RESEARCH ARTICLE

Changes and significance of plasma fibrinogen gamma-chain concentration in preeclampsia patients

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Abstract

Objective: To investigate the plasma fibrinogen gamma-chain concentration in preeclampsia patients and explore its value in preeclampsia prediction and auxiliary diagnosis.

Methods: Follow-up of pregnant women who regularly attended perinatal care at two hospitals in China was performed, and clinical data and plasma samples were collected at each examination until delivery. The gamma-chain concentration was detected by Western blotting, and Quantity One Software was used for gamma-chain grayscale value measurements.

Results: Forty-two patients with preeclampsia and 42 control patients completed the follow-up. In the control group, the gamma-chain concentration at 32 weeks of gestation was higher than that at 20 weeks of gestation, but the difference was not statistically significant ($p > 0.05$). In the experimental group, the gamma-chain concentration at preeclampsia diagnosis was significantly higher than that at 20 weeks of gestation ($p < 0.05$). Compared with the control group, the gamma-chain concentration was higher at 20 weeks of gestation in the experimental group, but the difference was not statistically significant. However, at 32 weeks of gestation and at the time of diagnosis, the gamma-chain concentration in the experimental group was significantly higher than that in the control group ($p < 0.05$). At 32 weeks of gestation and at the time of diagnosis, the AUCs from ROC curve analysis of plasma fibrinogen gamma-chain concentrations were 0.64 and 0.71, respectively.

Conclusion: Plasma fibrinogen synthesis and degradation were disrupted in preeclampsia patients before and after diagnosis, and gamma-chain concentration was significantly increased. Plasma fibrinogen gamma chain may be of some value in preeclampsia prediction and auxiliary diagnosis.

KEYWORDS
aided diagnosis, fibrinogen gamma chain, predict, preeclampsia, pregnancy

1 | INTRODUCTION

Preeclampsia is a special manifestation of "gestational hypertension syndrome" after 20 weeks of gestation and includes edema, hypertension, and proteinuria.[1] In severe cases, convulsions and coma may occur, and serious complications such as renal failure, heart failure, and early placental detachment can be caused; its incidence in primipara women is 3%–7%, and the incidence in multipara women is 1%–3%.[2] At present, a large number of studies have shown that endothelial dysfunction is an important factor in preeclampsia, which can trigger a series of inflammation and abnormal coagulation systems in various organs, thus causing corresponding clinical symptoms.[1-7]

According to the study of Blumenstein M et al,[8] through two-dimensional electrophoresis and relevant analytical techniques, 49 protein clusters ($p < 0.05$) were found in the plasma of pregnant women with preeclampsia, and 10 false positives were excluded through clinical information. The remaining 39 were identified by LC-MS/MS, and two protein clusters that accurately (>90%) classified women at risk of developing preeclampsia were identified. Western blot analysis confirmed that one of them was the fibrinogen gamma chain. The results of Blumenstein M et al suggested that the fibrinogen gamma chain might be an important marker for the occurrence of preeclampsia, but Blumenstein M failed to conduct quantitative analysis of the fibrinogen gamma chain.

Fibrinogen is a glycoprotein synthesized and secreted by liver cells, and it consists of an alpha chain (66,500), a beta chain (52,000), and a gamma chain (46,500) connected by disulfide bonds; fibrinogen is the coagulation factor with the highest content in plasma.[9] Under the action of plasmin, fibrinogen and fibrin can be decomposed into many soluble peptides, such as alpha, beta, and gamma single chains. Their degradation products and the synthesis and decomposition of these substances maintain a dynamic balance.[10] Under certain pathological conditions, the dynamic balance is broken, which leads to the abnormal contents of these substances in plasma. Currently, there is no screening test for preeclampsia with high sensitivity and specificity. We sought to identify plasma fibrinogen gamma-chain concentrations in preeclampsia patients and healthy pregnant women that may serve as predictive and auxiliary diagnostic biomarkers.

2 | SUBJECTS AND METHODS

2.1 | Subjects

2.1.1 | Preeclampsia group

From February 2018 to February 2020, follow-up of pregnant women undergoing regular perinatal care in the Affiliated Hospital of Jiujiang University or Shanghai First People's Hospital Baoshan Branch was performed, and plasma and clinical data for each perinatal care patient were collected until they were discharged (Figure 1).
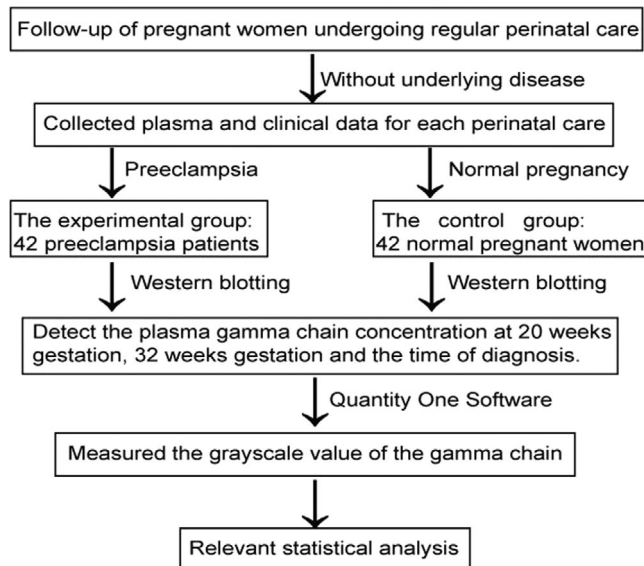

FIGURE 1 The flowchart of this study

Forty-two preeclampsia patients completed the whole follow-up. The diagnosis of preeclampsia endorsed by the International Society for the Study of Hypertension in Pregnancy (ISSHP) embraces new-onset hypertension (systolic >140 mmHg and diastolic >90 mmHg) accompanied by one or more other features: proteinuria (proteinuria— ≥300 mg per day or protein/creatinine ratio ≥30 mg/mmol [0.3 mg/mg]), other maternal organ dysfunction (including liver, kidney, neurological), or hematological involvement, and/or utero-placental dysfunction, such as fetal growth restriction and/or abnormal Doppler ultrasound findings of uteroplacental blood flow.[7] The study protocol was approved by the Human Ethics Committee of the Affiliated Hospital of Jiujiang University, and written informed consent was obtained from each participant.

2.1.2 | Control group

Forty-two normal pregnant women with the similar delivery gestational age as the preeclampsia group delivered in the Affiliated Hospital of Jiujiang University or Shanghai First People's Hospital Baoshan Branch during the same period were selected for inclusion in the control group and had no history of oral contraceptive use; heart, liver, kidney, or thyroid diseases; or diabetes, hypertension, or other diseases.

3 | MATERIALS AND METHODS

3.1 | Reagent materials

Sheep polyclonal fibrinogen antibody (HRP) (ab7539) produced by Abcam was purchased. The marker (0008806) was purchased from Fermentas, and protein electrophoresis, membrane transfer, and imaging systems (Bio-Rad) were used.

3.2 | Specimen collection

Normal maternal plasma samples were collected at 20 weeks of gestation, 32 weeks of gestation, and before delivery. The number of plasma specimen examinations in patients with preeclampsia increased with changes in condition. All plasma samples were collected from a vein of fasting participants in EDTA-coated tubes, mixed and centrifuged at 3500 g for 10 minutes, and stored in a low-temperature refrigerator at −80℃.

3.3 | Detection methods

Western blotting was used to detect the plasma gamma-chain concentration of fibrinogen in pregnant women in each group.[8] All operations were carried out in accordance with the instructions, and the grayscale value of the gamma chain was measured by Quantity One Software.

3.4 | Statistical analysis

SPSS 21.0 statistical software was used for statistical analysis of relevant experimental data. All results are from two-sample t tests. $p < 0.05$ was considered statistically significant.

4 | RESULTS

4.1 | Comparative analysis of general data of two groups of pregnant women.

All pregnant women with preeclampsia and the healthy control group had singleton pregnancies. There was no statistically significant difference in general data, such as maternal age, BMI at 20 weeks of gestation, and the ratio of primipara, between the preeclampsia and control groups (Table 1, $p > 0.05$).

TABLE 1 Comparative analysis of general data of pregnant women in the two groups

	Preeclampsia group	Control group	p
Age (years)	29.02 + 3.9	28.12 + 4.13	>0.05
BMI (kg/m^2) of 20 weeks' gestation	23.57 + 2.45	24.57 + 2.45	>0.05
Ratio of primipara (%)	71.4	52.4	>0.05
Delivery gestational age	36.83 + 1.545	37.07 + 1.583	>0.05
Birthweight (kg)	2.9 + 0.38	3.1 + 0.37	>0.05

Note: Data are expressed as mean ± SD.
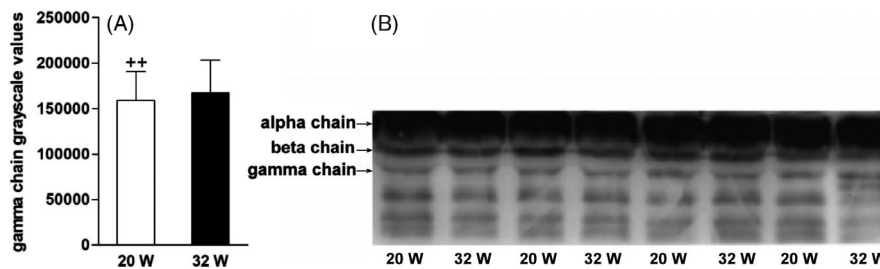Abbreviation: BMI, body mass index.

FIGURE 2 Comparison of plasma fibrinogen gamma-chain concentrations at 20 weeks of gestation and 32 weeks of gestation during normal pregnancy. (A) A total of 42 pairs of serum samples. All results are from two-sample t tests, ++$p > 0.05$. (B) Western blot results of 4 pairs of plasma fibrinogen gamma chains. 20 W: normal gestation at 20 weeks and 32 W: normal gestation at 32 weeks
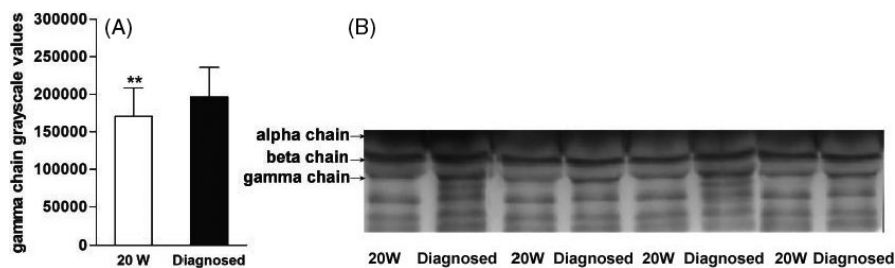

FIGURE 3 Plasma fibrinogen gamma-chain concentrations in preeclampsia patients at 20 weeks of gestation and at diagnosis of preeclampsia. (A) A total of 42 pairs of serum samples. All results are from two-sample t tests, **$p < 0.005$. (B) Western blot analysis of 4 pairs of plasma fibrinogen gamma chains

4.2 | Changes in plasma fibrinogen gamma-chain concentration during normal pregnancy

The analysis of grayscale values of plasma electrophoresis results of all 42 cases also showed that there was no statistically significant difference between 32 weeks of gestation and 20 weeks of gestation in women with a normal pregnancy (Figure 2, $p > 0.05$).

4.3 | Changes in plasma fibrinogen gamma-chain concentration in preeclampsia patients

During pregnancy, the plasma fibrinogen gamma-chain concentration in preeclampsia patients after diagnosis was significantly higher than that at 20 weeks of pregnancy (Figure 3A-B, $p < 0.005$).

4.4 | Comparison of plasma fibrinogen gamma-chain between preeclampsia patients and normal pregnant women at the same gestational weeks

At 20 weeks of gestation, there was no statistically significant difference in plasma fibrinogen gamma-chain concentration between preeclampsia patients and normal pregnant women at the same gestational weeks (Figure 4A-B, $p > 0.05$). At 32 weeks of gestation, the plasma fibrinogen gamma-chain concentration in preeclampsia patients was significantly higher than that in normal pregnant women at the same gestational weeks (Figure 4C-D, $p < 0.05$). At the time of diagnosis, the plasma fibrinogen gamma-chain concentration of preeclampsia patients was higher than that of normal pregnant women at the same gestational weeks, showing a significant difference (Figure 4E-F, $p < 0.005$).
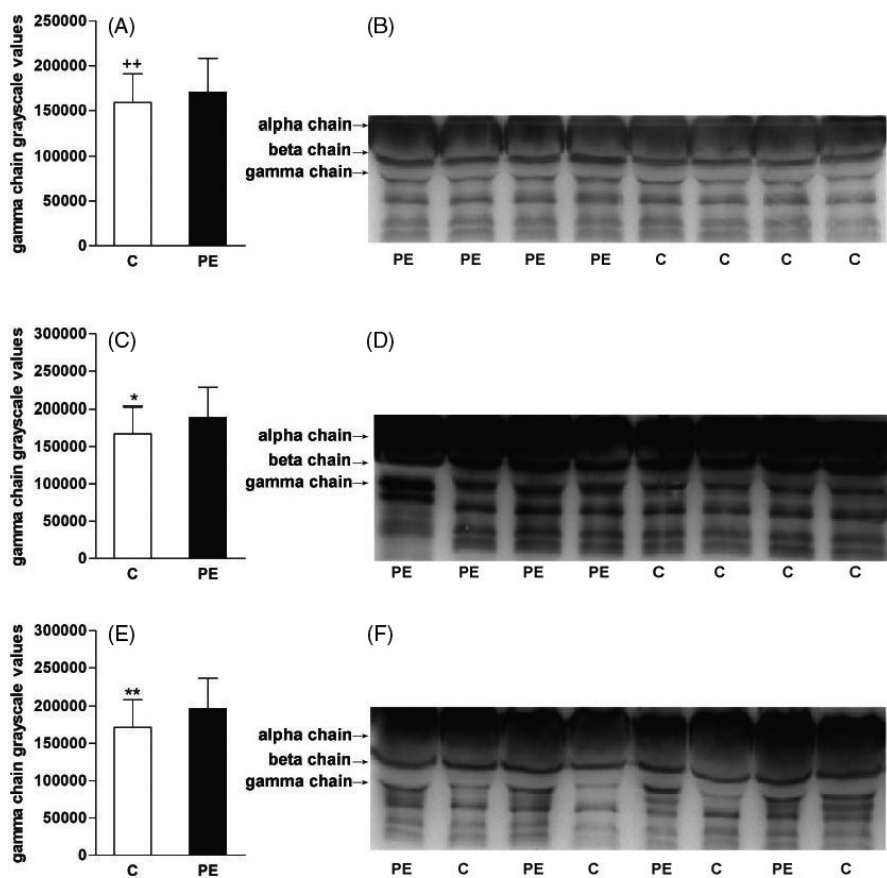

FIGURE 4 Plasma fibrinogen gamma-chain concentrations in pregnant women with preeclampsia compared with those of normal pregnant women at the same gestational weeks. (A-B) comparison of plasma fibrinogen gamma-chain concentration at 20 weeks of gestation; (C-D) comparison of plasma fibrinogen gamma-chain concentration at 32 weeks of gestation; (E-F) comparison of plasma fibrinogen gamma-chain concentrations at diagnosis of preeclampsia. A total of 42 pairs of serum samples, all results are from two-sided t tests, $++p > 0.05$, $*p < 0.05$, $**p < 0.005$, C: control group, PE: preeclampsia group
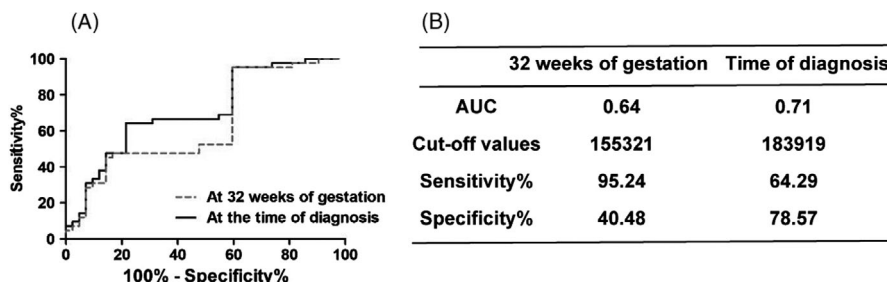

	32 weeks of gestation	Time of diagnosis
AUC	0.64	0.71
Cut-off values	155321	183919
Sensitivity%	95.24	64.29
Specificity%	40.48	78.57

FIGURE 5 ROC curve analysis of the plasma fibrinogen gamma-chain concentration at 32 weeks of gestation and at the time of diagnosis

4.5 | ROC curve analysis of the plasma fibrinogen gamma-chain concentration at 32 weeks of gestation and at the time of diagnosis

At 32 weeks of gestation, the AUC from ROC curve analysis of plasma fibrinogen gamma-chain concentrations was 0.64 (Figure 5, $p < 0.05$). At the time of diagnosis, the AUC from ROC curve analysis was 0.71 (Figure 5, $p < 0.001$).

5 | DISCUSSION

The pathogenesis of preeclampsia has not been fully elucidated and is believed to be related to various factors, among which vascular endothelial cell injury is the pathological basis of multisystem injury leading to preeclampsia, and abnormal coagulation and bleeding mechanisms are important clinical manifestations.[1,11] The results of this study showed that there was no significant change in plasma fibrinogen gamma-chain concentration at 20 weeks of gestation in preeclampsia patients compared with that of normal pregnant women. However, at 32 weeks of pregnancy, especially at the time of diagnosis, the plasma fibrinogen gamma-chain concentration was abnormally increased. These results suggest that under normal circumstances, plasma fibrinogen synthesis and cleavage in pregnant women maintain a dynamic balance and maintain the normal coagulation mechanism, while in the late gestation period of preeclampsia patients, especially when preeclampsia occurs, this balance is broken, and abnormal plasma fibrinogen gamma-chain synthesis or degradation occurs.

In this study, there was no significant change in plasma fibrinogen gamma-chain concentration between 20 and 32 weeks of gestation in normal pregnant women. The results showed that there was no significant correlation between the changes in plasma fibrinogen synthesis and degradation and gestational weeks. Compared with normal pregnant women, the concentration of plasma fibrinogen gamma chain in preeclampsia patients at 20 weeks of gestation (approximately 3 months before diagnosis) did not change significantly. However, at 32 weeks of gestation (2–7 weeks before diagnosis), the concentration of plasma fibrinogen gamma chain in preeclampsia patients increased significantly (Figure 4C-D, $p < 0.05$). It is suggested that 2–7 weeks before the diagnosis of preeclampsia, the synthesis and degradation of plasma fibrinogen are disordered, and the concentration of gamma chain is increased. When preeclampsia is diagnosed, the plasma fibrinogen gamma chain is further significantly increased (Figure 4E-F, $p < 0.005$). These results suggest that the synthesis and degradation of plasma fibrinogen are further disturbed and that the concentration of gamma chain is further increased in patients with preeclampsia.

In this study, at 32 weeks of gestation, the AUC from ROC curve analysis of plasma fibrinogen gamma-chain concentrations was 0.64 (Figure 5, $p < 0.05$), and its sensitivity and specificity were 95.24% and 40.48%, respectively, at the cutoff value. These results suggest that plasma fibrinogen gamma-chain concentrations may have some predictive value. At the time of diagnosis, the AUC from ROC curve analysis was 0.71 (Figure 5, $p < 0.001$), and its sensitivity and specificity were 64.29% and 78.57%, respectively, at the cutoff value, which suggests that plasma fibrinogen gamma-chain concentrations may be of some auxiliary diagnostic value.

In conclusion, the pathogenesis of preeclampsia is very complex, and the disorder of fibrinogen synthesis and degradation may be one of the important mechanisms. The plasma fibrinogen gamma chain was specifically elevated in pregnant women with preeclampsia, which may provide new clues for the prediction and auxiliary diagnosis of the occurrence of preeclampsia and is worth further investigation.

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