’agissait d’une étude de cohorte prospective menée à l’Hôpital universitaire d’enseignement de Lagos, Idi-Araba, Lagos, Nigeria. Les participants ont été inscrits au cours du premier trimestre de la grossesse, après quoi les données pertinentes ont été obtenues par l’entrevue. Des échantillons de sang à jeun ont été prélevés pour la mesure de la concentration maternelle d’homocystéine utilisant le méthode d’essais immunosorbent. Les résultats et les complications de grossesse ont été obtenus en reproduisant le prénatal, l’accouchement, et le médical nouveau-né medical Dossiers. Les naissances prématurées, le faible poids à la naissance (LBW) et la mort fœtale antépartum ont été utilisés comme variables de résultats confirmatoires dans l’analyse finale. Les statistiques descriptives pour toutes les données

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INTRODUCTION

In most developed countries, pregnancies are planned, complications are few, and outcomes are generally favorable for both mother and infant. Adverse outcomes are far more frequent in the developing world.\[^1\] The most severe adverse outcome of pregnancy is the death of the mother and/or her offspring. Even if both the mother and infant survive, pregnancy complications or problems at delivery or during the neonatal period can lead to severe maternal or infant morbidity.\[^2\] Vascular-related pregnancy complications are a major cause of these adverse maternal and fetal outcomes. The origin is thought to be related to early placental development, a process that involves trophoblast invasion and angiogenesis, but that is also dependent on vascular and endothelial function.\[^3\] Placental development in early pregnancy may be negatively influenced by the increased maternal homocysteine concentrations.\[^4\]

Homocysteine is the demethylated product of the essential amino acid methionine. It is metabolized by two main pathways: remethylation to methionine or transsulfuration to cystathionine and then to cysteine.\[^5\] A defect in either of the main pathways leads to an accumulation of homocysteine and this may be due to the congenital or acquired deficiencies of micronutrients, especially the B Vitamins (particularly folic acid).\[^6\] Hyperhomocysteinemia has been linked to fetal malformations and adverse pregnancy outcomes such as abruptio placentae, preeclampsia, neural tube defects, stillbirth, and recurrent miscarriage,\[^7^-^12\] but the direction of causality and the clinical importance of these relationships are uncertain. The effects of homocysteine on pregnancy outcomes have also been linked with other adverse antepartum events such as oligohydramnios and meconium staining of amniotic fluid,\[^5\] fetal growth restriction,\[^13\] impairment of placentafetal transport,\[^14\] and delivery of low-birth-weight (LBW) neonates\[^13, 15\] in the current pregnancy. There is still conflicting evidence on the extent to which elevated maternal homocysteine is a risk factor for pregnancy complications, so prospective, sufficiently powered studies from early pregnancy onward are required to establish this relationship. We, therefore, conducted this prospective cohort study that aimed to examine the impact of elevated maternal homocysteine concentrations in early pregnancy on certain vascular-related pregnancy complications of great clinical importance, such as spontaneous preterm birth, antepartum fetal death, and LBW in term neonates. This may subsequently allow the establishment of a clinical alarm system that will be useful as a marker to identify at-risk pregnant women to mitigate these undesirable pregnancy outcomes.

MATERIALS AND METHODS

Study design and setting

This was a prospective cohort study conducted at the Antenatal Clinics and Labor Ward Complex of the Lagos University Teaching Hospital (LUTH), Ile-Ife, Osun, Nigeria. LUTH has more than 800 beds and is located in the metropolis of Lagos in South-west Nigeria. The hospital provides services to patients from the neighboring states in South-west Nigeria. It is the largest hospital in Lagos State offering training, research, and clinical services, including prenatal, intrapartum, and postnatal care. Participants in the study were healthy pregnant women attending the antenatal clinics of the hospital between July and December 2015. The study was carried out after obtaining approval from the Health Research Ethics Committee of the LUTH, Lagos, Nigeria on May 15, 2015 (Approval number – ADM/DCST/HREC/018). Ethical principles according to the Helsinki Declaration were considered during the course of the research.

Study population and recruitment criteria

Eligible participants at enrolment were consenting women aged 18–45 years and with singleton pregnancy at gestational ages <14 weeks. Women with a history of diabetes or hypertension, HIV, current or previous history of smoking, and other described substance use were excluded from participation in the study.

Sample size determination and sampling techniques

The sample size (N) for the study was determined using the following formula:\[^16\]

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

Using data from a published study by Mascarenhas et al,\[^5\] the standard deviation = 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...
Elevated maternal serum homocysteine was defined as serum homocysteine level >15 μmol/L (reference range: 4–15 μmol/L).\(^7\) The coefficient of variation within and between assays of <5% was being used. Pregnancy outcome and complications were obtained by abstracting the antenatal, delivery, and newborn medical records. The gestational duration was based on the gestation deduced from participants’ last normal menstrual period confirmed or modified by ultrasound.

**Outcome variables of interest and data management**

Preterm births (delivery before 37 completed weeks), LBW (birth weight <2500 g), and antepartum fetal death were used as confirmatory outcome variables in the final analysis. Descriptive statistics for all data were computed using SPSS version 22.0 software (IBM, Armonk, NY, USA). Quantitative data were tested for normality with the Kolmogorov–Smirnov test. The 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 Chi-square 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 pregnancy outcome. \(P < 0.05\) was considered statistically significant.

### RESULTS

Initially, 200 participants with singleton pregnancies at <14 weeks’ gestation were enrolled. However, eight women withdrew their consent during the course of the study for personal or cultural reasons, 22 were lost to follow-up, and three women experienced mid-trimester pregnancy loss. Therefore, the final analysis included 167 women with

![Figure 1: Scatter plot of homocysteine concentrations in the cohorts of participants](image)

### Table 1: Distribution of participants’ baseline characteristics and maternal serum homocysteine levels (n=167)\(^a\)

| Characteristic       | Homocysteine levels | \(P\) |
|----------------------|---------------------|------|
|                      | Normal, \(n\) (%)   | High, \(n\) (%) |
| Age (years)          |                     |      |
| <30                  | 40 (71.4)           | 16 (28.6) | 0.684 |
| 30–34                | 57 (77.0)           | 17 (23.0) |
| >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)  | 0.866 |
| Secondary            | 36 (76.6)           | 11 (23.4) |
| Tertiary             | 87 (74.4)           | 30 (25.6) |
| Parity               |                     |      |
| 0–1                  | 95 (82.6)           | 20 (17.4) | 0.647\(^b\) |
| 2–4                  | 28 (71.8)           | 11 (28.2) |
| >4                   | 3 (100.0)           | 0 (0.0)  |
| Median parity (IQR)  | 2.0 (0.0–4.0)       | 2.0 (0.0–4.0) |
| Mean homocysteine level±SD (μmol/L) | 7.8±2.5 | 36.3±12.1 |
| Total                | 126 (75.4)          | 41 (24.6) |

\(^{a}\)Values are given as mean±SD, median (IQR), or \(n\) (%) unless indicated otherwise, \(^{b}\)Fisher’s exact test. SD=Standard deviation, IQR=Interquartile range
41 (24.6%) of these having a homocysteine concentration above the reference range (>15 µmol/L) and 126 (75.4%) having a normal homocysteine concentration. A scatter plot of serum homocysteine concentrations in the cohorts of study participants is shown in Figure 1. Using the 2.5 and 97.5 percentiles of the control cohort in the study population as the lower and upper reference limits, the reference range of serum homocysteine concentration in this study is 2.1–12.4 µmol/L.

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, 21 (12.6%) had preterm births. The preterm birth rate was seven times higher among women with maternal hyperhomocysteinemia than those with a normal homocysteine concentration (P = 0.001). Among the 146 term deliveries, 17 (11.6%) patients had neonates with LBW, and the rate among women with a high homocysteine concentration is five times higher than among women with a normal homocysteine concentration (P = 0.029). There were 2 (1.2%) intrapartum stillbirths recorded, and the rate did not differ between patients with high or normal homocysteine concentrations [Table 2]. After controlling for age, parity, and level of education, mothers with hyperhomocysteinemia had an approximately twelve-fold higher risk of having preterm births [Table 3] and a ten-fold higher risk of delivering a neonate with LBW at term, compared with women with a normal homocysteine concentration [Table 4]. Maternal age >34 years was also independently associated with an increased risk of preterm birth [Table 3].

**DISCUSSION**

In the present study, the prevalence of maternal hyperhomocysteinemia in early pregnancy was 24.6%, and elevated maternal homocysteine concentration was

| Characteristic | Total (n=167), n (%) | Homocysteine levels | RR (95% CI) | P |
|---------------|---------------------|---------------------|-------------|---|
| Pregnancy duration at birth (weeks) | | | | |
| Preterm (<37) | 21 (12.6) | 6 (4.8) | 15 (36.6) | 7.13 (2.11-13.89) | 0.001 |
| Term (≥37) | 146 (87.4) | 120 (95.2) | 26 (63.4) | 1.00 (reference) |
| Birth weight for term neonates (g) | | | | |
| <2500 | 17 (11.6) | 8 (6.7) | 9 (32.1) | 4.93 (2.07-9.37) | 0.029 |
| ≥2500 | 129 (88.4) | 110 (92.9) | 19 (67.9) | 1.00 (reference) |
| Antepartum fetal death | | | | |
| Yes | 2 (1.2) | 1 (0.8) | 1 (2.4) | 3.07 (0.91-17.12) | 0.118 |
| No | 165 (98.8) | 125 (99.2) | 40 (97.6) | 1.00 (reference) |

Values are given as n (%) unless indicated otherwise, n=146, Fisher’s exact test. CI=Confidence interval, RR=Risk ratio

| Characteristics | Bivariate analyses | Preterm birth | Multivariate analyses |
|-----------------|-------------------|--------------|----------------------|
| Age (years) | | | |
| <30 | 1.00 (reference) | Reference | 1.00 (reference) | Reference |
| 30-34 | 7.16 (4.69-15.52) | 0.039 | 0.94 (0.05-5.10) | 0.111 |
| >34 | 2.08 (1.17-7.25) | 0.006 | 5.12 (2.88-10.27) | 0.034 |
| Parity | | | |
| 0-1 | 1.00 (reference) | Reference | NA |
| 2-4 | 2.33 (0.76-7.71) | 0.651 | NA |
| >4 | 4.44 (1.08-10.22) | 0.037 | NA |
| Level of education | | | |
| Primary | 1.00 (reference) | Reference | 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 (reference) | Reference | 1.00 (reference) | Reference |
| High | 8.17 (3.40-15.68) | 0.027 | 12.23 (5.22-17.74) | 0.001 |

RR=Crude risk ratio, CI=Confidence interval, aRR=Adjusted risk ratio, NA=Not available
Table 4: Bivariate and multivariate analyses of the relationships between baseline characteristics and low‑birth weight in term neonates (n=146)

| Characteristics          | LBW                  |                 | Multivariate analyses |                 |
|--------------------------|----------------------|-----------------|-----------------------|-----------------|
|                          | RR (95% CI)          | P               | aRR (95% CI)          | P               |
| Age (years)              |                      |                 |                       |                 |
| <30                      | 1.00 (reference)     | Reference       | 1.00 (reference)      | Reference       |
| 30-34                    | 4.11 (0.77-8.15)     | 0.172           | 5.94 (0.64-16.18)     | 0.099           |
| >34                      | 2.08 (1.17-7.29)     | 0.006           | 2.12 (1.88-10.27)     | 0.227           |
| Parity                   |                      |                 |                       |                 |
| 0-1                      | 1.00 (reference)     | Reference       | 1.00 (reference)      | Reference       |
| 2-4                      | 2.33 (0.76-7.71)     | 0.235           | 1.99 (0.44-5.21)      | 0.087           |
| >4                       | 7.97 (1.08-10.22)    | 0.014           | 5.32 (0.97-9.65)      | 0.101           |
| Level of education       |                      |                 |                       |                 |
| Primary                  | 1.00 (reference)     | Reference       | 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 (reference)     | Reference       | 1.00 (reference)      | Reference       |
| High                     | 11.63 (4.32-17.84)   | 0.030           | 9.96 (8.87-21.04)     | 0.004           |

RR=Crude risk ratio, CI=Confidence interval, aRR=Adjusted risk ratio, LBW=Low‑birth weight, NA=Not available

significantly associated with preterm birth and LBW among term neonates. These findings could have implications for the future use of early pregnancy maternal serum homocysteine concentration as a marker of adverse delivery outcomes.

The prevalence of serum hyperhomocysteinemia recorded is similar to the rate of 22.2% found by Bergen et al.[38] but much lower than the rate of 50.0% reported by Visternicean in Moldova.[19] This variation may be due to the geographical/racial differences and lower cutoff value (12 µmol/L) for elevated serum homocysteine level chosen by Visternicean,[19] whereas the higher cutoff value for the current study (15 µmol/L) was based on the reference range for normal homocysteine levels proposed by Abbassi-Ghanavati et al.[17] However, the reference values for serum homocysteine recorded in this current study (2.1–12.4 µmol/L) are almost similar to that by Visternicean among Moldovan pregnant women[19] but lower than that reported by Abbassi-Ghanavati et al.[17] among Caucasian women in the United States. This suggests that our study could have reported a higher proportion of women with maternal hyperhomocysteinemia just as reported by Visternicean.[19]

Preterm birth rate recorded in this study was 12.6%, and this was more than double the incidence of 5.0% reported by our team among similar cohorts of participants in the same setting in Lagos.[20] This may be explained by the change in the profile of pregnant women seen in our tertiary hospital setting as more women now embrace institutional deliveries compared to what was obtainable at the time of our previous study in 2013. However, the incidence of preterm birth in this highly selected cohorts was only slightly higher than the rate quoted in the study by Edison et al. in South Carolina, USA (6.6%),[21] but within the range of 5%–25% reported in a study conducted in a regional tertiary hospital in Nigeria by Ezechukwu et al.[22] This was probably due to the similarities in the populations of participants used in all these studies. However, just like our study, Vollset et al.[23] and Kramer et al.[24] reported statistically significant relationships between maternal hyperhomocysteinemia and preterm births. About a tenth (11.6%) of the mothers in our study delivered LBW babies at term, and there was a statistically significant association between elevated maternal serum homocysteine in early pregnancy and delivery of LBW babies. This is similar to the findings from most previous studies[25,13,15] but at variance to the studies by Infante-Rivard et al. in 2003[25] and Dodds et al. in 2008.[26] The variations in the findings of these latter studies compared to the current study and others were largely attributed to the differences in their study designs. Two of the 167 women had an antepartum fetal death, and we reported no significant association between early pregnancy maternal hyperhomocysteinemia and the incidence of antepartum fetal death. This is similar to the findings from other previous studies.[12,27] The low incidence of fetal death reported may reflect the study population which comprised only of healthy, low risk, and predominantly low parous pregnant women. 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 number of folic acid tablets consumed by the mothers before their sample collection and homocysteine assay, 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 booking. It is also important to highlight that the association observed in the present study does not necessarily indicate causality. However, this is the only known study among Sub-Saharan African women that examined the possible effects of a high

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homocysteine concentration on pregnancy outcomes while carefully adjusting for confounding factors. This study thus provides valuable information for future robust longitudinal studies among pregnant women in Nigeria, which could inform future policies on the use of early pregnancy maternal homocysteine concentration as a predictive marker of adverse outcomes in pregnancy.

Conclusions
This study showed a relatively low prevalence of early pregnancy maternal hyperhomocysteinemia among women in Lagos. The significant associations observed between maternal hyperhomocysteinemia and adverse pregnancy outcomes could have implications on the future prevention of these adverse effects through the routine measurements of maternal serum homocysteine concentrations in at-risk pregnant women in early pregnancy. However, further robust and longitudinal research is needed to answer some of the major reservations that remain from the present study such as the overall impacts of the variously identified confounders on these relationships. This may also in the long term suggest the crucial role of nutritional folate fortification before and during pregnancy as the two pathways of homocysteine metabolism require folate (mostly) and Vitamin B6/B12. Therefore, mandatory prophylactic folate suplementations in at-risk women of reproductive age such as those in regions with a high burden of nutritional deficiencies, malaria, and sickle-cell anemia may have merit in the nearest future.

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Conflicts of interest
There are no conflicts of interest.

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