

## RESEARCH ARTICLE

# Estimation of homocysteine concentration as an indicator of foetal death in pregnant Chinese women with preeclampsia: A case-control study

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## Funding information

Health Commission of Guangxi Zhuang Autonomous Region, Grant/Award Number: Z20200744 and Z20210411

## Abstract

**Introduction:** This study evaluated whether changes in homocysteine concentrations in pregnant women with preeclampsia (PE) might be useful for predicting foetal death.

**Materials and methods:** This study evaluated 1,368 PE women at two Chinese centres. Medical records were reviewed to collect data regarding maternal age, homocysteine concentrations and other clinical parameters.

**Results:** Maternal serum homocysteine concentrations were significantly higher in the group with PE than control. Significant differences ( $p < 0.05$ ) were also observed between the foetal death and survival groups in terms of body mass index, neonatal weight, previous deliveries, gestation length and adverse pregnancy history. Multivariate logistic regression analysis revealed that upper-quartile homocysteine concentration was a significant risk factor of foetal death in the group with PE, and overall survival rate of patients with high homocysteine concentrations during pregnancy was significantly lower than those with low level ( $p < 0.05$ ).

**Conclusions:** Our results indicate that foetal death was associated with upper-quartile homocysteine concentrations in the group with PE, it can be an indicator of foetal death throughout the pregnancy.

## KEYWORDS

foetal death, homocysteine, preeclampsia, pregnant, prognostication

## 1 | INTRODUCTION

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 PE unless terminating pregnancy.<sup>1</sup> Although the pathogenesis of PE remains unclear,

plausible theories include endothelial dysfunction, inflammation and angiogenesis.<sup>2,3</sup> There is emerging evidence that women with PE have higher homocysteine concentrations than healthy pregnant women.<sup>4</sup> High level of homocysteine in early pregnancy was a risk factor for pregnancy loss, placental abruption, stillbirth and miscarriage,<sup>5</sup> and homocysteine concentrations may be useful when screening for PE,<sup>6</sup> although its prognostic value remains unclear.

Most studies were analysed either in pregnancy or at delivery, we need to know the progressive gestation changes of homocysteine

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level so as to interpret of these differences were a cause of foetal death in PE patients. Therefore, this case-control study aimed to determine whether inter-trimester changes in homocysteine concentrations could be detected in Chinese women with PE, and evaluate whether or not they present a danger of foetal death.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

This case-control study evaluated 1,497 pregnant Chinese women with PE who underwent physical examination, peripheral blood examination and urine protein 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, the present study defined PE based on the International Society for the Study of Hypertension in Pregnancy criteria: new onset of hypertension and proteinuria after the 20th gestational week in a woman with previously normal blood pressure and proteinuria, none of the women had pre-existing hypertension, diabetes, or cardiovascular disease. 95 patients were excluded because chronic disease or other reasons during the pregnancy, from 1,402 included patients, 34 patients were lost to follow up during pregnancy because of being transferred or out of touch. A total of 1368 PE patients completed the study (mean age:  $32.610 \pm 5.252$  years), and 38 PE women had the abortion in the second trimester because of stillbirth, the structure of this study was demonstrated in the flow chart (Figure 1), 1377 randomized healthy pregnant women were selected as control group.

### 2.2 | Methods

Serum homocysteine concentrations had been determined via regular blood testing during all trimesters for all patients. Proteinuria was measured using a dipstick test and blood pressure was measured using a mercury sphygmomanometer. Hypertension was defined as maternal systolic blood pressure of  $\geq 140$  mmHg and/or diastolic

blood pressure of  $\geq 90$  mmHg measured on two occasions separated by  $\geq 6$  h. 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  $\geq 28$  weeks of pregnancy. The study's case-control protocol was approved by the ethics committee at the Maternal and Child Health Hospital of Guangxi Zhuang Autonomous Region, and the study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

### 2.3 | Blood samples and tests

Fasting venous blood samples were collected using separating gel coagulation-promoting tubes (Becton, Dickinson and Company) in different trimesters, confirm foetal survival was carried out prior to the blood draw by Doppler ultrasound, which were centrifuged at 1400 g for 5 min. The tubes were stored vertically and the serum was generally separated within 60 min. The homocysteine concentrations were determined using an enzymatic cycling method and the AU2700 system (Beckman Coulter).

### 2.4 | Anthropometric characteristics

The women's body weight and height were measured using standard techniques, and body mass index (BMI) was calculated as  $\text{kg}/\text{m}^2$ . Standardized questionnaires had been used to collect routine clinical data regarding previous abortions, previous deliveries and adverse pregnancy history. Data regarding the Apgar score at 5 min and neonatal weight were collected by clinicians at the patient's bedside, the cut-off values for quartile distribution of homocysteine concentrations ( $\mu\text{mol}/\text{L}$ ) in the study were determined by SPSS in with pregnant women with PE, first trimester: I  $< 5.76$ , II 5.76–6.75, III 6.76–8.21, IV  $> 8.21$ , second trimester: I  $< 5.93$ , II 5.93–7.27, III 7.28–9.03, IV  $> 9.03$  and the third trimester: I  $< 7.12$ , II 7.12–8.88, III 8.89–12.00, IV  $> 12.00$ .

### 2.5 | Statistical analysis

Results were reported as number (%) or mean  $\pm$  standard deviation, data not normally distributed were shown as median (25th–75th percentiles), 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.

The associations of foetal death with each of the parameters were evaluated by logistic regression analysis, they were expressed as the odds ratio (OR) and its 95% confidence interval (95% CI), homocysteine concentrations were log-transformed for statistical analysis. Receiver operating characteristic (ROC) curves were constructed to evaluate the ability of homocysteine concentrations to predict the foetal and neonatal outcomes. Kaplan-Meier analysis

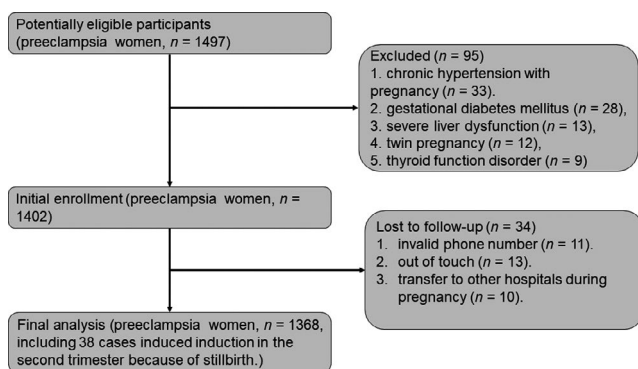


FIGURE 1 Patient disposition throughout the study

was used to assess the prognostic value of homocysteine concentration during every trimester. All statistical analyses were performed using SPSS software (version 13.0; IBM Corp.). Differences were considered statistically significant at two-sided  $p$  values of  $< 0.05$ .

### 3 | RESULTS

#### 3.1 | Biochemical and clinical characteristics

Biochemical and clinical characteristics of PE patients and control groups appear in Table 1. Compared with control, PE patients were characterized by increased serum concentration of homocysteine in second and third trimester. Higher value of age, BMI, abortion times and delivery times, decreased level of neonatal weight, Apgar score and gestation length. Moreover, Pregnant women with PE had significant higher rate of the adverse pregnancy history. No significant difference was observed in homocysteine concentration in the first trimester.

#### 3.2 | Progressive gestation changes of homocysteine level

Homocysteine concentrations were markedly elevated until at the third trimester in the control group (Figure 2A), both foetal death and survival group had increasing homocysteine concentrations throughout their pregnancy in PE women ( $p < 0.05$ , Figure 2B,C). The ROC curve results revealed that homocysteine concentration was an indicator of foetal death ( $p < 0.05$ , Figure 2C–E), high area under the curve values were observed in all trimesters.

#### 3.3 | Changes in homocysteine concentrations in PE women

The biochemical and clinical characteristics of the foetal death group and the survival group are shown in Table 2. During every trimester, women who experienced foetal death had significantly higher homocysteine concentrations than women who did not experience foetal death ( $p < 0.05$ ). Furthermore, significant inter-group differences were observed in terms of BMI, neonatal weight, previous deliveries, gestation length and adverse pregnancy history ( $p < 0.05$ , Table 2).

#### 3.4 | Factors that predicted foetal death in PE

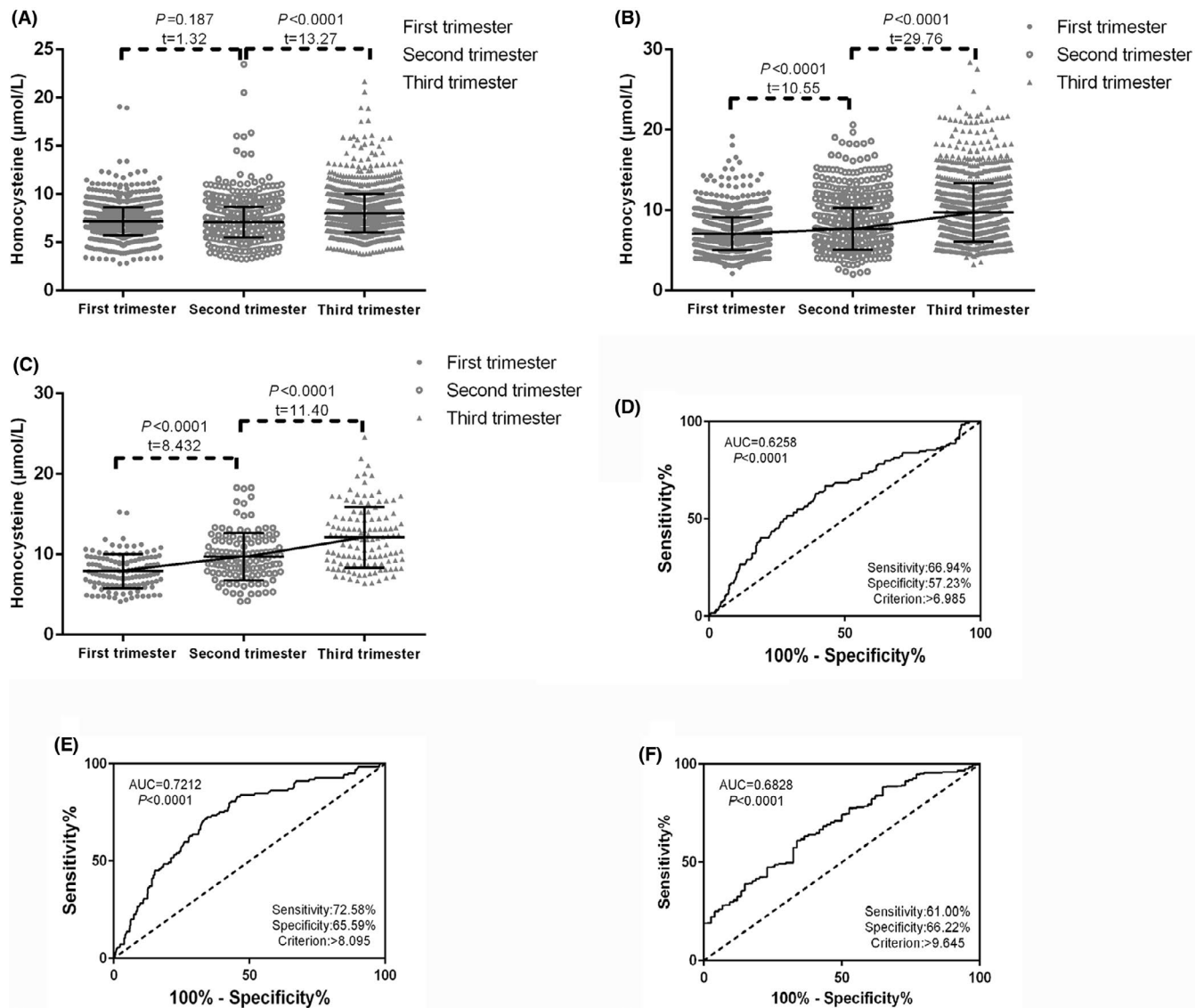
The incidence of foetal death increased evidently by quartiles of homocysteine during all the trimesters, from 5.71% quartile I to 14.97% quartile IV in the first trimester, from 3.01% quartile I to 17.14% quartile IV in the second trimester and from 1.04% quartile I to 9.24% quartile IV in the third trimester (Figure 3A,B). The Kaplan–Meier survival curve showed that the overall survival rate of patients with high homocysteine concentrations during pregnancy was significantly lower than those with low level, survival time was calculated from the date of pregnancy until delivery or death (Figure 3C–E). Logistic regression, as appropriate, was used to investigate associations between the posterior probability of foetal death and risk factors, after adjusting of BMI, previous deliveries and adverse pregnancy history, multivariate logistic regression analysis revealed that upper-quartile homocysteine concentration was a significant risk factor of foetal death in the group with PE (Table 3).

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

Variables	Preeclampsia	Control	$t/\chi^2$	$p$
	$N = 1,368$	$N = 1,377$		
Age (years)	$32.61 \pm 5.25$	$30.79 \pm 4.68$	9.59	$<0.001$
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	$<0.001$
Third trimester ( $\mu\text{mol/L}$ )	$9.85 \pm 3.72^a$	$8.00 \pm 1.98$	16.768	$<0.001$
Body mass index ( $\text{kg/m}^2$ )	$28.36 \pm 3.64$	$26.22 \pm 3.09$	11.685	$<0.001$
Neonatal weight (g)	$2083.04 \pm 868.41$	$3163.75 \pm 362.84$	42.491	$<0.001$
Apgar score (5 min)	$8.98 \pm 2.80$	$9.98 \pm 0.29$	13.188	$<0.001$
Previous abortions	1 (0, 2)	0 (0, 1)	-	$<0.001$
Previous deliveries	0 (0, 1)	1 (0, 1)	-	$<0.001$
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	$<0.001$
Foetal death (n)	124 (9.06%)	1 (0.0001%)	127.654	$<0.001$

Note: Data are shown as mean  $\pm$  standard deviation, number (percentage), or median (25th–75th percentiles).

<sup>a</sup> $n = 1310$ .



**FIGURE 2** The mean  $\pm$  standard deviation values for homocysteine concentrations. (A) Control group, according to foetal survival status among pregnant women with preeclampsia (B) survival group, (C) foetal death group). Receiver operating characteristic curve analysis was used to identify homocysteine cut-off values during the first trimester (D), second trimester (E) and third trimester (F) according to foetal survival status

## 4 | DISCUSSION

This study of 1,368 pregnant Chinese women aimed to examine whether homocysteine concentrations could predict foetal death. Women with PE had higher rates of foetal death.<sup>7</sup> 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 PE.<sup>8</sup> Previous studies have examined whether hyperhomocysteinemia in pregnancy is related to adverse outcomes, such as small size for gestational age at birth, PE, recurrent abortions, low birth weight and intrauterine growth restriction.<sup>4,9</sup> Among our patients with PE, homocysteine concentrations gradually and significantly increased throughout the pregnancy,

these results are consistent with previously reported findings in Spain and India populations.<sup>10,11</sup>

Hyperhomocysteinemia increases the oxidative stress and collagen accumulation that leads to vascular fibrosis, and results in endothelial damage,<sup>9,12</sup> which is a non-protein amino acid that is formed after cleavage of the terminal methyl group from methionine, genetic polymorphism of the enzyme methylenetetrahydrofolate reductase (MTHFR), which catalyses the conversion of 5,10-methylene THF to 5-methyl THF in the folate cycle, also increases homocysteine.<sup>13,14</sup> A study of Turkey revealed homocysteine levels were not correlated with disease severity,<sup>7</sup> but another study in Shanghai showed homocysteine in severe PE were significantly higher than those in the median.<sup>15</sup> Therefore, it remains unclear whether increased homocysteine concentrations are a pathological factor, and how

TABLE 2 Comparing the clinical characteristics of patients with preeclampsia

Variables	Survival	Foetal death	$t/\chi^2$	$p$
	$N = 1,244$	$N = 124$		
Age (years)	$32.67 \pm 5.24$	$32.00 \pm 5.36$	1.34	0.182
Homocysteine				
First trimester ( $\mu\text{mol/L}$ )	$7.04 \pm 2.05$	$7.88 \pm 2.13$	4.22	<0.001
Second trimester ( $\mu\text{mol/L}$ )	$7.65 \pm 2.59$	$9.70 \pm 2.95$	8.29	<0.001
Third trimester ( $\mu\text{mol/L}$ )	$9.72 \pm 3.65$	$12.17 \pm 4.13$	5.58	<0.001
Body mass index ( $\text{kg/m}^2$ )	$28.47 \pm 3.62$	$27.26 \pm 3.68$	3.49	0.001
Neonatal weight (g)	$2204.89 \pm 795.12$	$860.65 \pm 579.52$	23.7	<0.001
Previous abortions	1 (0, 2)	1 (0, 2)		0.194 <sup>a</sup>
Previous deliveries	0 (0, 1)	1 (0, 1)		0.046 <sup>a</sup>
Gestation length (weeks)	$36.26 \pm 3.45$	$28.81 \pm 4.31$	22.38	<0.001
Adverse pregnancy history (n)	104 (8.36%)	18 (14.52%)	5.26	0.022

Note: Data are shown as mean  $\pm$  standard deviation, number (percentage), or median (25th, 75th percentiles).

<sup>a</sup>Mann-Whitney U test.

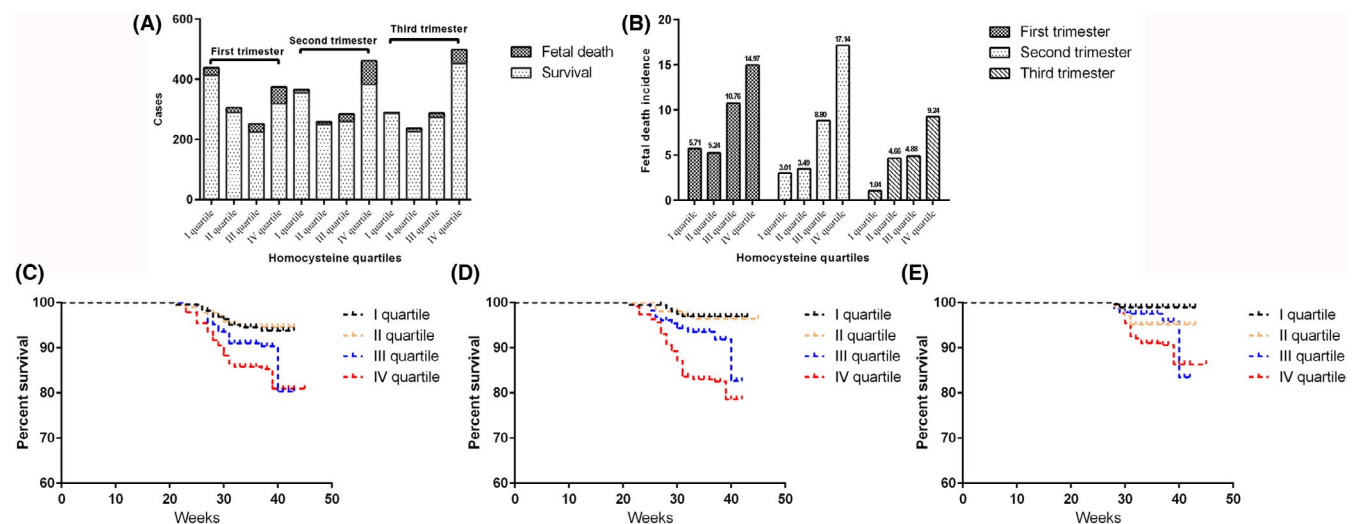


FIGURE 3 The relationship between homocysteine concentrations and foetal death. The amount of survival and foetal death cases in pregnant women with preeclampsia (PE) by quartiles of homocysteine during different trimesters (A). The incidence of foetal death in pregnant women with PE by quartiles of homocysteine during all the trimesters (B). Survival curves of foetal in pregnant women with PE by quartiles of homocysteine during the first (C), second (D) and third (E) trimesters

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, but only 5.88% of the participants were Asians in this study,<sup>16</sup> another study also revealed that elevated homocysteine concentrations were significantly associated with pregnancy loss.<sup>17</sup> But extensive studies only focused on the level of homocysteine concentrations in a single time point during pregnancy, the clinical significance of its dynamic alternation remains unclear.

In the present study, we found the dynamic alternation of homocysteine level was different between PE women and control group, serum homocysteine concentrations were comparable between PE women and control at the first trimester, however,

homocysteine concentrations elevated significantly as gestation advances in PE women, and we only observed a significant increase of homocysteine concentrations at the third trimester in the control group. It suggests that homocysteine play a role in the etiopathogenesis of PE. We furthermore found that serum homocysteine concentrations were significantly higher in foetal death group as compared to survival group in PE women, it indicates that homocysteine may contribute to the increased risk of foetal death, and foetal death was also associated with body mass index, neonatal weight, previous delivery, gestation length and adverse pregnancy history.

We observed that elevated homocysteine concentrations were a risk factor for foetal death in PE women. It influenced the foetal prognosis during all trimesters, and the incidence of foetal death

**TABLE 3** Logistic regression analysis of foetal death during the different trimesters according to elevated serum homocysteine concentrations

	OR	Univariable analysis HR (95% CI)	p Value	OR	Multivariable analysis HR (95% CI)	p Value
Age (years)	0.98	0.94–1.01	0.173	-	-	-
Log homocysteine						
First trimester	4.36 <sup>a</sup>	2.25–8.45	<0.001	0.32 <sup>b</sup>	0.10–0.97	0.043
Second trimester	10.12 <sup>a</sup>	5.63–18.17	<0.001	15.85 <sup>b</sup>	1.13–60.74	<0.001
Third trimester	6.1 <sup>a</sup>	3.15–11.80	<0.001	1.21 <sup>b</sup>	0.43–3.43	0.715
BMI	0.91	0.86–0.96	0.001	0.044	0.01–0.30	0.001
Previous abortions	1.09	0.94–1.28	0.259	-	-	-
Previous deliveries	1.48	1.11–1.95	0.007	1.14	0.96–1.35	0.135
Adverse pregnancy history (n)	1.86	1.09–3.19	0.024	1.472	0.70–3.11	0.311

<sup>a</sup>Crude OR of log homocysteine.

<sup>b</sup>Adjusted OR of log homocysteine.

increased impressively with the evaluation of homocysteine concentrations, the ROC curve results revealed that homocysteine concentrations might be an indicator of foetal death. The incidence of foetal death increased evidently by quartiles of homocysteine during all the trimesters, in order to further prove the reliability of its predictive value, foetal survival follow-up was carried out. The patients were divided into four groups based on the level of homocysteine concentrations, high level of homocysteine concentration is correlated with a poor clinical outcome in PE women since in the first trimester, it indicated that hyperhomocysteinemia was correlated with disease severity in Chinese PE women since in the early stage of pregnancy. After adjusting for BMI, previous deliveries and adverse pregnancy history, multivariate logistic regression indicated that the high homocysteine concentration was an independent risk factor of foetal death in the first and second trimesters, rather the third trimester.

The present study is limited by a lack of data regarding maternal lifestyle factors, we were unable to collect data like smoking habits, alcohol consumption, vitamin B12 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 PE.

In conclusion, the present study confirmed that foetal death was associated with maternal age and previous delivery, which agree with previously reported findings. Furthermore, among Chinese women with PE, incidence of foetal death increased progressively from quartile I to IV of homocysteine levels, and upper-quartile homocysteine concentrations were significantly associated with the risk of foetal death in the early stage of pregnancy.

#### ACKNOWLEDGEMENTS

We are grateful to the support of Department of Medical Laboratory of the Maternal & Child Health of Guangxi Zhuang Autonomous Region and The People's Hospital of Guangxi Zhuang Autonomous Region.

#### CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

#### AUTHOR CONTRIBUTIONS

Conception and design: JJP and QWY, administrative support: HXH and FCHY, provision of study materials or patients: JJP, QWY LXJ and WJL, collection and assembly of data: JJP, QWY LXJ and WJL, data analysis and interpretation: JJP, QWY and HXH, manuscript writing: JJP and QWY.

#### DATA AVAILABILITY STATEMENT

The data generated and analysed in the presented study are not publicly available for the reason to preserve individuals' privacy under the European General Data Protection Regulation, but are available from the corresponding author on request.

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**How to cite this article:** Qin W, Hu X, Fu C, et al. Estimation of homocysteine concentration as an indicator of foetal death in pregnant Chinese women with preeclampsia: A case-control study. *J Clin Lab Anal*. 2022;36:e24312. doi:[10.1002/jcla.24312](https://doi.org/10.1002/jcla.24312)