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Relationship between Serum sEng, PLGF, Gal-1 Levels and Pregnancy Outcome in Pregnant Hypertensive Mothers during Pregnancy

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ABSTRACT

The objective of this study was to observe the relationship between serum levels of soluble fms-like tyrosine kinase-1 (sEng), placental growth factors (PLGF), and galectin-1 (Gal-1) in hypertensive pregnant women during pregnancy and the pregnancy outcome. The study used convenience sampling method to select 375 pregnant women with hypertension in pregnancy attending the clinic from March 2020 to April 2023, and general information questionnaire, enzyme-linked immunosorbent assay was used to determine the serum sEng, PLGF, Gal-1 levels of the pregnant women at the time of admission to the hospital. The pregnant women were followed up for 6 months and 319 cases were completed, and the pregnancy outcome was defined by the presence of preterm labour, intrauterine foetal growth retardation, and placenta previa. Pearson correlation, logistic regression, and ROC curve analyses were used to determine the correlation and diagnostic efficacy of each index with pregnancy outcome. Serum sEng, PLGF, and Gal-1 levels were positively correlated with preterm labour, intrauterine fetal growth retardation, and placental abruption. Binary logistic regression analysis determined that serum sEng, PLGF, and Gal-1 levels were independent risk factors for preterm labour and intrauterine fetal developmental delay; initial diastolic blood pressure at admission and serum Gal-1 levels were independent risk factors for placental abruption; and serum sEng, PLGF, and Gal-1 were good diagnostic factors for preterm labour and intrauterine fetal serum sEng, PLGF, and Gal-1 had good diagnostic efficacy for preterm labour and IUGR. It was concluded that there is a close relationship between serum levels of sEng, PLGF, and Gal-1 in hypertensive pregnant women and their pregnancy outcome. These three substances can be used as biomarkers of maternal preterm labour and foetal growth retardation, and their elevated levels can help to assess the pregnancy outcome of hypertensive pregnant women during pregnancy.

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YW and LC conducted the experiments in this study. LC and XJ contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words
Hypertension, Pregnancy, sEng, PLGF, Gal-1, Pregnancy outcome

INTRODUCTION

Hypertension in pregnancy (HP) is the most common maternal factor causing neonatal deaths, accounting for 24.9% of neonatal deaths in the study population (Kumar *et al*., 2021). Soluble fms-like tyrosine kinase-1 (sEng) (El-Tarhouny *et al*., 2014), placental growth factors (PLGF) (Yanachkova *et al*., 2023), and galectin-1 (Gal-1) (Schrader *et al*., 2022) are three important biomarkers that have been widely used in the study of gestational diabetes and other pregnancy complications. Among them, sEng is a receptor antagonist of vascular endothelial growth factor receptor that inhibits the action of VEGF. It has been found that in HP, elevated levels of sEng characterise impaired endothelial cell function known as endothelial dysfunction (Nadarajah *et al*., 2009). PLGF is a growth factor produced by the placenta that promotes angiogenesis (Tan *et al*., 2022). In HP, elevated levels of PLGF may be an articulatory mechanism that responds to placental vascular injury and insufficiency, and placental vascular disruption is the cause of pregnancy complications. Gal-1 belongs to the calcium channel proteins, which promotes inflammatory cell excitation and activation, and thus participates in inflammatory responses (Loh *et al*., 2023). In HP, elevated levels of Gal-1 may indicate an increased inflammatory response. levels are elevated in HP, which may suggest an increased inflammatory response. However, the relationship between maternal serum sEng, PLGF and Gal-1 levels and pregnancy outcome (PO) in HP remains at the basic research level, and empirical studies are lacking. Systematic observation of the relationship between these three markers and PO in HP will provide a

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basis for developing effective countermeasures.

There are still questions about the three markers roles in HP and how they relate to outcomes in the following areas: how the three markers differ in amounts and are defined in pregnant HP patients. A single marker has been the subject of the great majority of investigations, and nothing is known about how the three markers' levels compare. The majority of research have only focused on one marker and have not thoroughly examined the compound effect of numerous markers. It is yet unknown if the three markers can correctly predict PO from changes in level. Studies on the predictive power of the three markers have produced conflicting and unsatisfactory results. The purpose of this study was to look at the connection between PO in HP pregnant women and serum levels of sEng, PLGF, and Gal-1. The hypothesis of the study was that there is an association between the levels of the three biomarkers and pregnancy outcomes. Higher levels of the three markers can monitor and predict adverse outcomes. With the help of resolving the above uncertainties, the study hopes that positive results will facilitate clinical practice and improve the accuracy of early assessment of prognosis in hypertensive pregnant women during pregnancy.

MATERIALS AND METHODS

General information

Convenience sampling method was used to select 375 pregnant women with HP who visited our hospital from March 2020 to April 2023.

The inclusion criteria were (1) meeting the diagnostic criteria for HP (Butalia *et al*., 2018); (2) 24-40 weeks of gestation; (3) serum samples required for the study were available at the time of admission to the hospital; (4) agreed to participate in this study and signed an informed consent form. Exclusion criteria were (1) combination of other complications during pregnancy such as foetal growth retardation and toxic syndrome of pregnancy; (2) Pregnancy termination (zero salivary acid syndrome, abortion, etc.); (3) combination of other serious diseases such as diabetes mellitus, hyperlipidaemia, hypertension, etc.; (4) smoking or multiple births; (5) refusal to participate in this study; (6) lost visit in the middle of the study or unable to determine the final PO. The follow-up was completed in 319 cases with 6 months of follow-up.

General information survey

The hospital developed its own general information questionnaire, which included indicators such as age, education, gestational week, number of pregnancies, delivery history, and initial measurement of systolic and diastolic blood pressure on admission.

Serological indicator tests

Venous serum samples were collected from all pregnant women on admission, 5ml of venous blood was collected using EDTA anticoagulation tubes and stored in low temperature. After centrifugation for 10 min, the separated serum was stored in a -80°C cryogenic refrigerator for backup. sEng assay: Double Antibody Sandwich ELISA kit (Xinbosai Biological, cat#MEK0237) was used. Sample lysate was PBS buffer. Matrix was milk protein. The spiking sequence was as follows: standard, probe, sample, secondary antibody, POD-labelled conjugate, TMB chromogenic substrate, OD value was measured at 450 nm. PLGF assay: A Quantikine ELISA kit (R&D Systems, cat#Dpgp00) was used. Sample lysate was Reagent Diluent. The matrix was nitrocellulose. The reagent spiking sequence was: standard, probe, sample, HRP-labelled secondary antibody, TMB chromogenic substrate. OD was measured at 450 nm. Gal-1 assay: Human Galectin-1 Duoset ELISA Kit (R&D Systems, cat#DY1197) was used. The sample solvent was 0.5% BSA. the matrix was polycarbonate. The reagent spiking sequence is: standard, probe, sample, HRP-labelled secondary antibody, TMB chromogenic substrate, OD value was measured at 450 nm and converted to quantitative data. Quality control: Repeat the measurement of the same sample 3 times, the relative standard deviation is within 10% and the results are considered reproducible.

PO follow-up

All mothers were managed with routine obstetric monitoring and follow-up for 6 months after admission to the hospital, and adverse PO conditions (preterm labour, intrauterine foetal growth retardation, placenta previa) occurring during the follow-up period were considered as endpoint events of the study. Preterm labour, intrauterine foetal growth retardation, and placental abruption were collected by full-time obstetric follow up staff.

Statistical methods

The study was statistically analysed using SPSS 24.0 software. The levels of the three biomarkers were expressed as mean±standard deviation, the presence of preterm labour, intrauterine foetal growth retardation or placental abruption was compared with the group without the aforementioned PO using the independent samples t-test, and the data on gender and history of delivery were tested using the $\chi 2$ test. Pearson correlation analysis was used to determine the correlation between the levels of the three biomarkers and PO. Binary logistic regression analysis was also performed for different birth outcomes to identify the relationship between demographic and clinical factors associated with the outcomes. ROC curves were

Table I. General Information of the sample.

	Premature (n=79)	Non-premature (n=240)	Growth retardation (n=63)	Non-growth retardation (n=256)	Placental abruption (n=52)	Non- placental abruption (n=267)
Age (years)						
	30.56±3.85	30.33±3.13	30.41±3.73	30.38±3.22	30.02±3.73	30.45±3.24
Education						
A	43	118	27	131	26	135
B	36	112	36	125	26	132
Pregnancy (weeks)						
	28.87±2.86	28.69±2.98	28.73±2.73	28.73±3.00	28.92±2.97	28.70±2.95
Pregnancy (times)						
	2.04±0.82	1.99±0.84	1.94±0.88	2.02±0.82	2.00±0.84	2.00±0.83
History of childbirth						
Yes	40	107	28	119	24	123
No	39	113	35	137	28	144
Pressure at admission (mmHg)						
SBP	161.97±14.69	159.48±15.62	161.24±15.44	159.82±15.42	160.96±13.56	159.93±15.76
DBP	103.30±9.12	100.63±9.14	103.54±8.85	100.74±9.21	103.96±9.62	100.77±9.03

A, Junior high school and below; B, Senior high school and above.

plotted to assess the accuracy and sensitivity of the three biomarkers in predicting different POs. The visualisations of this study were done using GraphPad 8.0.2 software.

RESULTS

As shown in Table I, the initial diastolic blood pressure at admission was higher in those with adverse PO (preterm labour, intrauterine foetal growth retardation, placental abruption) than in those without adverse PO, and the difference was statistically significant (P<0.05).

Serum sEng, PLGF, and Gal-1 levels were higher in the three PO mothers with preterm labour, intrauterine foetal growth retardation, and placenta previa, and the difference was statistically significant (Fig. 1). After pearson correlation analysis, it was found that serum sEng, PLGF, and Gal-1 levels were positively correlated with preterm labour (r=0.635, 0.706, 0.747, all P<0.001); serum sEng, PLGF, and Gal-1 levels were positively correlated with intrauterine foetal delay (r=0.577, 0.701, 0.747, all P<0.001); serum sEng, PLGF, and Gal-1 levels were positively correlated with placental abruption (r=0.171, 0.111, 0.192, all P< 0.001); serum sEng, PLGF, and Gal-1 levels were positively correlated with placental abruption (r=0.171, 0.111, 0.192, all P<0.05).

Using the occurrence of preterm labour as the outcome variable, the initial diastolic blood pressure at admission, serum sEng, PLGF, and Gal-1 levels were determined to be independent risk factors for preterm labour after substituting them into binary logistic regression analyses (P<0.05); using the occurrence of intrauterine foetal growth retardation as the outcome variable, the relevant factors were found to be independent risk factors for intrauterine foetal growth retardation (P<0.05); and using the occurrence of placental abruption as the outcome variable, the relevant factors were found to be independent risk factors for intrauterine foetal growth retardation (P<0.05). sEng, PLGF, and Gal-1 levels were independent risk factors for intrauterine fetal growth retardation (P < 0.05); with the occurrence of placental abruption as the outcome variable, after substituting the relevant factors, it was found that the initial diastolic blood pressure measured at admission and serum Gal-1 levels were independent risk factors for placental abruption (P<0.05) (Table II).

As shown in Figure 2A, the sensitivity of serum sEng, PLGF, and Gal-1 levels for preterm labour was 79.75%, 78.48%, and 84.81%, respectively; the specificity was 93.33%, 93.33%, and 94.58%, respectively; and the areas under the ROC curves were 0.894, 0.937, and 0.966, with the correlation criteria of >6.43ng/ml, > 48.01 pg/ml, >83.75 ng/ml. As can be seen in Figure 2B, the sensitivity of serum sEng, PLGF, and Gal-1 levels for IUGR was 80.95%, 85.71%, and 90.48%, respectively; the specificity was 90.23%, 91.80%, and 79.69%, respectively; and the

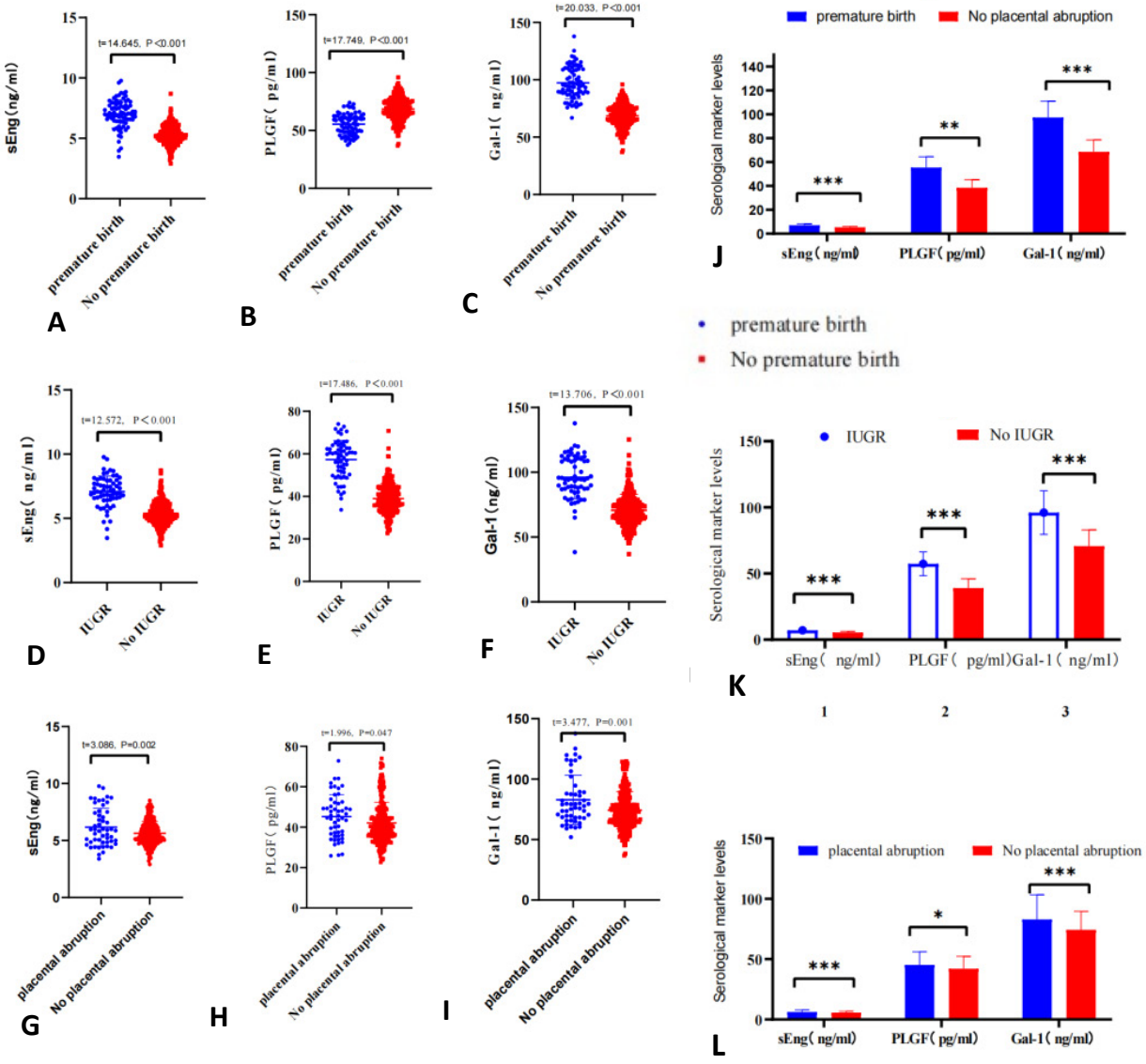

Fig. 1. Differences in serum sEng, PLGF, and Gal-1 levels among PO women in labour. A, D and G, the differences in serum sEng level; B, E and H, the differences in serum PLGF level; C, F and I, the differences in serum Gal-1 level; J, K and L, three PO mothers with preterm labour, intrauterine foetal growth retardation, and placental abruption, respectively.

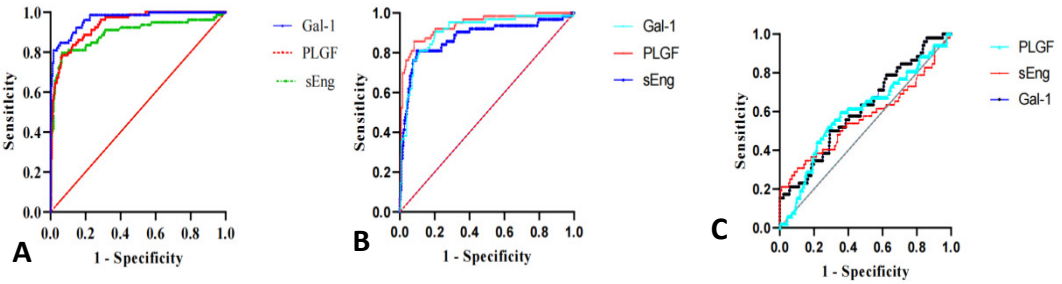

Fig. 2. Results of ROC curve analysis for different PO maternities. A, preterm labour; B, IUGR; C, placental abruption.

Table II. Logistics regression analysis.

Ending variables	Element	B	SE	OR	Wald	P	95%CI
Prematurity	Diastolic blood pressure	0.009	0.039	1.009	0.053	0.818	0.936~1.088
	sEng	0.874	0.322	2.395	7.346	0.007	1.274~4.505
	PLGF	0.147	0.040	1.158	13.337	0.000	1.070~1.253
	Gal-1	0.197	0.042	1.218	21.553	0.000	1.121~1.323
	Constant	-30.022	5.733	0.000	27.426	0.000	-
Intrauterine foetal growth retardation (IUGR)	Diastolic blood pressure	-0.026	0.028	0.974	0.909	0.340	0.923~1.028
	sEng	0.794	0.230	2.213	11.887	0.001	1.409~3.475
	PLGF	0.196	0.035	1.216	31.851	0.000	1.136~1.302
	Gal-1	0.043	0.017	1.043	6.194	0.013	1.009~1.079
	Constant	-16.553	3.100	0.000	28.519	0.000	-
Placental abruption	Diastolic blood pressure	0.040	0.018	1.041	4.833	0.028	1.004~1.079
	sEng	0.241	0.156	1.273	2.405	0.121	0.938~1.727
	PLGF	-0.016	0.019	0.984	0.782	0.377	0.949~1.020
	Gal-1	0.025	0.011	1.025	4.838	0.028	1.003~1.048
	Constant	-8.390	2.017	0.000	17.309	0.000	-

area under the ROC curve was 0.876, 0.939, 0.907, and the correlation criteria were >6.39 ng/ml, >48.72 pg/ml, >78.63 ng/ml. As can be seen from Figure 2C, the sensitivity of serum PLGF and Gal-1 levels for placental abruption was 30.77%, 59.62%, and 50.00%, respectively; the specificity was 89.51%, 64.42%, 70.79%; the area under the ROC curve was 0.570, 0.596 and 0.615, and the relevant criteria were >6.99ng/ml, >43.36pg/ml and >78.79ng/ml.

DISCUSSION

The results of the present study showed that serum sEng, PLGF and Gal-1 levels in HP pregnant women were highly correlated with preterm labour and fetal growth retardation. Elevated levels of these substances independently predicted PO in HP pregnant women. This suggests that serum sEng, PLGF and Gal-1 may reflect the role of the degree of vascular endothelial dysfunction in the evolution of HP pathogenesis. Consistent with the findings of Maynard *et al*. (2003) and Cho *et al*. (2003), the present study also found that serum sEng and Gal-1 levels predicted placental abruption events in HP pregnant women. This study is the first to show that increased PLGF can also predict preterm labour and fetal growth retardation. This confirms that the association of PLGF with HP is not simply a reactive increase (Tissot Van Patot *et al*., 2012). Although the results are encouraging, the study has a limited sample size and is a single-centre study. More prospective large-sample studies are needed to further elucidate the value of these substances in predicting PO. In addition, the relationship between these substances and the pathogenesis of HP still needs to be explored in depth.

It was found that sEng, PLGF and Gal-1 levels all acted as predictors of PO in pregnant women with HP and helped in earlier detection of at-risk mothers and fetuses. Previously, maternal endothelial dysfunction caused by placental factors has been considered as the pathophysiological cause of gestational diabetes mellitus. The development of HP syndromes results in high rates of maternal and infant morbidity and mortality. Of these, preeclampsia is the most common. In the last decade, the role of sEng in preeclampsia has shed light on the underlying mechanisms of this disorder (Sircar *et al*., 2015). In the last decade, increased production of PLGF has been identified as a placental factor contributing to maternal endothelial dysfunction and systemic vascular dysfunction, with an increased risk of maternal placental dysplasia, which is more likely to trigger IUGR (Tomimatsu *et al*., 2019; Albonici *et al*., 2020). Meanwhile, a strong and complex relationship exists between fetal growth restriction and gestational hypertension (Marasciulo *et al*., 2021). The occurrence of placental abruption during pregnancy is associated with an imbalance between oxidative activity and antioxidant defences, placental oxidative stress is a feature leading to increased production of pro-inflammatory cytokines, and Gal-1 is an important effector of placental abruption (Tenório *et al*., 2019; Kosińska-Kaczyńska, 2022). It can be seen that sEng, PLGF, and Gal-

1 are closely associated with preterm labour and IUGR in HP pregnancies. And placental abruption is more closely related to Gal-1. In the future, high-risk pregnant women can be regularly monitored according to the levels of these three substances to optimize the frequency of follow-up and pregnancy management. An abnormal rise in the level of one or more of these markers can indicate a risk of fetal growth retardation or preterm delivery, requiring timely adjustment of the treatment plan. Measures to strictly control blood pressure and load in pregnant women can help to mitigate complications.

From a scientific research perspective, these results reveal the role of these three substances in the pathogenesis of HP. sEng, PLGF and Gal-1 are all associated with endothelial dysfunction (Margioula-Siarkou *et al*., 2022), vascular damage and inflammatory response, representing three important aspects of HP, respectively (Aminuddin *et al*., 2022). Changes in their levels can reflect exacerbation or treatment progress, providing a basis for research into new targets and therapeutic approaches. Although, further studies are needed to confirm the results, this study shows that the detection of these three substances has the potential to provide valuable information for clinical work (Deshpande *et al*., 2021). If corresponding guidelines for clinical application are established, it could help to improve the pattern of pregnancy management in pregnant women with HP. However, this study still has some shortcomings and cannot adequately represent the whole HP population with some selective bias. The study design was a cross-sectional study, and it was not possible to determine whether increased levels of sEng, PLGF (Azimirad, 2021) and Gal-1 were causative factors of HP and the related mechanistic outcomes. Only a correlation between them and PO could be indicated. The study lacked control variables. Other underlying diseases and medication use in pregnant women were not moderated, which may affect the levels of molecular markers.

These experimental results suggest that sEng, PLGF and Gal-1 levels can all be used as valid biomarkers to predict PO in HP. However, further exploration is needed in order to achieve clinical application. Later studies could monitor the dynamic changes of these three substances during the follow-up period and evaluate their temporal relationship with adverse pregnancy outcomes. Their molecular mechanisms in endothelial dysfunction, inflammatory response and vascular injury should also be explored in depth to clarify their role in hypertension pathology (Shah, 2007). In addition, the stability and reproducibility of these three markers need to be repeatedly verified in larger samples. Other validated biomarkers for predicting adverse PO could also be sought, and it could be explored whether combined monitoring of multiple markers could further improve accuracy. In summary, despite the encouraging results, there is a need to rely on large-sample, prospective studies to further clarify the promise of these molecular markers in clinical management. The goal of the study is to search for potential therapeutic targets, aiming for more accurate and personalized clinical diagnosis and intervention.

CONCLUSION

There is a close relationship between the serum levels of sEng, PLGF, and Gal-1 in HP pregnant women and their PO, and these three substances can be used as biomarkers of maternal preterm labour and IUGR, and their elevated levels can help to assess the preterm labour and IUGRPO of HP pregnant women, and only the Gal-1 level has a significant effect on the preterm abruption of the placenta.

Funding

Not applicable.

IRB approval

This study was approved by the Advanced Studies Research Board of the Sixth Medical Center of PLA General Hospital, Beijing, China.

Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of the Sixth Medical Center of PLA General Hospital, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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