

Original Research Article

Serum Sestrin2 and PIGF levels in pregnant women with pre-eclampsia and their correlations with the severity of the disease: A case-control study

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Abstract

Purpose: Sestrin2, a metabolic regulator with antioxidant activity, might have some certain predictability for the occurrence and severity of pre-eclampsia (PE). The purpose of this work was to explore the levels of serum Sestrin2 and PIGF in pregnant women with PE and their correlation with the severity index of the disease.

Methods: This was a retrospective case-control study of pregnant women with PE who planned to give birth in our hospital from 05/2017 to 05/2019. Pearson correlation was used to analyze Sestrin2 and PIGF levels with PE severity. Receiver operating characteristic (ROC) curves were used to evaluate the predictive value of Sestrin2 and PIGF for PE.

Results: There were 52 women in the control group, 46 in the mild PE group, and 36 in the severe PE group. As the severity of PE increased, the median levels of Sestrin2 increased (8.1, 9.8, and 11.8 ng/ml), and the levels of PIGF decreased (156, 117, and 67 pg/ml) in the three groups of women (all $P < 0.05$). The levels of Sestrin2 and PIGF were strongly correlated with mean arterial pressure, proteinuria, newborn birth weight and gestational weeks at delivery (all $P < 0.001$). Sestrin2 and PIGF had high efficiency of diagnosing PE (cut-off: 8.90 ng/ml, Area Under Curve [AUC]=0.979; cut-off: 122.50 pg/ml, AUC=0.963). Additionally, Sestrin2 and PIGF showed high value of severity prediction (cut-off: 11.15 ng/ml, AUC=0.857; cut-off: 68.50 pg/ml, AUC=0.837).

Conclusion: Sestrin2 and PIGF are correlated with the severity of PE. Both Sestrin2 and PIGF had high value for PE diagnosis and severity prediction.

Keywords: pre-eclampsia; Sestrin2; placental growth factor; oxidative stress; severity

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INTRODUCTION

Hypertensive disorder during pregnancy seriously threatens the health of pregnant women and newborns. About 2%-10% of pregnancies in the world are affected by it [1-5].

The incidence of pre-eclampsia (PE) is higher among women with hypertensive disorders, for a prevalence of about 3% among pregnant women [2-5]. The severity and onset of PE are closely related to the prognosis of pregnant women. Currently, the pathogenesis of PE is not fully

understood, but the possible causes include abnormal placental implantation, angiogenic factors, cardiovascular maladaptation and vasoconstriction, genetic predisposition, immunologic phenomena, and vascular endothelial damage, among others [2, 3].

PE results in reduced organ perfusion due to vasospasms and activation of the coagulation cascade [6], leading to early placental ischemia and hypoxia [2, 3]. Late placental ischemia-reperfusion injury leads to a placental oxidative stress reaction and the release of various inflammatory factors into circulation, triggering a systemic inflammatory reaction and maternal diseases [7]. The placental growth factor (PlGF) can promote vascular regeneration and improve blood circulation [8-10]. As a member of the Sestrin family, Sestrin2 is a metabolic regulator, and its expression increases in the presence of DNA damage, hypoxia, oxidative stress, and other adverse metabolic conditions. Sestrin2 has antioxidant activity [11, 12], suggesting that it might have some certain predictability for the occurrence and severity of PE [13, 14].

At present, there are many studies on the relation of PE with PlGF [15-17], but there are few regarding the association of Sestrin2. Therefore, the aim of this study was to explore the levels of serum Sestrin2 and PlGF in pregnant women with PE and their correlation with the severity index of the disease. The results could provide a novel biomarker for the prediction, diagnosis, and management of PE.

METHODS

Study design and patients

This was a retrospective case-control study of pregnant women with PE who planned to give birth in our hospital between May 2017 and May 2019. This study was approved by the Academic Ethics Committee of the Tianjin Central Hospital of Gynecology Obstetrics, China.

The inclusion criteria were: 1) met the diagnostic criteria of PE and severe PE according to the Guidelines for the Diagnosis and Treatment of Hypertensive Disorder Complicating Pregnancy (2015) issued by the Chinese Medical Association [18]; 2) pre-pregnancy blood pressure <140/90 mmHg; 3) >20 weeks of gestation; 4) single-fetus pregnancy and live birth; 5) mother of 20-40 years of age; and 6) no history of hypertension treatment. The exclusion criteria were: 1) history of chronic diseases such as hypertension, heart disease, diabetes, nephropathy, and autoimmune diseases; 2) history of proteinuria occurring before 20 weeks of pregnancy; 3) multiple gestation pregnancy; 4)

premature rupture of membranes; or 5) fetal malformation.

Women with a normal pregnancy matched for age, gestational age, and body mass index (BMI) with the women with PE during the same period were selected as the control group.

Diagnostic criteria

The diagnostic criteria for PE were: 1) systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 mmHg after 20 weeks of pregnancy; and 2) accompanied by urinary protein ≥ 0.3 g/24 h, or urinary protein/creatinine ≥ 0.3 , or random urinary protein + or above; or abnormal changes in heart, liver, lung, kidney, other important organs, or blood.

The diagnostic criteria for severe PE were: 1) systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg; 2) central nervous system symptoms such as visual impairment or headache; 3) persistent upper abdominal pain; 4) abnormal elevation of liver enzymes; 5) progressive renal function impairment: urinary protein >2.0 g/24 h; oliguria or serum creatinine (sCr) >106 $\mu\text{mol/L}$; 6) heart failure or pulmonary edema; 7) ascites, pleural effusion, or pericardial effusion; 8) platelets decreased continuously and less than $100 \times 10^9/\text{L}$; 9) microvascular hemolysis; and 10) fetal growth restriction or placental abruption, fetal death in utero, etc.

Patients who met the diagnostic criteria for PE but not severe PE were divided into mild PE group.

Management

Oral antihypertensive drugs were given, mainly labetalol 50-100 mg, 3 times/day, or nifedipine 10 mg, 3 times/day. If necessary, intravenous nicardipine was given at ≥ 1 mg/h, and the dose was adjusted according to blood pressure. The first choice for spasmolysis was intravenous magnesium sulfate, with a loading dose of 20 ml of 25% magnesium sulfate dissolved in 100 ml of 5% glucose over 15-20 min, with a maintenance dose of 1-2 g/h. The total amount of magnesium sulfate was no more than 25 g/day, administered for 6-12 h per day and less than 5 days. For sedation, oral diazepam 2.5-5 mg or 10 mg intramuscular was given before going to bed. For diuresis, intravenous furosemide was given in case of edema, heart failure, and renal insufficiency. Albumin was supplemented before diuretics in case of hypoproteinemia, and mannitol was given in case of cerebral edema. Pregnant women with <34 weeks of gestation and who were expected to give birth within 1 week were given glucocorticoids (dexamethasone 6 mg for intramuscular injection, once

every 12 h, four injections) to promote fetal lung maturation. According to the patients' conditions, gestational weeks, and fetal conditions, the pregnancy was terminated in due course.

Data collection

All pregnant women routinely underwent an obstetric examination, including blood pressure, body weight, uterine height, abdominal circumference, fetal heart rate, fetal position, and obstetric ultrasound to evaluate fetal growth. The patients with PE had a weekly prenatal check-up after diagnosis. ECG, fetal heart monitoring, blood biochemistry (including liver function, renal function, blood sugar, blood lipid, electrolyte, and myocardial enzyme), coagulation function, 24-h urine proteins, ultrasound examination of the fetus, placenta, and amniotic fluid were carried out routinely. Color Doppler ultrasound fetal blood flow monitoring was conducted every two weeks. The patients were guided to monitor their blood pressure in the morning, at noon, in the afternoon, and before going to bed. Patients with severe PE were hospitalized for management. In severe PE, fetal heart monitoring was conducted at least once a day, ultrasound examination of the fetus, placenta, and amniotic fluid, and color Doppler ultrasound fetal blood flow monitoring were carried out every 5-7 days.

For measuring blood pressure, before measurement, the patient rested quietly for at least 5 min, sitting or lying, limbs relaxed, and the cuff at the same level as the heart. Usually, the right upper limb was used, and both arms were used if necessary. At the first occurrence of hypertension, retests were carried out at intervals of 4 h or more, and the diagnosis was only made if both times reach the criterion for hypertension. For severe hypertension, i.e., systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg, the patients could be diagnosed by repeated measurement over several minutes.

Urine was collected for 24 h urine for 24-h urine protein examination. For patients in mild PE and severe PE group, 5 ml of venous blood was drawn and stored in EDTA at the diagnosis of PE, while patients in control group were sampling at matching gestational week of 30-37 weeks. After centrifugation at 4°C and 3000rpm for 15 min, serum was taken and stored at -80°C. Serum Sestrin2 and PIGF levels were determined by ELISA.

Statistical analysis

Data processing was performed using SPSS 22.0 (IBM, Armonk, NY, USA). Continuous data were expressed as means \pm standard deviations or median (range) based on the Kolmogorov-

Smirnov test of normal distribution. ANOVA or Kruskal-Wallis test was performed with the Bonferroni or Dunnett post hoc test to examine the group differences. Spearman correlation analysis was used to analyze the correlation between Sestrin2, PIGF and PE severity. The difference in serum sestrin2 and PLGF levels among the three groups of patients is shown by box plots. Receiver operating characteristic (ROC) curves were used to evaluate the predictive value of Sestrin2 and PIGF for PE (control vs. PE, irrespective of severity) and PE severity (mild vs. severe PE). The area under the curve (AUC) was calculated, as well as sensitivity and specificity. Two-sided P-values ≤ 0.05 were considered statistically significant.

RESULTS

Characteristics of the patients

A total of 134 pregnant women were included: 52 cases in the control group, 46 in the mild PE group, and 36 in the severe PE group. There were no significant differences in age, gestational age at blood sampling, BMI, and platelets among the three groups (all $P > 0.05$) (Table 1). The mean arterial pressure (MAP) at blood sampling, sCr, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and proteinuria of pregnant women with mild PE were significantly higher than those in the control group (all $P < 0.05$), and those indexes in the severe PE group were significantly higher than those in the mild PE group; newborn birth weight, gestational age at delivery, and duration between blood sampling and delivery were smaller when comparing mild PE and control group, as well as severe PE and mild PE group (all $P < 0.05$) (Table 1).

Serum Sestrin2 and PIGF levels

The median levels of Sestrin2 in the control, mild PE, and severe PE groups were 8.1 (7.0, 9.5), 9.8 (8.0, 11.4), and 11.8 (9.5, 14.9) ng/ml, while the median levels of PIGF were 156 (123, 198), 117 (70, 156), and 67 (36, 148) pg/ml, respectively. Patients in the severe PE group had significantly higher levels of Sestrin2 and lower levels of PIGF than those in the control and mild PE groups (all $P < 0.05$) (Figure 1).

Correlations between PE severity and Sestrin2 and PIGF levels

The levels of Sestrin2 were strongly and positively correlated with MAP at blood sampling ($r = 0.875$, $P < 0.001$) and proteinuria ($r = 0.908$, $P < 0.001$), while strongly and negatively correlated with newborn birth weight ($r = -0.813$, $P < 0.001$) and gestational weeks at delivery ($r = -0.869$, $P < 0.001$) (Table 2).

Table 1: Comparison of the characteristics of pregnant women

Characteristics	CG (n=52)	MP (n=46)	SP (n=36)	P value			
				P	CG vs. MP	MP vs. SP	CG vs. SP
Age (years)	30 (23,36)	29 (24,36)	29 (23,38)	0.950			
Gestational weeks at blood sampling (weeks)	33.7 (30.3,36.7)	33.7 (30.3,36.3)	34.0 (28.9,36.3)	0.996			
BMI at blood sampling (kg/m ²)	28.55 (26.30,31.90)	28.45 (26.50,31.90)	28.55 (26.90,32.30)	0.826			
MAP at blood sampling (mmHg)	80.98 (72.00,97.00)	110.2 (97.50,120.50)	128.40 (108.50,148.20)	<0.001	<0.001	<0.001	<0.001
Platelet ($\times 10^9/L$)	214 (125,289)	189 (100,298)	206 (65,299)	0.544			
Serum creatinine ($\mu\text{mol/L}$)	54.95 \pm 9.02	62.61 \pm 10.23	79.79 \pm 14.92	<0.001	0.003	<0.001	<0.001
Alanine transaminase (U/L)	20 (8,36)	21 (10,55)	35 (12,235)	<0.001	0.415	<0.001	<0.001
Aspartate transaminase (U/L)	25 (6,39)	29 (11,60)	47 (19,267)	<0.001	0.048	<0.001	<0.001
Lactate dehydrogenase (U/L)	199.7 (99.5,230.5)	219.9 (156.9,250.2)	529.0 (245.9,783.2)	<0.001	0.001	<0.001	<0.001
Urinary proteins (g/24 h)	0 (0,0)	1.51 (0.32,1.99)	4.30 (2.11,7.82)	<0.001	<0.001	<0.001	<0.001
Newborn birth weight (g)	3300 (2800,3900)	3000 (2100,3700)	2325 (1500,3200)	<0.001	<0.001	<0.001	<0.001
Gestational weeks at delivery (weeks)	39.3 (37.3,41.4)	37.4 (35.1,39.1)	36.1 (32.1,38.1)	<0.001	<0.001	0.001	<0.001
Duration of delivery and blood sampling (weeks)	5.6 \pm 1.5	3.8 \pm 1.0	2.1 \pm 1.2	<0.001	<0.001	<0.001	<0.001

BMI: body mass index; MAP: mean arterial pressure; CG: control group; MP: mild pre-eclampsia group; SP: severe pre-eclampsia group. The data displayed as mean \pm deviation; others displayed as median (range)

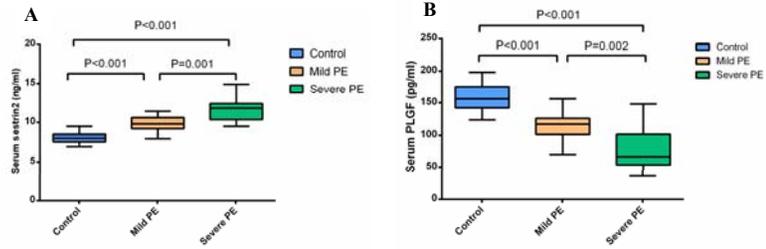
**Figure 1:** Comparison of serum Sestrin2 (A) and placental growth factor (PIGF) (B) levels in pregnant women

Table 2: Correlation between Sestrin2 and PIGF levels and PE severity-related indicators

Indicator	Sestrin2		PLGF	
	r	P	r	P
MAP at blood sampling (mmHg)	0.875	<0.001	-0.850	<0.001
Platelet ($\times 10^9/L$)	-0.302	<0.001	0.306	<0.001
Serum creatinine ($\mu\text{mol/L}$)	0.755	<0.001	-0.891	<0.001
Alanine transaminase (U/L)	0.564	<0.001	-0.595	<0.001
Aspartate transaminase (U/L)	0.683	<0.001	-0.655	<0.001
Lactate dehydrogenase (U/L)	0.738	<0.001	-0.690	<0.001
Urinary proteins (g/24 h)	0.908	<0.001	-0.879	<0.001
Newborn birth weight (g)	-0.813	<0.001	0.853	<0.001
Gestational weeks at delivery (weeks)	-0.869	<0.001	0.900	<0.001
Duration of delivery and blood sampling (weeks)	-0.682	<0.001	0.677	<0.001

PIGF: placental growth factor; MAP: mean arterial pressure

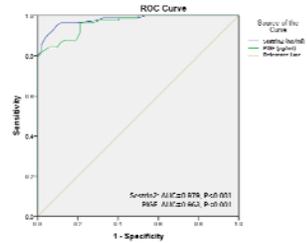


Figure 2: Receiver operating characteristic (ROC) curve of pre-eclampsia predicted by Sestrin2 and placental growth factor (PIGF)

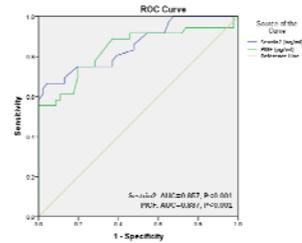


Figure 3: Receiver operating characteristic (ROC) curve of mild and severe pre-eclampsia predicted by Sestrin2 and placental growth factor (PIGF)

The levels of PIGF were strongly and positively correlated with newborn birth weight ($r=0.853$, $P<0.001$) and gestational weeks at delivery ($r=0.900$, $P<0.001$), while strongly and negatively correlated with MAP at blood sampling ($r=-0.850$, $P<0.001$) and proteinuria ($r=-0.879$, $P<0.001$) (Table 2).

ROC analysis

Figure 2 and Figure 3 present the ROC curve analyses. For the discrimination between controls and PE, when using a cut-off of 8.90 ng/ml for Sestrin2 led to 95.1% sensitivity, 90.4% specificity, and an AUC of 0.979, and using a cut-off of 122.50 pg/ml for PIGF yielded 80.5% sensitivity, 100% specificity, and an AUC of 0.963.

For the discrimination between mild and severe PE, when using a cut-off of 11.15 ng/ml for Sestrin2 led to 66.7% sensitivity, 95.7% specificity, and an AUC of 0.857, and using a cut-off of 68.50 pg/ml for PIGF yielded 55.6% sensitivity, 100% specificity, and an AUC of 0.837.

DISCUSSION

There are many studies on the relation of PE with PIGF [15-17], but there are few regarding the association of Sestrin2. Therefore, this study aimed to explore the levels of serum Sestrin2 and PIGF in pregnant women with PE and their correlation with the severity index of the disease. The results suggest that Sestrin2 and PIGF are correlated with the severity of PE. Both Sestrin2 and PIGF had high value for PE diagnosis and severity prediction while the sensitivity of Sestrin2 is higher than PIGF.

Through the analysis of the three groups of pregnant women, it was found that the levels of MAP at blood sampling, sCr, ALT, AST, LDH, proteinuria were higher, and a lighter newborn birth weight, a younger gestational age at delivery, and a shorter duration between blood sampling and delivery in patients with mild PE or severe PE than that of healthy pregnant women. The above symptoms were more severe in serious PE than in mild PE, as supported by a study by Li et al. [19], suggesting that PE can cause many adverse patient and delivery outcomes with the aggravation of the disease.

The basic pathological changes in PE are the differentiation of maternal placental trophoblasts and abnormal remodeling of spiral arterioles, which lead to the decrease of uterine and placental blood flow [2-5]. Long-term hypoxia will

lead to mitochondrial dysfunction and increased reactive oxygen species (ROS) production [20], which will imbalance the antioxidant defense mechanisms. Subsequently, the increase of ROS, endoplasmic reticulum stress (ERS), and placental oxidative stress [21] result in multiple organ injury to the mother and infant and threatening the life of the mother and infant in serious cases. The regeneration effect of PIGF on small blood vessels has been confirmed [8-10], as well as the antioxidant effect of Sestrin2 and its potential effect in immune-inflammatory reaction [11, 12].

At present, multiple studies have shown that the expression of Sestrin2 is increased in hypoxia and oxidative stress [22-24], which can significantly reduce the content of ROS and various inflammatory factors in cells, thus protecting the cells from the effects of oxidative stress and ischemia. Jegal et al. [25] showed that the up-regulation of Sestrin2 expression could inhibit mitochondrial damage and related apoptosis, which is consistent with the results of this study. The ischemic and anoxic environment of PE pregnant women will lead to placental ERS, which will affect physiological processes such as energy metabolism and protein synthesis, modification, and transport [26]. Once ERS exceeds the range of the body's self-regulation ability, it will cause cell apoptosis. Ye et al. [27] found that ERS can induce the expression of Sestrin2 and alleviate the damage of diseases caused by ERS. Those results are supported by those by Tayyar et al. [13], who showed that Sestrin2 is involved in PE, particularly in severe PE. Furthermore, the severity of PE is related to the antioxidant level [20], suggesting that the study of Sestrin2 might provide observation indicators for the early prediction of severe PE, as supported by Tayyar et al. [13].

During the development of PE, placental neovascularization and vasodilation function are closely related to the disease [28]. Related angiogenic factors play an important role. PIGF can maintain the integrity and permeability of normal vascular inner wall structure, provide sufficient nutrients for infants, promote neovascularization, improve blood supply, and relieve the ischemia and hypoxia state of severe PE. PIGF can promote the differentiation of placental trophoblasts, enhance their invasiveness, reduce the apoptosis of trophoblasts, effectively promote the recasting of uterine spiral arteries, and improve the blood supply [29].

Soluble fms-like tyrosine kinase-1 (sFlt-1), as a common anti-angiogenic factor, can aggravate the progression of PE and inhibit the binding of PIGF to the vascular endothelial growth factor receptor. Guo et al. [30] found that Sestrin2 can inhibit the effect of sFlt-1 on antagonizing angiogenesis and activate adenosine phosphate-dependent protein kinase (AMPK), while AMPK may activate and increase VEGF, promote neovascularization, and improve the condition of patients. Sestrin2 can induce the expression of nitric oxide synthase, increase the production of NO, and improve the vasodilation function of blood vessels, thus protecting the cells from ischemia [31]. Therefore, the Sestrin2 levels of the pregnant women in the three groups increased with the increase of PE severity, and PIGF levels decreased in turn.

The correlation analysis showed that the levels of Sestrin2 and PIGF were each significantly correlated with various biomarkers of the health status and PE severity, suggesting that Sestrin2 may participate in the occurrence and development of PE, and its level has a certain reference value for judging the severity of PE. The ROC curve showed that both Sestrin2 and PIGF had high value for PE diagnosis and severity prediction. Relevant studies showed that the severity of PE is related to antioxidant status [32]. The increase of the Sestrin2 levels reflects the oxidative stress level of pregnant women with severe PE, and Sestrin2 could inhibit the antagonistic effect of sFlt-1 on blood vessels and promote PIGF to play its role against PE. Therefore, Sestrin2 has higher sensitivity. This result suggests the importance of Sestrin2 levels in PE diagnosis and severity judgment.

This study has limitations. PE is a relatively common condition in pregnant women, and the sample size of the present study was relatively small. In addition, all participants were from a single hospital covering a limited geographical area. The results should be validated in larger populations and other ethnicities. Because of the retrospective nature of the study, the correlation analyses were limited to the data available in the charts.

CONCLUSION

Sestrin2 and PIGF are significantly correlated with the severity of PE and with various biomarkers of the health status and PE severity. The sensitivity of Sestrin2 is higher than PIGF's for PE diagnosis and severity prediction. These results suggest that Sestrin2 could be a novel biomarker for the screening, diagnosis, and management of PE. Future studies could look

into whether the development of PE can be prevented by increasing the concentration of Sestrin2.

DECLARATIONS

Acknowledgement

None provided.

Conflicts of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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