ORIGINAL ARTICLE

Methylenetetrahydrofolate Reductase Enzyme Level and Antioxidant Activity in Women with Gestational Hypertension and Pre-eclampsia in Lagos, Nigeria

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Abstract

Background Deficiencies of enzymes in the folate cycle may lead to the generation of homocysteine, a toxic metabolic intermediate with pro-oxidant effect and ability to induce oxidant stress and lipid peroxidation as part of the pathophysiological process in gestational hypertension (GH) and pre-eclampsia (PE).

Aim The aim of this study is to assess the reliability of plasma homocysteine (hcy) 5, 10 methylenetetrahydrofolate reductase (MTHFR) enzyme and oxidative stress parameters as indicators of aetio-pathogenesis and severity of gestational hypertension and pre-eclampsia.

Subjects and Methods This was a comparative cross-sectional study conducted over 6 months. Two hundred pregnant women were recruited from two sites. They were divided into gestation hypertension ($n = 40$), pre-eclampsia ($n = 60$) and control groups ($n = 100$). Parameters evaluated for statistical analysis were MTHFR enzyme level, plasma homocysteine and malondialdehyde (MDA) levels, with glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities.

Results Mean plasma hcy level and MDA were significantly higher in pre-eclampsia and gestational hypertension when compared to control group ($p < 0.05$). However, MTHFR enzyme level, GSH, SOD and CAT were significantly higher in normotensive females when compared to PE and GH subgroups ($p < 0.05$). Pre-eclampsia was significantly associated with an increased risk of lipid peroxidation (OR = 4.923; $p = 0.007$).

Conclusion Pre-eclampsia and gestational hypertension are associated with marked homocysteine metabolic derangement and increased lipid peroxidation induced by oxidative stress and reduced MTHFR enzyme activity which may be the significant risk factors in the aetio-pathogenesis of GH and PE.

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Introduction

Hypertensive disorders of pregnancy (HDP) are leading causes of maternal and perinatal mortality and morbidity globally and may account for up to 40% of maternal deaths in developing countries [1, 2]. HDP global prevalence is varied [3]. In contrast, the case fatality rate of HDP is largely reduced in high-income countries, due to early detection and effective management of cases [4–6]. Nigeria has one of the highest rates of maternal mortality in the world, and HDP remains a focal point of study as a major cause of maternal and perinatal mortality [7]. Clinically, HDP are characterized by elevated blood pressure with or without proteinuria and a wide range of pathophysiological organ and system disturbances [8–10].

HDP are now considered to arise from a myriad of metabolic, immunologic, genetic and environmental influences [11–17]. The role of oxidative stress in the pathophysiology of PE has increasingly been postulated [18–20]. The placenta continuously generates ROS, and the overproduction of ROS results in accelerated placental ageing. Therefore, enzymatic mechanisms have naturally evolved to control or mitigate effects of ROS through the generation of antioxidants such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) [21].

Available evidence similarly suggests that disorders of homocysteine metabolism may be involved in the pathophysiology of HDP [22–25]. These metabolic processes are regulated by complex array of enzymes and biochemical compounds including folate and vitamin B12 which function as substrates and cofactors. Methylenetetrahydrofolate reductase (MTHFR) is a rate-limiting enzyme in the remethylation of homocysteine. Methylenetetrahydrofolate reductase catalyses the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. In addition to dietary deficiencies of vitamins, mutations in the genes coding for enzymes involved in homocysteine metabolism may alter enzymatic levels or activities and may lead to elevated homocysteine concentrations [26]. Accumulated homocysteine level is a risk factor associated with endothelial dysfunction, a key feature in the pathogenesis of HDP. Homocysteine is readily oxidised to homocysteine thiolactone resulting in the formation of free radicals and lipid peroxidation.

This study investigated the pattern and interactions of MTHFR enzyme, plasma homocysteine, oxidative stress markers (MDA) and antioxidants in the aetio-pathogenesis of gestational hypertension and pre-eclampsia among pregnant Nigerian women.

Materials and Methods

Study Design, Sites and Period

This comparative cross-sectional analytical study was carried out at the Lagos University Teaching Hospital, Idi-Araba, Lagos, and General Hospitals under the Lagos State Health Service Commission after obtaining ethical clearance from the Health Research and Ethics Committee of both institutions. The study was conducted from April 2016 to October 2016.

Study Population

The study population consisted of three groups (I, II and III) of pregnant women aged 18 to 44 years, at a gestational age of 20 weeks and above. Group I included 100 apparently

healthy normotensive pregnant women (control). Group II included 40 participants with clinically diagnosed hypertension during pregnancy without proteinuria (gestational hypertension), while group III included 60 pregnant participants with clinically diagnosed hypertension and proteinuria (Pre-eclampsia) in accordance with the 2014 revised classification by the International Society for the Study of Hypertension in Pregnancy (ISSHP) [10]. After full explanation of the research objectives, informed written consent was obtained from each participant before recruitment into the study. Exclusion criteria included existence of kidney disease, diabetes mellitus, connective tissue disorders, and prior history of thromboembolism, repeated miscarriage, abruption placenta and preterm delivery.

Data and Sample Collection

Interviewer-administered questionnaires were used to obtain information on baseline characteristics, demographic data, nutritional and reproductive history including obstetric history. Medical record of each participant was also reviewed. Blood pressure readings were taken in sitting position with a mercury sphygmomanometer, and hypertension was defined as a blood pressure reading ≥ 140/90 mmHg. Urinalysis was done using the DUS reagent strips for urinalysis by DFI Company Ltd, Republic of Korea. Participants with detectable proteinuria (≥ 2 + on dipstick) were considered significant for pre-eclampsia in addition to elevated blood pressure reading. For each participant with hypertension, an apparently healthy normotensive participant was recruited matched for maternal age, gestational age and parity. From each participant, about 10 ml of venous blood was collected in two separate EDTA bottles for analysis of plasma homocysteine (Hcy), MTHFR enzyme level, oxidative stress parameter (MDA) and specific antioxidants.

Determination of Plasma Homocysteine and MTHFR Enzyme Level

Plasma samples were isolated by centrifugation (2000g for 15 min) and stored at -80 °C until analyses. The plasma from the EDTA sample was used for the estimation of homocysteine, based on enzyme immunoassay technique using the AxisR homocysteine EIA kit, LOT No: 802896074 (Axis-Shield Diagnostics Ltd, Scotland, UK) and final readings with ELX 800TM absorbance microtiter reader (Biotek Ltd, UK serial No: 205808). Detection range for the plasma homocysteine was of 2–50 µmol/l. The MTHFR enzyme level was determined using commercial enzyme-linked immunosorbent assay (ELISA) kits by MyBiosource, Inc. San Diego (USA) with a detection range of 46.88–3000 pg/ml.

Antioxidant Enzymes Assay

Antioxidant enzyme activities were determined spectrophotometrically. Superoxide dismutase activity (SOD) was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Navneet Omprakash Soni [27]. Serum catalase activity (CAT) was determined according to the method of Beers and Sizer as described by Fatai et al. [28]. By measuring the decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide in a H_2O_2 UV recording spectrophotometer. The reduced glutathione (GSH) was estimated according to the method of Sedlak and Lindsay, as described by Sonja et al. [29]. Malondialdehyde (MDA) was determined using the method of Buege and Aust, as described by Malek et al. [30]. Plasma protein was determined using Mindray BS120 auto analyser (Mindray Medical International Limited, Shenzhen, China.

Statistical Analyses

All statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). For clinical data, Pearson χ^2 test and Student t test (or one-way ANOVA) were used for comparing proportions in categorical variables and mean differences for continuous variables, respectively, with confidence level at $p < 0.05$. The Pearson zero-order correlation (r) was used to describe initial unadjusted association between GH/PE and outcome variables, while partial correlation was used to adjust for confounding variable. Multinomial logistic regression model was used to describe the effects and interactions of the dependent variables (gestational hypertension, pre-eclampsia and normotensive pregnancy outcome) on multiple unrelated outcome variables.

Results

Baseline characteristics of study participants are shown in Table 1. There were no statistical significant differences in the maternal age, parity and gestational age of the study participants ($p > 0.05$, respectively). Significant mean difference in BMI was observed among participants ($p = 0.014$). Post hoc analysis (Sidak) showed that BMI of participants with pre-eclampsia (31 ± 4.5 kg/m^2) and GH (30 ± 4.2 kg/m^2) was higher compared with normotensive pregnant controls at 27 ± 4.4 kg/m^2 ($p < 0.05$, respectively), but no significant mean difference was observed in BMI between GH (30 ± 4.2 kg/m^2) and PE participants (31 ± 4.5 kg/m^2; $p > 0.05$).

Table 1 Baseline characteristics of study participants

Variables	Study groups			p value
	Group I	Group II	Group III	
	$n = 100$	$n = 40$	$n = 60$	
	n (%)	n (%)	n (%)	
Age group (years)				
< 30	40 (40.0)	20 (40.0)	30 (50.0)	0.7268**
30–40	51 (51.0)	13 (32.5)	24 (40.0)	
> 40	9 (9.0)	7 (17.5)	6 (10.0)	
Mean maternal age ± SD (years)	30.3 ± 4.7	29.4 ± 3.4	30.5 ± 4.3	0.569*
Parity				
0	7 (7.0)	2 (5.0)	9 (15)	0.227**
1–2	48 (48.0)	18 (45.0)	31 (51.7)	
≥ 2	45 (45.0)	20 (50.0)	20 (33.3)	
Trimester of pregnancy				
Second trimester	15 (15.0)	3 (21.0)	17 (28.0)	0.184**
Third trimester	85 (85.0)	37 (79.0)	43 (72.0)	
Mean gestational age ± SD (weeks)	31.5 ± 5.1	32.8 ± 5.1	30 ± 4.2	0.552*
BMI (kg/m^2)				
Normal	53 (31.0)	8 (20.0)	15 (25.0)	0.002*
Overweight	26 (28.0)	8 (20.0)	18 (30.0)	
Obese	21 (41.0)	24 (60.0)	27 (45.0)	
Mean BMI ± SD (Kg/m^2)	27 ± 4.4	30 ± 4.2	31 ± 4.5	0.014*

BMI body mass index

*Significant p value for ANOVA; **Significant p value for Chi-square statistics

Mean plasma homocysteine level differed significantly across participants ($p = 0.000$) as shown in Table 2. Post hoc analysis shows significantly higher mean plasma homocysteine level for women with pre-eclampsia (21.9 ± 7.9 µmol/l) compared to those with gestational hypertension (17.8 ± 5.9 µmol/l; $p < 0.05$) and also higher mean plasma homocysteine in pre-eclamptic group (21.9 ± 7.9 µmol/l) compared to participants in the control group (11.9 ± 2.4 µmol/l; $p < 0.05$). Participants in the GH group also had significantly higher plasma homocysteine (17.8 ± 5.9 µmol/l) compared with control group (11.9 ± 2.4 µmol/l; $p < 0.05$). Mean MTHFR enzyme level also differs across the different groups ($p = 0.020$); post hoc analysis indicated significantly lower mean MTHFR enzyme level among women with pre-eclampsia in group I (74.1 ± 11.14 pg/ml) compared to control group (100.0 ± 7.94 pg/ml; $p < 0.05$), but there is no significant difference between pre-eclamptic women (74.1 ± 11.14 pg/ml) and women with GH(82.1 ± 15.25 pg/ml; $p > 0.05$). The mean plasma protein level also differed significantly among the study groups ($p = 0.018$). Mean GSH activity progressively declined across the participant groups ($p = 0.021$). Mean GSH enzymes activity in control group (0.519 ± 0.06 µmol/ml/mg protein) was significantly higher compared to levels among participants with PE ($0.370 \pm .036$ µmol/ml/mg protein) and participants with GH ($0.368 \pm .037$ µmol/ml/mg protein) using Post hoc Sidak ($p < 0.05$), but no difference in GSH activities was observed between PE and GH ($p > 0.05$). CAT enzyme activity was significantly different across the three groups ($p = 0.013$). Post hoc evaluation of CAT mean activity only indicated statistical significant difference between normotensive females (7.73 ± 0.332 µmol/ml/mg protein) and females with PE (6.168 ± 0.402 µmol/ml/mg protein; $p < 0.05$). Superoxide dismutase activities significantly varied across the three groups ($p = 0.006$). Post hoc evaluation only indicated significantly lower values of SOD activities in PE (1.767 ± 0.072 µmol/ml/mg protein) and GH (1.666 ± 0.071 µmol/ml/mg protein) participants compared

to control (2.149 ± 0.153 µmol/ml/mg protein; $p < 0.05$). Mean MDA level differs across the three groups of participants, but in a post hoc analysis, significant increase in MDA levels (or lipid peroxidation) was observed for PE participants only (0.034 ± 0.007 µmol/ml/mg protein) when compared to 0.018 ± 0.005 µmol/ml/mg protein among control group participants ($p < 0.05$).

Gestational hypertension was associated with significant reduction in SOD activity as shown in Table 3 ($B = -2.189$; OR $= 0.112$, 95% CI $0.023 - 0.545$; $p = 0.007$). Conversely, catalase and GSH activities were not significantly associated with GH ($p > 0.05$, respectively). Gestational hypertension was not significantly associated with increase in lipid peroxidation (OR $= 5.642$, 95% CI $0.529 - 1.916$; $p = 0.053$). Decreased MTHFR enzyme level was significantly associated with GH compared to control ($B = -0.034$; OR $= 0.966$, 95% CI $0.938 - 0.995$; $p = 0.024$).

Pre-eclampsia was associated with significant reduction in SOD activity ($B = -1.642$; OR $= 0.194$, 95% CI $0.044 - 0.850$; $p = 0.030$). Conversely, no significant association was observed between CAT and GSH activities and

Table 3 Association between GH, PE and outcome variables

Parameters	B	OR	95% CI	p value
GH				
CAT	-1.023	0.977	0.722–1.321	0.879
GSH	-2.459	0.890	0.003–2.236	0.140
SOD	-2.189	0.112	0.023–0.545	0.007*
MDA	5.642	3.184	0.529–1.916	0.053
MTHFR	-0.034	0.966	0.938–0.995	0.024*
PE				
CAT	-0.200	0.819	0.516–1.190	0.879
GSH	-1.796	0.166	0.008–3.409	0.244
SOD	-1.642	0.194	0.044–0.850	0.030*
MDA	8.790	4.923	2.235–10.850	0.007*
MTHFR	-0.040	0.961	0.929–0.994	0.020*

*Significant OR

Table 2 Comparing mean values of variables among subgroup of participants

Variables	PE (Mean ± SD)	GH (Mean ± SD)	Control (Mean ± SD)	p value
Hcy (µmol/L)	21.9 ± 7.9	17.8 ± 5.9	11.9 ± 2.4	0.000*
MTHFR (pg/ml)	74.1 ± 11.14	82.1 ± 15.25	100.0 ± 7.94	0.020*
Plasma protein (g/L)	64 ± 8.8	72 ± 7.9	78 ± 7.6	0.018**
GSH (µmol/ml/mg protein)	$0.370 \pm .036$	$0.368 \pm .037$	0.519 ± 0.06	0.021*
CAT (µmol/ml/mg protein)	6.168 ± 0.402	6.796 ± 0.487	7.735 ± 0.332	0.013*
SOD (µmol/ml/mg protein)	1.767 ± 0.072	1.666 ± 0.071	2.149 ± 0.153	0.006*
MDA (µmol/ml/mg protein)	0.034 ± 0.007	0.024 ± 0.009	0.018 ± 0.005	0.044*

GH gestational hypertension, *PE* pre-eclampsia

*Significant p value for ANOVA; **Significant p value for kruskal wallis statistics

occurrence of PE (OR = 0.819 and 0.166; $p > 0.05$, respectively). Lipid peroxidation in PE significantly increased 4.9-fold above control values ($B = 8.790$; OR = 4.923, 95% CI $2.235 - 10.850$; $p = 0.007$). Decreased MTHFR enzyme level was significantly associated with PH compared to control ($B = -0.040$; OR = 0.961, 95% CI $0.929 - 0.994$; $p = 0.020$).

Plasma homocysteine correlated negatively and significantly with reduced glutathione level and catalase activity (Table 4), but positively with MDA in the PE group ($r = -0.226$; $p = 0.025$; $r = -0.332$; $p = 0.00$; and $r = 0.342$; $p = 0.001$, respectively). No significant correlation was observed between plasma homocysteine levels and SOD in the zero-order Pearson correlation ($r = 0.136$; $p = 0.182$). A negative significant correlation was observed between plasma homocysteine and MTHFR level among PE participants ($r = -0.265$; $p = 0.013$). Partial correlation adjusting for the effect of BMI in PE participants showed loss of the significant correlation between plasma homocysteine and GSH earlier observed ($r = -0.202$; $p = 0.086$). However, significant positive correlation was now observed between plasma homocysteine and SOD ($r = 0.245$; $p = 0.036$). In the GH group, only MDA correlated positively and significantly with plasma homocysteine level ($r = 0.462$; $p = 0.047$); even after adjusting for BMI, the significant positive correlation was maintained ($r = 0.496$; $p = 0.037$).

Discussion

Several reports have shown that homocysteine metabolic derangement and endothelial damage are important in the pathogenesis of hypertensive disorders of pregnancy [18, 19, 21]. Hyperhomocysteinemia caused by altered micronutrients such as folic acid and vitamin B12 is associated with increased production of reactive oxygen species that generate oxidative stress [29].

This study showed significant alterations in homocysteine levels and antioxidant enzyme activities among subjects with PE and GH when compared to healthy controls. These findings are consistent with available evidence that attribute the pathogenesis of HDP to several factors such as oxidative

stress, endothelial damage and altered homocysteine metabolism [18, 21]. Significant elevation in mean plasma homocysteine observed in women with PE and GH in this study is a consistent finding in the literature [18, 19, 21]. Of note was the progressive reduction in plasma MTHFR enzyme level observed with severity of HDP among participants and the observed significant association between rising plasma homocysteine and lower levels of plasma MTHFR among participants. The studies have reported reduced (qualitative) MTHFR enzyme activity associated with MTHFR C677T mutations in some populations with pre-eclampsia [31], but data on quantitative 5-methylenetetrahydrofolate reductase enzyme deficiency and possible association with HDP are rare. Mutations or anomalies resulting in MTHFR enzyme level reduction might significantly disrupt enzymatic conversion of 5, 10 methylenetetrahydrofolate to 5, methyltetrahydrofolate, an important substrate for the 1 carbon methylation of homocysteine to methionine. This may result in the systemic accumulation of homocysteine, a condition which might further get worse in the presence of micronutrient deficiency. Under physiological conditions, levels of maternal serum homocysteine normally decrease with gestation. This may either be due to increase in utilization by foetus, a physiological response to pregnancy, or decrease in albumin. This physiological decrease in homocysteine may even be from increased plasma volume and increased demand for methionine by both the mother and foetus [20]. When added to plasma, homocysteine is readily oxidized, leading to the formation of oxygen radicals and lipid peroxidation [32]. Malondialdehyde (MDA), a metabolite of lipid peroxides, is then detectable in plasma and can be used as an indicator of lipid peroxidation [33].

Findings from this study indicated a significant decrease in mean antioxidant activities. PE was associated with significant decrease in SOD activity and significant lipid peroxidation. These variations may be associated with the degree of disease severity, where a delicate balance between antioxidant enzyme consumption (as a result of increasing production of ROS) and compensatory increase in antioxidant level is eventually disrupted especially with the onset of lipid peroxidation and organ damage. Reports in the

Table 4 Variables and correlations with PE and GH

Variables	PE		GH	
	Unadjusted	Adjusted for BMI	Unadjusted	Adjusted for BMI
	r (p value)	r (p value)	r (p value)	r (p value)
GSH	− 0.226 (0.025)	− 0.202 (0.086)	− 0.049 (0.844)	− 0.055 (0.830)
SOD	0.136 (0.182)	0.245 (0.036)	− 0.147 (0.548)	− 0.117 (0.645)
CAT	− 0.332 (0.001)	− 0.265 (0.023)	− 0.171 (0.484)	− 0.208 (0.487)
MDA	0.342 (0.001)	0.322 (0.030)	0.462 (0.047)	0.496 (0.037)
MTHFR	− 0.265 (0.013)	− 0.242 (0.037)	− 0.205 (0.399)	− 0.235 (0.348)

literature have documented varying pattern of antioxidants in HDP [32, 34, 35].

Rising plasma homocysteine was significantly correlated with increased lipid peroxidation in both PE and GH. It is documented that elevation of plasma homocysteine causes an increased formation of hydrogen peroxide which will eventually lead to decreased activity of the principal antioxidant enzymes which includes glutathione peroxidase, superoxide dismutase and catalase thus promoting the generation of oxidative stress [36]. Reports of association between elevated Hcy and SOD have similarly been documented in subjects with cardiovascular disorders [37]. However, some authors have stated that in the acute phase, elevated homocysteine concentration may actually cause the release of heparan sulphate-bound extracellular SOD into the blood and thus constitute a protective mechanism with the effect of combating oxidative stress [36]. The correlation between plasma homocysteine and antioxidant activity in PE and GH subjects observed in this study was strongly confounded by BMI. In this study, BMI was observed to be significantly higher among participants with GH and PE compared to normotensive pregnant females in the control group. However, the initial observed significant correlation between Hcy and GSH in the PE group was lost after adjustment for BMI. In contrast, the correlation between Hcy and SOD increased significantly on controlling for BMI.

These findings point to the fact that other factors including nutritional and environmental, might play significant role in Hcy metabolism and oxidative stress in pre-eclampsia. Both PE and GH were associated with significant increase in mean MDA level and a significant 4.9-fold increase in the risk of lipid peroxidation in women with pre-eclampsia compared to normotensive pregnant females. This significant increase in lipid peroxidation activity has been similarly reported in a previous study [35]. Lipid peroxidation activity positively correlated with elevated Hcy in both GH and PE. These findings point to the fact that derangement in homocysteine metabolism and lipid peroxidation are early events and key factors in the pathogenesis of pre-eclampsia. The observed correlation between plasma homocysteine and MTHFR level indicates possible role for MTHFR deficiency in the pathogenesis of pre-eclampsia or in disease progression.

Conclusion

Plasma homocysteine and MTHFR enzyme levels play significant role in the aetio-pathogenesis of GH and PE; elevated values in pregnancy may be possible indicator for HDP. Moreover, oxidative stress parameters may be associated with disease severity and progression.

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About the Author

Dr. V. O. Osunkalu is a pathologist with haematology as subspecialty and focuses on nutritional anaemia and haematological genetics. He had his undergraduate degree in medicine and surgery (MB.ChB) from the Obafemi Awolowo University, Ile-Ife, in 1991 and had a master's degree in public health from the University of Lagos, Nigeria and a master's degree in cell biology and genetics from the University of Lagos. He also holds the fellowship degree of the National Postgraduate Medical College of Nigeria (FMCPath) in 2005. Furthermore, he holds the membership of the West Africa Postgraduate Medical College in general pathology (FMCP) in 2006. He is currently doing a Ph.D. in genetics domiciled in the department of cell biology and genetics, University of Lagos. Over the years, he has been involved in the teaching of haematology at the 300 level in the College of Medicine, Idi-araba, and teaching of haematology and blood transfusion and supervision of research projects for the postgraduate students. He has conducted several research in the area of nutritional anaemia especially in folate metabolic disorders/hyperhomocysteinemia and molecular aspects of folate metabolism in pregnancy. He has mentored over 30 students in areas of research methodology, statistical analysis and proposal writing.

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