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CLINICAL ARTICLE

Associations between serum placental leucine aminopeptidase and pregnancy outcomes

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ABSTRACT

Objective: To investigate the association between serum concentrations of placental leucine aminopeptidase (P-LAP) and hypertensive disorders in pregnancy (HDP), gestational diabetes mellitus (GDM), and perinatal mortality. *Methods:* In a prospective study, women with singleton pregnancies and affected by HDP, GDM, or fetal death, and those who were healthy, were enrolled at Shenzhen Seventh People's Hospital, Shenzhen, China, between January 2014 and July 2015. Serum P-LAP concentrations at delivery/fetal death were compared among the groups. The predictive value of serum P-LAP levels in fetal death was evaluated. *Results:* Serum P-LAP concentrations were (mean ± SEM) 74.02 ± 8.45 U/L in the HDP group (n = 38), 72.57 ± 12.03 U/L in the GDM group (n = 35), and 3.76 ± 3.02 U/L in the fetal death group (n = 14), all of which were significantly lower than the mean concentration of 107.11 ± 30.68 U/L in the control group (n = 30; $P = 0.031$, $P = 0.042$, and $P < 0.001$, respectively). On the basis of the receiver operating characteristic curve, low serum P-LAP levels had high sensitivity and specificity for predicting fetal death (100% and 78.9%, respectively, for a serum P-LAP cutoff of 47.07 U/L). *Conclusion:* Serum P-LAP levels were reduced among patients with HDP and GDM, and extremely low among patients affected by fetal death. Serum P-LAP is potentially a viable predictor of fetal death.

1. Introduction

Oxytocin is considered the most important uterotonic peptide hormone and has a key role in the regulation of labor [1]. During pregnancy, placental leucine aminopeptidase (P-LAP) is the only oxytocinase to degrade oxytocin in human serum and the placenta [2]. This cystine aminopeptidase belongs to the oxytocinase subfamily of the M1 family of aminopeptidases [2]. It is commonly soluble in maternal serum and is membrane-bound in the placenta [3]. P-LAP is also involved in fetal development, maintenance of homeostasis during pregnancy, blood pressure regulation, memory retention, antigen presentation, and cancer development [4,5].

Hypertensive disorders of pregnancy (HDP) and gestational diabetes mellitus (GDM) are common complications during pregnancy and cause serious maternal and fetal problems [6]. HDP is defined as a systolic blood pressure of 140 mm Hg or higher, or a diastolic blood pressure of 90 mm Hg or higher. It affects 7%–10% of pregnancies [7], and is a major cause of mortality and morbidity of fetuses, mothers, and newborns [8]. GDM, which is also associated with maternal and neonatal adverse outcomes [9], is characterized by pregnancy-induced insulin resistance or glucose intolerance: women without a previous history of diabetes exhibit high levels of blood glucose during the third trimester, resulting in maternal hyperglycemia [9,10]. Although varying criteria have been reported, GDM is diagnosed by a fasting plasma glucose level of 126 mg/dL (7.0 mmol/L) or more, or a casual plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher [11]. Women with GDM have an increased risk of hypertensive disorders, including gestational hypertension, pre-eclampsia, eclampsia, and fetal death [12].

Fetal death, or stillbirth, is a severe obstetric outcome, occurring in approximately 0.5% of all pregnancies. The cause of fetal death is complex, and 9.5%–50.2% of stillbirths are classified as unexplained [13]. Although many factors are known to contribute or interact with HDP, GDM, and fetal death, the pathogenesis of these pregnancy-related disorders is not definitively understood.

It has been suggested that P-LAP could be a viable molecular target in the treatment of gynecologic malignancies because it has been associated with poor outcomes in gynecologic cancers and is involved in regulating tumor cell progression, invasion, and angiogenesis via degradation or inactivation of peptides such as oxytocin, angiotensin, and endothelin-1 [14]. Moreover, P-LAP is a placenta-specific enzyme [15]. Serum P-LAP levels increase with length of pregnancy and diminish after delivery [16]. P-LAP is also likely to be involved in both the onset of preterm labor and pre-eclampsia via the degradation of fetoplacental peptides [17].

Data are lacking regarding the potential links between serum P-LAP levels and abnormal pregnancy outcomes such as HDP, GDM, and fetal

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death. The aim of the present study was therefore to investigate the associations between serum P-LAP levels and HDP, GDM, and fetal death.

2. Materials and methods

The present prospective study included pregnant women who delivered at the Department of Obstetrics, Shenzhen Seventh People's Hospital, Shenzhen, China, between January 1, 2014, and July 31, 2015. The study population comprised women affected by HDP, GDM, or fetal death, and healthy women with full-term delivery. The inclusion criteria were: a normal menstrual cycle and a known length of pregnancy; delivery at 28–41 weeks for women in the HDP, GDM, and fetal death groups, and at 37–40 weeks for those in the control group; natural conception; singleton pregnancy; no other complications; and no oxytocin use. The Ethics Committee of Shenzhen Seventh People's Hospital approved the study, and written consent was obtained from all participants.

Women with HDP were diagnosed on the basis of a systolic blood pressure of 140 mm Hg or more, or a diastolic blood pressure of 90 mm Hg or more, as determined by the mean of at least two measurements on the same arm taken 15 minutes or more apart [7]. Women with HDP had pregnancy-induced hypertension or complications of proteinuria, mild or severe pre-eclampsia and eclampsia (with blood pressure restoring to the normal range at 12 weeks or less after delivery), or had been diagnosed with hypertension before pregnancy.

GDM was diagnosed in accordance with the modified criteria of the International Association of Diabetes and Pregnancy Study Group [9,11]: a fasting blood glucose level of 5.1 mmol/L or higher, or a blood glucose level of 10.0 mmol/L or higher at 1 hour, or 8.5 mmol/L or higher at 2 hours on a 75-g 2-hour oral glucose tolerance test indicated GDM. Any individual abnormal value during pregnancy was considered to be a positive sign of GDM.

Fetal death was defined as decrease of fetal movement and fundal growth after 28 weeks of gestation, and was verified by the absence of a fetal heart beat on ultrasonography.

All healthy women attending the hospital for full-term delivery in the study period were approached to participate in the present study. Those who consented, had no complications (e.g. hypertension, pre-eclampsia, hepatitis, diabetes, or other chronic diseases), and met the other study inclusion criteria were included.

Venous blood samples were obtained from patients with HDP, GDM, or fetal death immediately after admission to the study hospital, and were collected from healthy pregnant women at full-term delivery. Serum samples were purified by a conventional procedure and stored at –70°C until analysis. After admission, general information including age, number of pregnancies, body weight, and height was collected from all participants. After delivery, recorded data included manner of delivery (natural or cesarean), newborn sex, parity, week of delivery, and 1-minute and 5-minute Apgar scores.

Serum P-LAP levels were measured using L-leucine-*p*-nitroanilide as a substrate in the presence of 20 mM l-methionine, as described previously [4,18], via a kit for the detection of leucyl-cystinyl aminopeptidase (Wuhan USCN Business, Wuhan, China). The P-LAP activity in all samples was assayed in duplicate, and the results were expressed as IU/mL. The lower detection limit of the assay was 5 IU/mL.

Within each group, the participants were divided into three subgroups according to the week of delivery or fetal death: $28–36^{+6}$ weeks, 37–40 weeks, and 40^{+1} weeks.

Statistical analyses were done with SPSS version 17.0 (SPSS Inc, Chicago, IL, USA). All data are presented as mean ± standard error of the mean or number (percentage). Serum P-LAP levels at similar pregnancy lengths among different groups or at different pregnancy lengths within one group were compared by Student *t* test. Percentages among different groups were compared by Pearson χ^2 test. The P-LAP data from the four groups were used to generate a receiver operating characteristic (ROC) curve. The area under the curve (AUC) was calculated to predict the risk of fetal death on the basis of serum P-LAP levels. $P<0.05$ was considered to be statistically significant.

3. Results

Overall, 117 women were enrolled: 38 women had HDP, 35 had GDM, 14 experienced fetal death, and 30 were healthy and underwent full-term delivery (control group). Age, number of pregnancies and deliveries, body weight, and height were similar across all four groups (Supplementary Material S1). There were no significant differences in type of delivery or neonate sex ratios among the four groups (Table 1). Among the women affected by fetal death, however, fetal body weight was lower and the length of pregnancy at delivery was shorter than among women in each of the other three groups (Table 1). The newborn Apgar scores at 1 and 5 minutes did not differ significantly among the control, HDP, and GDM groups, but were regarded as 0 in the fetal death group (Table 1).

Mean serum P-LAP concentration was 74.02 ± 8.45 U/L in the HDP group, 72.57 ± 12.03 U/L in the GDM group, 3.76 ± 3.02 U/L in the fetal death group, and 107.11 ± 30.68 U/L in the control group. Serum P-LAP levels in the HDP, GDM, and fetal death groups were significantly lower than were those in the control group ($P=0.031$, $P=0.042$, and $P<0.001$, respectively) (Fig. 1). Moreover, serum P-LAP levels were significantly higher in the HDP and GDM groups than in the fetal death group (both $P<0.001$) (Fig. 1). Serum P-LAP levels increased significantly between $28–36^{+6}$ weeks and 37–40 weeks in the HDP ($P=0.035$) and GDM ($P=0.012$) groups (Fig. 2A). However, the P-LAP levels decreased significantly after 40 weeks as compared with 37–40 weeks in the HDP ($P=0.047$) and control ($P=0.048$) groups (Fig. 2A). Serum P-LAP levels among women in the fetal death group remained low at all pregnancy lengths (Fig. 2A).

Serum P-LAP levels were compared among the different groups at similar gestational ages. Serum P-LAP levels in the fetal death group were significantly lower than those in the other three groups at every gestational age ($P<0.001$). Serum P-LAP levels after 37 weeks were significantly lower in the HDP and GDM groups than in the control group ($P=0.046$ and $P=0.041$, respectively, at 37–40 weeks; and $P=0.042$ and $P=0.048$, respectively, after 40 weeks) (Fig. 2B). Notably, serum P-LAP levels at any of the three gestational ages were statistically similar in the HDP and GDM groups (Fig. 2B).

To explore the predictive value of serum P-LAP in perinatal mortality, a ROC curve was generated using the serum P-LAP levels from the four groups. The AUC was calculated as 0.75, suggesting that serum P-LAP might be predictive of perinatal mortality (Fig. 3). For a serum P-LAP cutoff value of 47.07 U/L, the sensitivity and specificity for predicting perinatal mortality were 75.0% and 44.7%, respectively. The sensitivity and specificity for predicting perinatal mortality gradually improved with decreasing serum P-LAP levels: the sensitivity and specificity for predicting perinatal mortality were, respectively, 98.6% and 68.4% for a serum P-LAP cutoff of 12.82 U/L, 100% and 78.9% for a cutoff of 4.71 U/L, and 100% and 100% for a cutoff of 0.11 U/L.

4. Discussion

The present study found that serum P-LAP levels were lower among women with HDP or GDM than among healthy pregnant women, and were extremely low among those with fetal death. Notably, serum P-LAP levels in the HDP and GDM groups gradually increased from 28 to 40 weeks of pregnancy, but significantly decreased after 40 weeks.

The total content of fetus-derived oxytocin has been shown to increase from early gestation, while maternal serum P-LAP levels also increase with the progression of pregnancy [1,17]. Similar findings were observed among patients with HDP and GDM in the present study, for whom serum P-LAP levels gradually increased from 28 to 40 weeks. Changes in maternal serum P-LAP can be a useful biomarker not only for monitoring fetoplacental function among patients with

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