Changes in Serum Homocysteine Concentrations and their Prognostic Value in Pregnant Chinese Women with Preeclampsia : A Retrospective Study

Wanyuan Qin

People's Hospital of Guangxi Zhuang Autonomous Region

Xuehua Hu

Maternal and child health of Guangxi zhuang autonomous region

Chunyun Fu

Maternal and child health of Guangxi zhuang autonomous region

Yuqun Tang

Maternal and child health of Guangxi zhuang autonomous region

Xiangjun Lu

Maternal and Child Health of Guangxi zhuang autonomous region

Zuoxin Deng

Maternal and Child health of Guangxi zhuang autonomous region

Qiang Huang

Maternal and child health of Guangxi zhuang autonomous region

Xiaofen Wei

Maternal and child health of Guangxi zhuang autonomous region

Yan Chen

Maternal and Child health of Guangxi zhuang autonomous region

Junlong Wang

Maternal and child health of Guangxi zhuang autonomous region

Kangkai Huang

Maternal and child health of Guangxi zhuang autonomous region

Junpeng Jing (✉ jingjun.peng@163.com)

Maternal and Child Health of Guangxi Zhuang Autonomous Region <https://orcid.org/0000-0003-3675-0623>

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Abstract

Background: This study evaluated whether changes in homocysteine concentrations in pregnant women with preeclampsia might be useful for predicting pregnancy-related outcomes.

Methods: This retrospective study evaluated 2,745 pregnant Chinese women (1,368 women with preeclampsia and 1377 healthy pregnant controls) at two Chinese centres. Medical records were reviewed to collect data regarding maternal age, homocysteine concentrations, gestational weight gain, body mass index (BMI), neonatal weight, Apgar score, previous abortions, previous deliveries, adverse pregnancy history, and pregnancy-related outcomes.

Results: Maternal serum homocysteine concentrations were significantly higher in the preeclampsia group (vs. the control group) during the second trimester ($P<0.0001$) and the third trimester ($P<0.0001$). Foetal death was also significantly more common in the preeclampsia group. Significant differences ($P<0.05$) were also observed between the preeclampsia and control groups in terms of maternal age, gestational weight gain, BMI, neonatal weight, Apgar score, previous abortions, previous deliveries, gestation length, adverse pregnancy history, and pregnancy outcomes. Women with preeclampsia had elevated homocysteine concentrations that increased from the first trimester until the third trimester. Among women with preeclampsia, the risk of foetal death was significantly associated with maternal age, BMI, neonatal weight, Apgar score, and serum homocysteine concentrations during the second trimester ($P=0.022$) and the third trimester ($P=0.034$).

Conclusion: Our results indicate that foetal death in women with preeclampsia was associated with maternal age, BMI, neonatal weight, and Apgar score. Furthermore, foetal death was associated with high maternal homocysteine concentrations during the second and third trimesters.

Background

Preeclampsia (PE) is defined as the onset of hypertension and proteinuria (> 300 mg/day) after 20 weeks of gestation, which changes the placental microenvironment and releases a series of maternal circulation factors, this serious pregnancy-specific syndrome affects multiple organs, is detected in 5–8% of pregnancies worldwide, and there are no effective tools for the treatment of preeclampsia unless terminating pregnancy [1]. Although the pathogenesis of preeclampsia remains unclear, plausible theories include endothelial dysfunction, inflammation, and angiogenesis [2, 3], there is emerging evidence that women with preeclampsia have higher homocysteine concentrations than healthy pregnant women [4].

The vascular endothelium can be harmed by elevated concentrations of homocysteine [5], which is a non-protein amino acid that is formed after cleavage of the terminal methyl group from methionine [6, 7]. Furthermore, high level of homocysteine in early pregnancy was a risk factor for pregnancy loss, abruptio placenta, stillbirth, and miscarriage [8], and homocysteine concentrations may be useful when screening for preeclampsia [9], although its prognostic value remains unclear. Therefore, this retrospective study aimed to determine whether inter-trimester changes in homocysteine concentrations could be detected in Chinese women with preeclampsia, and whether those concentrations were associated with foetal death.

Methods

Patients

This retrospective study evaluated 2,745 pregnant Chinese women who underwent examinations between January 2016 and December 2019 at the Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region and the People's Hospital of Guangxi Zhuang Autonomous Region. Serum [homocysteine](#) concentrations had been determined via regular blood testing during all trimesters for all patients, who included 1,368 patients with preeclampsia (mean age: 32.610 ± 5.252 years) and 1,377 healthy pregnant women (mean age: 30.790 ± 4.682 years). The present study defined preeclampsia based on the International Society for the Study of Hypertension in Pregnancy criteria: new onset of hypertension and proteinuria after the 20th gestational week or new onset of hypertension without proteinuria but with haematological complications, renal insufficiency, impaired liver function, neurological symptoms, or uteroplacental dysfunction. Proteinuria was measured using a dipstick test and blood pressure was measured using a mercury sphygmomanometer. Hypertension was defined as maternal systolic blood pressure (SBP) of ≥ 140 mmHg and/or diastolic blood pressure (DBP) of ≥ 90 mmHg measured on two occasions separated by ≥ 6 h. None of the women had pre-existing hypertension, diabetes, or cardiovascular disease. The first trimester was defined as <12 weeks of pregnancy, the second trimester was defined as 12–27 weeks of pregnancy, and the third trimester was defined as ≥ 28 weeks of pregnancy. The study's retrospective protocol was approved by the ethics committee at the Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, Informed consent obtained from study participants was written, and all procedures complied with the Declaration of Helsinki.

Blood samples and tests

Fasting venous blood samples were collected using separating gel coagulation-promoting tubes (Becton, Dickinson and Company), which were centrifuged at 3,000 rpm for 5 min. The tubes were stored vertically and the serum was generally removed within 60 min. If the serum could not be removed within 60 min, the tubes were stored vertically in a freezer at -20°C until use. The [homocysteine](#) concentrations were determined using an enzymatic cycling method and the AU2700 system (Beckman Coulter, USA).

Anthropometric characteristics

The women's body weight and height were measured using standard techniques, and body mass index (BMI) was calculated as kg/m^2 . Standardized questionnaires had been used to collect routine clinical data regarding previous abortions, previous deliveries, adverse pregnancy history, and gestational weight gain. Data regarding the Apgar score at 5 min and neonatal weight were collected by clinicians at the patient's bedside.

Statistical analysis

Results were reported as number (%) or mean $\pm$ standard deviation, with comparisons performed using the chi-squared test, independent samples t test, or Mann-Whitney U test, as appropriate. Changes in homocysteine concentrations between the trimesters were evaluated using the paired t test. Receiver operating characteristic (ROC) curves were constructed to evaluate the ability of homocysteine concentrations to predict the foetal and neonatal outcomes. Multivariable analyses with the Cox proportional hazards model were used to estimate relationships with survival outcomes. All statistical analyses were performed using SPSS software (version 13.0; IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at two-sided *P*-values of <0.05 .

Results

Biochemical and clinical characteristics

The biochemical and clinical characteristics of the control group and the preeclampsia group are shown in Table 1. Relative to the control group, the preeclampsia group had higher serum homocysteine concentrations during the second and third trimesters, although no significant difference was observed in the first trimester concentrations. Furthermore, significant inter-group differences were observed in terms of maternal age, BMI, previous abortions, previous deliveries, gestational weight gain, neonatal weight, and gestation length. The preeclampsia group also had significantly higher rates of adverse pregnancy history and foetal death.

Table 1
Comparing the clinical characteristics of the control group and the group with preeclampsia

	Preeclampsia N = 1,368	Control N = 1,377	t/χ^2	P
Age (years)	32.61 $\pm$ 5.25	30.79 $\pm$ 4.68	9.590	< 0.0001
Homocysteine				
First trimester ($\mu\text{mol/L}$)	7.12 $\pm$ 2.08	7.17 $\pm$ 1.47	7.670	0.443
Second trimester ($\mu\text{mol/L}$)	7.85 $\pm$ 2.70	7.10 $\pm$ 1.58	8.914	< 0.0001
Third trimester ($\mu\text{mol/L}$)	9.90 $\pm$ 3.69	8.00 $\pm$ 1.98	16.768	< 0.0001
Gestational weight gain (kg)	12.43 $\pm$ 5.03	13.04 $\pm$ 3.77	3.592	< 0.0001
Body mass index (kg/m^2)	28.36 $\pm$ 3.64	26.22 $\pm$ 3.09	11.685	< 0.0001
Neonatal weight (g)	2083.04 $\pm$ 868.41	3163.75 $\pm$ 362.84	42.491	< 0.0001
Apgar score (5 min)	8.98 $\pm$ 2.80	9.98 $\pm$ 0.29	13.188	< 0.0001
Previous abortions	1.02 $\pm$ 1.14	0.75 $\pm$ 0.97	16.680	< 0.0001
Previous deliveries	0.55 $\pm$ 0.62	0.62 $\pm$ 0.64	3.055	< 0.0001
Gestation length (weeks)	35.59 $\pm$ 4.14	39.21 $\pm$ 1.11	31.287	0.002
Adverse pregnancy history (n)	122 (8.92%)	36 (2.61%)	50.270	< 0.0001
Foetal death (n)	124 (9.06%)	1 (0.0001%)	127.654	< 0.0001
Data are shown as mean $\pm$ standard deviation or number (percentage).				

Changes in homocysteine concentrations and relationships with foetal death

Women in the control group had significantly lower homocysteine concentrations during the second trimester, relative to during the first or third trimesters ($P < 0.05$, Fig. 1A). Women with preeclampsia had increasing homocysteine concentrations throughout their pregnancy (Fig. 1B), as well as significantly higher **homocysteine** concentrations than the control group ($P < 0.001$, Fig. 1C).

During every trimester, women who experienced foetal death had significantly higher homocysteine concentrations than women who did not experience foetal death ($P < 0.05$, Fig. 2A). The receiver operating characteristic curve results revealed that homocysteine concentrations might predict the risk of foetal death ($P < 0.05$, Fig. 2B–D). High area under the curve values were observed in all trimesters, although the largest area was observed for the second trimester (0.7212), where a homocysteine concentration of $> 8.095 \mu\text{mol/L}$ provided 72.58% sensitivity and 65.59% specificity for predicting foetal death (Fig. 2C).

Factors that predicted foetal death in preeclampsia

The Cox proportional hazards model was used to analyse homocysteine concentrations and other factors (Table 2). The results revealed that foetal death was significantly associated with high homocysteine concentrations during the second trimester (OR = 1.813, 95% CI: 1.088, 3.021; $P = 0.022$), high homocysteine concentrations during the third trimester (OR = 1.784, 95% CI: 1.046, 3.045; $P = 0.034$), age ($P < 0.0001$), BMI ($P < 0.05$), neonatal weight ($P < 0.0001$), and Apgar score ($P < 0.0001$). However, foetal death was not significantly associated with gestational weight gain, homocysteine concentration during the first trimester, previous abortions, previous deliveries, or adverse pregnancy history (Table 2). Survival curves were created using the Cox proportional hazards model, which revealed increased risks of foetal death at homocysteine concentrations of $> 8.095 \mu\text{mol/L}$ during the second trimester (Fig. 3A, C) and $> 9.515 \mu\text{mol/L}$ during the third trimester (Fig. 3B, D).

Table 2

Cox proportional hazard regression models for foetal death during the different trimesters according to elevated serum homocysteine concentrations

	1st trimester ($> 6.985 \mu\text{mol/L}$)			2nd trimester ($> 8.095 \mu\text{mol/L}$)			3rd trimester ($> 9.515 \mu\text{mol/L}$)		
	OR	P	(95% CI)	OR	P	(95% CI)	OR	P	(95% CI)
Age	1.087	0.003	1.029–1.148	1.077	0.008	1.019–1.137	1.078	0.006	1.022–1.138
Homocysteine	1.072	0.753	0.697–1.648	1.813	0.022	1.088–3.021	1.784	0.034	1.046–3.045
Gestational weight gain	1.05	0.063	0.997–1.106	1.037	0.192	0.982–1.096	1.025	0.401	0.967–1.086
Body mass index	1.089	0.004	1.027–1.154	1.105	0.001	1.042–1.172	1.091	0.002	1.035–1.163
Neonatal weight	0.998	< 0.0001	0.997–0.998	0.998	< 0.0001	0.997–0.998	0.998	< 0.0001	0.997–0.998
Apgar score (5 min)	0.581	< 0.0001	0.527–0.639	0.592	< 0.0001	0.538–0.652	0.587	< 0.0001	0.534–0.646
Previous abortion	1.051	0.567	0.887–1.244	1.041	0.641	0.879–1.232	1.058	0.504	0.896–1.25
Previous delivery	0.801	0.221	0.562–1.143	1.041	0.293	0.575–1.181	0.792	0.196	0.556–1.128
Adverse pregnancy history	1.249	0.462	0.691–2.256	1.614	0.119	0.884–2.947	1.784	0.096	0.909–3.219

Discussion

This study of 2,475 pregnant Chinese women aimed to examine whether homocysteine concentrations could predict foetal death. Relative to the control group, the group with preeclampsia had elevated serum homocysteine concentrations during the second and third trimesters. Furthermore, the group with preeclampsia was older, had higher BMI values, and were more likely to have had previous abortions and deliveries, with lower values for neonatal weight, gestation length, and gestational weight gain. Moreover, the women with preeclampsia had higher rates of adverse pregnancy history and foetal death, which agrees with the higher foetal mortality rate reported by Ahmad et al. among patients with preeclampsia [10].

Homocysteine is a naturally occurring amino acid and its metabolism disorder was associated with increased risks of vascular disease. Hyperhomocysteinemia results in endothelial damage, it increases the oxidative stress related to preeclampsia [11]. Previous studies have examined whether hyperhomocysteinemia in pregnancy is related to adverse outcomes, such as small size for gestational age at birth, preeclampsia, recurrent abortions, low birth weight, and intrauterine growth restriction [4, 12]. Among our patients with preeclampsia, homocysteine concentrations gradually and significantly increased throughout the pregnancy, while among the control patients the serum homocysteine concentrations decreased during the second trimester and slightly recovered during the third trimester. These results are consistent with previously reported findings in Spain and India populations [13, 14], another study of central south China revealed that the homocysteine concentration in normal pregnant women was only significantly elevated during the third trimester (vs. during the first and second trimester) [15], but the reference intervals of homocysteine for pregnant women were inconsistent with our study. Serum homocysteine concentrations reportedly decrease during normal pregnancy, which might be related to a normally increased glomerular filtration rate and associated haemodilution, and it was possible decreases in homocysteine concentration during pregnancy were mainly endocrine-based [16]. So, the significant increasing of homocysteine concentrations throughout the pregnancy in patients with preeclampsia reflects a disturbance in homocysteine metabolism.

Hyperhomocysteinemia during pregnancy may play a significant role in the pathogenesis of preeclampsia, as an increase in homocysteine concentration significantly increased the odds of any placenta-mediated complication, which may lead to adverse outcomes [17]. Hyperhomocysteinemia also increases the oxidative stress and collagen accumulation that leads to vascular fibrosis, and results in endothelial damage [12]. A study of Turkey revealed homocysteine level in the control group was significantly lower than in the severe and mild preeclampsia groups, respectively, and homocysteine levels were not correlated with disease severity [6], but another study in Shanghai showed homocysteine in severe preeclampsia were significantly higher than those in the median [18]. Furthermore, homocysteine is a significant biomarker for overall health status. Nevertheless, it remains unclear whether increased homocysteine concentrations are a pathological factor or merely an indicator of disease, and how this specifically contributes to the increased risk of foetal death. Nevertheless, one study revealed that women in the top decile of serum homocysteine concentrations (based on the gestational age-specific distribution) had an increased risk of foetal death, although high homocysteine concentrations did not appear to be suitable for predicting preeclampsia later in pregnancy or subsequent foetal death [17], another study also revealed that elevated homocysteine concentrations were significantly associated with pregnancy loss [19]. We observed that elevated homocysteine concentrations were a risk factor for foetal death in women with preeclampsia, although our

results also suggest that homocysteine concentrations influence the foetal prognosis during the second and third trimesters. For examine, among the women with preeclampsia, we observed that foetal death was associated with homocysteine concentrations of $> 8.095 \mu\text{mol/L}$ during the second trimester and $> 9.515 \mu\text{mol/L}$ during the third trimester.

The Cox proportional hazards model indicated that foetal death was also associated with maternal age, BMI, neonatal weight and Apgar score. A previous report has also indicated that older maternal age is associated with morbidity in the child [20]. Maternal weight is also an important predictor of pregnancy outcomes, as pregnant women must accumulate subcutaneous fat to ensure that their foetus can grow and develop [21]. However, we found that a high BMI was a significant risk factor for foetal death among women with preeclampsia, the excess bodyweight increased the risk of foetal death in preeclampsia patients. Neonatal weight and Apgar score are standardized tools for predicting foetal outcomes [22], and our findings also confirm that they may help predict the risk of foetal death among women with preeclampsia.

The present study is limited by a lack of data regarding maternal lifestyle factors, we were unable to collect data VB12 and folic acid supplement use. These factors might influence homocysteine concentrations and should be considered in future studies regarding the relationship between homocysteine concentrations and poor outcomes in cases of preeclampsia.

Conclusion

In conclusion, the present study confirmed that foetal death was associated with maternal age, BMI, neonatal weight, and Apgar score, which agree with previously reported findings. Furthermore, among Chinese women with preeclampsia, elevated homocysteine concentrations during the second and third trimesters were significantly associated with the risk of foetal death.

Abbreviations

Body mass index: BMI, Receiver operating characteristic: ROC, Vitamin B12: VB12.

Declarations

Ethics approval and consent to participate:

The study's retrospective protocol was submitted to and approved by the ethics committee of the Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region.

Consent to publish:

Not applicable.

Availability of data and materials:

All relevant data are included in the report and its associated files.

Competing interests:

None.

Funding:

Not applicable.

Authors' contributions:

JJP and QWY contributed to study design, data analysis, manuscript drafting, and critical discussion. TYQ, LXJ, DZX, WJL, CHY, WXF, HQ and HKK contributed to data collection, HXH and FCHY contributed to data interpretation, manuscript drafting, and critical discussion. All authors were involved in drafting the work or revising it critically.

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Figures

Figure 1

Changes in homocysteine concentrations in pregnant women. The paired t test was used to compare homocysteine changes between the control group (A) and the group with preeclampsia (PE) (B). The mean $\pm$ standard deviation values for homocysteine concentration in pregnant women (C).

Figure 2

Homocysteine concentrations according to foetal survival status among pregnant women with preeclampsia (PE) (A). Receiver operating characteristic curve analysis was used to identify homocysteine cut-off values during the first trimester (B), second trimester (C), and third trimester (D).

Figure 3

Survival curves created using a Cox proportional hazards model according to homocysteine concentrations of >8.095 $\mu\text{mol/L}$ during the second trimester (A, C) and >9.515 $\mu\text{mol/L}$ during the third trimester (B, D).