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ORIGINAL ARTICLE

Maternal Serum PLGF, PAPPA, β-hCG and AFP Levels in Early Second Trimester as Predictors of Preeclampsia

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SUMMARY

Background: **Identifying women at risk of preeclampsia (PE) by maternal serum screening is conducive to prompt gestational management and thereby improve both maternal and perinatal outcomes. The purpose of the present study was to evaluate the association between the concentrations of maternal serum placental growth factor (PLGF), pregnancy associated plasma protein-A (PAPPA), free β-human chorionic gonadotropin (β-hCG), and α-Fetoprotein (AFP) and the development of preeclampsia early in the second trimester.**

Methods: **Forty pregnant women subsequently developed mild PE, 21 pregnant women subsequently developed severe PE, and 61 cases of normotensive controls were included. Maternal serum concentrations of PLGF, PAPPA, β-hCG, and AFP were measured at 15 - 20 weeks of gestation.**

Results: **Serum PLGF level was lower in women who subsequently developed PE than in normotensive controls. However, the significant difference was only found between the severe PE and control groups (p = 0.015). Serum PAPPA, β-hCG, and AFP levels were not significantly different between the PE and control groups.**

Conclusions: **Serum PLGF level was lower in women who subsequently developed severe PE early in the second trimester, suggesting its role in prediction of PE.**

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KEY WORDS

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INTRODUCTION

Preeclampsia (PE) is a specific syndrome in pregnancy associated with high blood pressure and proteinuria, which is a major cause of maternal and perinatal morbidity and mortality [1]. Nowadays, the precise etiology of PE still remains unknown, while it is widely accepted that abnormalities in the development of placental vasculature existed before the appearance of clinical symptoms [2,3]. Identifying women at risk of PE by maternal serum screening is conducive to prompt gestational management, and then improve both maternal and perinatal outcomes. A number of recognized biochemical markers were investigated for this purpose, such as placental growth factor (PLGF), pregnancy associated

plasma protein-A (PAPPA), free β-human chorionic gonadotropin (β-hCG), and α-fetoprotein (AFP). PLGF belongs to the vascular endothelial growth factor (VEGF) system, which plays a significant role in the regulation of vascular development in the placenta. Decreased expression of PLGF may interrupt the function of the uteroplacental unit and, thus, contribute to many adverse obstetric outcomes [4,5]. PAPPA is a syncytiotrophoblast-derived, insulin-like growth factor binding protein protease which is believed to play an important role in placental growth and development. Low serum PAPPA levels have been shown to be associated with a higher incidence of pregnancy loss, hypertension, PE, preterm delivery, fetal growth restriction, and fetal death [6]. AFP and β-hCG have long been recognized as screening markers for Down's syndrome in the second trimester, and early placental dysfunction may influence maternal serum AFP and β-hCG levels [7].

Systematic review suggested that low-dose aspirin administrated at or before 16 weeks of gestation reduced the risk of PE [8], so some researches attempted to extend first trimester aneuploidy screening programs to PE screening in the last decade [9-12]. Nevertheless, in China, many pregnant women only accepted maternal serum screening in the second trimester. Pregnant women who have missed the first trimester screening could benefit from closer monitoring and interventions if pregnant women with a high risk for PE could be distinguished early in the second trimester. However, predictive values of serum biochemical markers early in the second trimester were different in previous studies, some results were even contradictory. Many factors affect the levels of maternal serum biochemical markers, such as gestational age, maternal weight, multiple gestation, and maternal race. For example, compared to white women, the risk of PE appears to be lower in Asian and Hispanic women and higher in African-American women [13]. Therefore, it is valuable to study the predictive efficiency of biochemical markers for PE in local population.

The aim of present study is to examine the association between maternal serum PLGF, PAPPA, β-hCG, and AFP concentration and the development of PE in local population early in the second trimester, and to investigate whether those markers could be used as an additional test for pregnant women who have missed the first trimester screening.

MATERIALS AND METHODS

Study population

Between January 2013 and April 2014, 79 PE patients accepted second trimester maternal serum screening for aneuploidy as well as giving birth at the Department of Obstetrics and Gynecology of Nanjing Drum Towel Hospital, China. Among them, 4 cases were excluded because of *in vitro* fertilization pregnancy or twin pregnancy, and 14 pregnant women with gestational diabetes mellitus were also excluded. Of 61 PE patients included in this study, 40 cases were diagnosed with mild PE and 21 cases with severe PE. For each PE woman, one normotensive control was selected, matched according to gestation age (± 3 days), maternal weights at the collection of serum specimen (± 2 Kg), and storage time of samples at -70°C (± 2 months). Gestation age in all pregnancies was calculated based on crown-rump length at first trimester ultrasound or biparietal diameter at second trimester ultrasound. All normotensive controls had no pregnancy complications. All subjects were Chinese, no smoking habit, no infectious diseases or aneuploid fetus recognized in pregnancy. The data came from computerized inpatient medical records, which included information about clinical diagnosis, the course of pregnancy, and delivery. A protocol for the study was approved by the ethics committee of Nanjing Drum Tower Hospital. All participants provided written informed consent. The diagnosis of PE required two recordings of systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg at least 4 hours apart in previously normotensive women after 20 weeks of gestation and proteinuria of 300 mg or more in 24 hours [14]. Severe PE was defined as BP > 160/110 mmHg with either a urine dipstick showing 3 - 4+ in a random urine sample or proteinuria > 5.0 g/24 hours, or including elevated eclampsia, pulmonary edema, oliguria (less than 500 mL per 24 hours), serum creatinine, fetal growth restriction, oligohydramnios, and symptoms suggesting significant end-organ involvement (headache, visual disturbances, or epigastric or right upper quadrant pain). Women who met the criteria of PE but not severe PE were diagnosed as mild PE.

Blood samples and biochemical analysis

Blood samples were drawn from each woman between 15 + 0 and 20 + 6 weeks of gestation. Serum was separated by centrifugation at 3,000 rpm for 8 minutes. The β-hCG and AFP levels were measured as a part of the routine screening for fetal aneuploidy, while PLGF and PAPPA levels were studied retrospectively on the same serum samples.

Serum concentrations of PLGF, PAPPA, β-hCG, and AFP were measured using an AutoDELFIA 1235 System and matching reagents (Perkin-Elmer Life Science, Turku, Finland). The sensitivity of PLGF, PAPPA, β-hCG, and AFP in this assay was 5.6 pg/mL, 5 mU/L, 0.2 ng/mL and 0.1 U/mL, and intra-assay variabilities were 3.2% - 6.7%, 1.3% - 2.4%, 2.6% - 3.4%, and 1.7% - 1.8%, respectively.

Statistical analysis

Statistical analysis was done using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). The values were expressed as mean ± standard deviation (SD). Statistical evaluation was performed with Student's *t*-test. A p-value < 0.05 was considered as statistically significant.

Table 1. Baseline characteristics and PlGF, PAPPA, β-hCG, and AFP levels of the study population.

	Severe PE (n:21)	Normal Controls of Severe PE (n:21)	p	Mild PE (n:40)	Normal Controls of Mild PE (n:40)	p
Maternal age (years)	29.41 ± 3.99	29.20 ± 3.17	0.857	29.04 ± 3.72	28.41 ± 2.57	0.380
Gestational age at sampling (day)	122.19 ± 8.63	121.29 ± 7.77	0.737	121.23 ± 6.63	121.50 ± 6.71	0.837
Maternal weight at sampling (kg)	60.55 ± 9.05	60.57 ± 8.77	0.993	61.29 ± 8.01	61.15 ± 8.06	0.936
BMI (kg/m^2)	23.36 ± 3.14	22.50 ± 3.18	0.398	23.85 ± 2.89	22.86 ± 3.04	0.162
Gestational age at delivery (week)	35.86 ± 3.48	39.29 ± 1.31	< 0.001 *	38.80 ± 1.24	39.70 ± 1.525	< 0.001 *
Birth weight (g)	2495.24.67 ± 856.59	3481.43 ± 315.33	< 0.001 *	3309.50 ± 527.99	3479.25 ± 436.92	0.135
PLGF (pg/mL)	101.88 ± 63.02	169.83.96 ± 83.81	0.015 *	132.83 ± 74.10	133.30 ± 61.01	0.975
PAPPA (mU/L)	14838.10 ± 4232.16	15156.67 ± 4832.07	0.827	15088.50 ± 5643.49	15183.50 ± 4955.29	0.938
β-hCG (ng/mL)	18.55 ± 10.95	20.29 ± 17.57	0.613	18.22 ± 11.70	16.51 ± 9.20	0.473
AFP (U/mL)	47.19 ± 15.98	38.82 ± 11.63	0.065	38.09 ± 13.15	41.81 ± 19.16	0.306

Data are expressed as mean ± SD.
BMI - body mass index, PE - preeclampsia, PLGF - placental growth factor, PAPPA - pregnancy associated plasma protein-A, β-hCG - free β-human chorionic gonadotropin, AFP - α-fetoprotein.
*** p-value < 0.05 is considered as statistically significant.**

RESULTS

There were no significant differences between study groups in regards to maternal age, gestational age, maternal weight, and BMI at sampling ($p > 0.05$). Gestational age at delivery was significantly lower in women who developed PE than those in the control group ($p < 0.001$), and the fetus birth weight in the severe PE group ($p < 0.001$) was significantly lower (Table 1). Four cases of severe PE and one case of mild PE were associated with intra-uterine growth restriction.

Mean serum PLGF levels were lower in women who subsequently developed severe PE or mild PE than for the normotensive controls, while a significant difference was only found between severe PE group and control group ($p = 0.015$). Although decreased PAPPA levels were observed in both the severe PE group ($p = 0.827$) and the mild PE group ($p = 0.938$), there were no significant differences. Serum β-hCG and AFP levels were also not significantly different between PE group and control group (Table 1, Figure 1).

DISCUSSION

Previous studies have demonstrated that gestational age, maternal weight, multiple gestation, maternal race, and smoking history were influential factors for the levels of maternal serum markers, which should be corrected for evaluating the onset risk of target disease [15]. In our study, control groups matched severe PE group or mild PE group according to gestational age and maternal weights. All objects were Chinese, single gestation, got pregnant naturally, and had no smoking history. Pregnancy-related information collected from inpatient medical records to ensure clinical diagnostic criteria of PE was identical. Therefore, the influence of these factors on maternal serum marker levels was mainly avoided in this study.

Our study indicates that serum PLGF levels decreased in PE women early in the second trimester in local population, significantly in severe PE. Su et al. reported that maternal serum PLGF level was significantly decreased in PE women early in the second trimester [16], and Levine et al. observed similar changes, nevertheless, the severity of PE could not be distinguished in the two studies [17]. Serum PLGF levels show a curvilinear relationship with gestational age with an increase in the first and second trimesters, reaching a maximum at approximately 30 gestational weeks and subsequently decreasing [18]. In the placenta, placental growth factor is produced mainly by the cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast. Lower PLGF levels in PE pregnancy are mainly due to hypoxic trophoblast downregulated placental growth factor expression [19]. In this study, PLGF levels were significantly

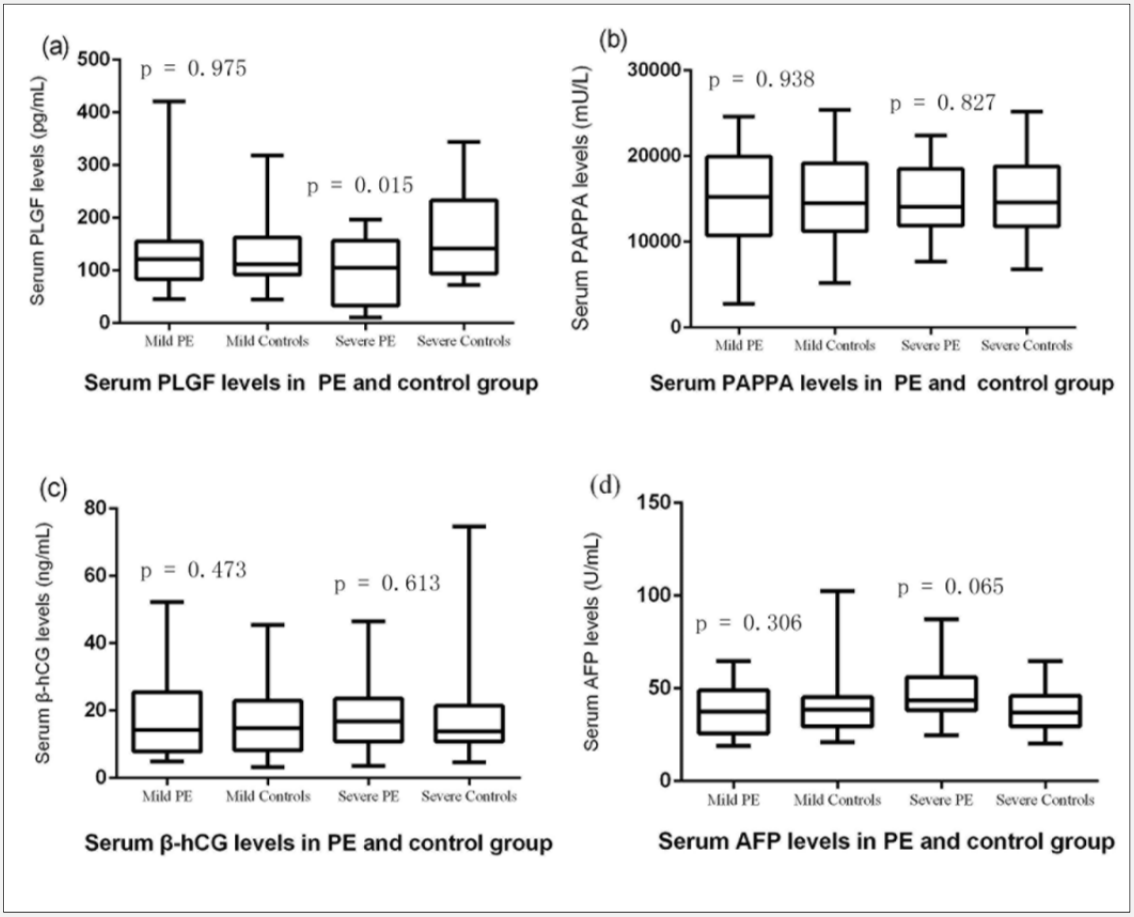

Figure 1. Maternal serum marker levels between preeclampsia and normotensive pregnancies.

decreased in severe PE rather than mild PE, which may be due to severe PE usually being associated more critical placental damage.

Our results suggested that PAPPA levels were lower early in the second trimester but without significant differences in both the severe and mild PE group. For PAPPA, a screening marker for aneuploidy in the first trimester, there were relatively few studies on the predictive value in the second trimester, some showing discrepancies. D'Anna et al. reported that serum PAPPA levels were lower in women destined to develop PE in the second trimester [20], while Bestwick et al. argued that there were no significant differences [21]. The same problems also existed in studies on β-hCG and AFP as predictors for PE. Our results indicated that measurements of β-hCG or AFP early in the second trimester were not useful in screening for the development of PE. Davidson et al. reported that human chorionic gonadotropin levels were increased by 24% in women who later developed preeclampsia, and AFP levels were not significantly different at 15 - 20 gestational weeks [22]. In contrast, Gu et al. found that AFP levels were lower, and β-hCG levels showed no statistical differences in women who subsequently developed PE [23]. We have no exact explanations for the differences between studies on PAPPA, β-hCG, and AFP levels as predictors of PE early in the second trimester. In our opinion, differences in the severity of PE among studies may be one explanation. Early-onset PE or severe PE were usually associated with more serious impaired placental trophoblastic invasion, and, as a result, more serious varieties of maternal serum markers might be detected. Therefore, different constituent ratios of early-onset PE or severe PE among previous studies may lead to different results. On the other hand, differences in ethnic composition of the study population may be one reason. It was reported that hCG and AFP levels in Black/African American women were about 10% to

15% higher than in Caucasian women [15].
Relatively small sample size is the main limitation of this study, which could cause a bias in the observations and statistical power. However, this shortage is partially made up by selecting a uniform population and differentiating the severity of PE. Further studies with a larger number of participants are required in order to obtain a median of the serum marker levels according to pregnancy week, as well as the sensitivity and specificity of serum screening markers.

CONCLUSION

Serum PLGF levels are decreased in local women who subsequently developed severe PE, suggesting that PLGF is a useful screening marker for severe PE early in the second trimester. By contrast, PAPPA, β-hCG, and AFP levels measured early in the second trimester may not be helpful in the prediction of PE. However, a large prospective study is needed to explore its use as an early predictor for the condition.

Declaration of Interest:
None.

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