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Relationship between first-trimester serum placental protein-13 and maternal characteristics, placental Doppler studies and pregnancy outcome

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Abstract

Objective: To examine potential correlations between maternal serum placental protein-13 (PP-13) and first trimester maternal and placental factors, and to evaluate the association of this marker with adverse pregnancy outcome.

Methods: Serum samples from prospectively enrolled patients between 11 and 13 weeks and 6 days were analyzed for PP-13 using an ELISA assay. The relationships between maternal serum PP-13 levels and gestational age, maternal age, ethnicity, parity, smoking status, body mass index (BMI), mean arterial blood pressure, uterine and umbilical artery Doppler parameters were examined. The association between first-trimester PP-13 levels and subsequent pre-eclampsia and delivery of a small for gestational age (SGA) neonate was also investigated, after excluding patients who received aspirin.

Results: In 908 patients, PP-13 levels ranged from 8.0 to 537.5 pg/mL. A significant negative correlation was identified between PP13 and BMI (Spearman rho −0.20, $P<0.0001$). Smoking significantly decreased PP-13 ($P<0.01$). No relationship was identified with the other parameters. In a subgroup of 668 low-risk patients who did not receive aspirin, PP-13 levels were not associated with development of pre-eclampsia, SGA or the combination of them.

Conclusion: First-trimester PP-13 levels are significantly correlated with BMI and smoking. These correlations appear independent of uterine and umbilical artery resistance. In low risk patients, PP-13 levels fail to predict the risk for pre-eclampsia or SGA.

Keywords: Fetal Doppler ultrasound; placenta; PP-13; pre-eclampsia; SGA; small-for-gestational age.

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Introduction

In normal pregnancy, early placental growth, angioneogenesis and synthesis of trophic substances are important for physiologic changes that can be observed in the mother, placenta and fetus as early as 8–12 weeks' gestation [1]. There is a significant decline in mean arterial pressure, decreasing blood flow impedance in the uterine and umbilical arteries, and exponential placental and fetal growth. Abnormalities in this early placental development lead to incomplete physiological adaptation and are precursors of placental dysfunction placing women at risk for hypertensive gestational disorders and fetal growth restriction [2, 3]. Identification of markers of suboptimal early placental development opens possibilities for an integrated screening approach. In this context it has been demonstrated that the combination of maternal factors, biochemical markers and ultrasound evaluation offers superior prediction but requires careful consideration of interactions in the construction of a screening model [4, 5]. Accordingly, recognition of interactions between first-trimester markers is important in the construction of effective risk prediction strategies.

Placental protein 13 (PP-13) is a 32-kDa protein located in the brush border at the maternal-fetal interface of the syncytiotrophoblast layer that is involved in placental implantation and spiral artery remodeling [6, 7]. Decreased levels and impaired responsiveness to PP-13 are thought to play a role in the pathogenesis of placental dysfunction [8, 9]. While several screening studies have demonstrated significantly lower levels of PP-13 in the first trimester in pregnancies with subsequent pre-eclampsia

and fetal growth restriction [3, 6, 10–15], some other reports failed to find such association [16–19]. In these studies, predictive accuracy varied among ethnic groups, patient demographics, and case control studies. In addition, maternal factors such as the ABO blood type appear to affect measurements of PP-13 concentration [20]. In view of these findings it was the aim of this study to evaluate the relationships between PP-13 levels and maternal and placental factors in the first trimester of pregnancy and to determine the association with adverse pregnancy outcome in women who did not receive aspirin treatment in pregnancy.

Materials and methods

Study population and design

This is a nested multi-site prospective observational study of women with a singleton gestation presenting for first-trimester screening at four hospitals in the Baltimore metropolitan area between 2007 and 2010. The protocol was approved by the Institutional Review Boards of each participating center. Ethical review was not required for this study. On arrival to the fetal medicine unit, all patients were informed about the study and after the first-trimester ultrasound confirmed a live singleton intrauterine pregnancy at 11 weeks and 0 day to 13 weeks and 6 days gestational age, a request was made to join the study.

After written informed consent, maternal demographics, medical and obstetric history and self-reported smoking status were recorded. Gestational age was calculated from the last menstrual period and confirmed by ultrasound crown-rump length (CRL) measurement. Doppler ultrasound of the maternal uterine and fetal umbilical arteries was performed using the trans-abdominal approach. The uterine artery Doppler waveform was assessed semi-quantitatively using the pulsatility index (PI) and qualitatively for the presence of a diastolic notch. The umbilical artery Doppler waveform was also analyzed using the PI and noting the presence of forward end diastolic velocity. Ultrasound output was set to yield thermal and mechanical index values below 0.8 in the region of interest, following the as low as reasonably achievable (ALARA) principle [21].

After completion of the ultrasound examination, maternal height and weight were measured. Following seated rest for 5 min a single automated blood pressure measurement was obtained (Dinamap Pro1000, General Electric Medical Systems, Milwaukee, WI, USA). For this measurement the cuff size was adjusted to the maternal arm circumference and the arm was supported at the level of the heart. The mean arterial blood pressure [MAP=(systolic blood pressure+2×diastolic blood pressure)/3] was calculated.

All values were entered into a database under the assigned unique patient identifier. A 5 ml blood sample was then collected into a serum separation tube using occlusive venipuncture. The tubes were marked with the patient identifier, centrifuged at 8000 rpm for 15 min and stored at −4°C within 60–90 min of the blood draw and stored at −80°C in 1 cc aliquots.

Pregnancy outcome and corresponding source documentation was collected by study personnel. Cases of spontaneous or therapeutic abortion, intrauterine demise, major fetal anomaly or aneuploidy were excluded from the analysis. Delivery circumstances, birth-weight and birth-weight percentile were ascertained. Small for gestational age (SGA) neonates were defined as those having a birth-weight (BW) below the 10th percentile by reference standards. Pre-eclampsia was defined as hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) on at least two occasions, 6 h to 7 days apart, in a woman with previously normal blood pressure, associated with proteinuria (>300 mg protein in 24 h collected urine, without prior proteinuria) after 20 weeks of gestation [22].

PP-13 immunoassay

Frozen stored serum samples were thawed slowly and brought to room temperature for examination by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA). They were diluted 1:3 and overlaid onto a 96-well microplate coated with PP13 specific mouse mono-clonal antibody (MAb) and incubated overnight at room temperature. The microplates were then washed and the complex was marked with a second PP13 specific MAb conjugated with biotin. The complex was amplified with the biotin-extravidin-horseradish-peroxidase complex. The assay was then developed with tetra-methyl-benzidine substrate, as previously described [9]. The optical density was measured at 450 nm against a 650 nm background. Concentrations were determined by extrapolation from a standard curve constructed using recombinant PP-13 standards (0–400 pg/mL). The laboratory staff performing the PP-13 assays had only sample codes and was blinded to pregnancy outcome. Both sonographers, who acquired the Doppler measures, and physicians, who observed the patients, were blinded to the results of PP-13 assays to avoid any observer bias. The performance of PP-13 ELISA kit and assay were previously assessed in a validation study on samples from 56 pregnant and 56 non-pregnant patients. Samples were tested in duplicates. The specificity of the assay was 90.5%. The coefficients of variation (CV) were set sequentially to fit the following PP-13 values: <9% for PP13 above 25 pg/mL, up to 16% for PP13 between 12.5 and 24.9 pg/mL and up to 23% for PP13 values below 12.5 pg/mL. At this level, the optical density (OD) was 0.01–0.04 OD and therefore 0.01 OD unit difference corresponds to 20% CV which is only up to 1 pg/mL PP13.

Statistical analysis

The relationships between maternal serum levels of PP-13 and gestational age, maternal age, ethnicity, parity, smoking, body mass index (BMI), mean arterial blood pressure (MAP), uterine and umbilical Doppler parameters were examined. All Doppler indices were converted into z-scores using reference values derived from pregnancies with documented normal outcome from our study cohort. The relationship of PP-13 levels with gestational age and maternal characteristics was explored using a non-parametric correlation analysis (Spearman's coefficient), since neither raw nor log-transformed PP-13 values fitted a Gaussian distribution.

In order to evaluate the correlation with pregnancy outcomes, the measured PP-13 concentrations were then converted into multiples of the median (MoM). A Mann-Whitney test was used to estimate the association between first-trimester PP-13 MoM and: a)

development of pre-eclampsia, b) delivery of an SGA neonate and c) a composite outcome of pre-eclampsia or SGA, after excluding patients who received aspirin or enoxaparin in pregnancy. At our Institutions aspirin treatment is initiated in the first trimester in patients considered at high risk for pre-eclampsia or intrauterine growth restriction based on obstetric history (previous pre-eclampsia or SGA), first-trimester maternal characteristics (chronic hypertension, pregestational diabetes, high BMI) and the presence of bilateral notch in first-trimester uterine artery Doppler [23].

Statistical analysis was performed with SPSS 20.0 (SPSS Inc, Chicago, IL, USA). A P-value <0.05 was considered statistically significant.

Results

A total of 942 patients were consecutively enrolled. Of these, 34 met one of the exclusion criteria. The characteristics of 908 women included in the analysis are reported in Table 1. The mean gestational age at enrollment was 12.6 weeks. The median maternal age was 31 years and women were predominantly of Caucasian or African-American ethnicity. Over one third of women were nulliparous. Pre-existing medical conditions were infrequent and 53 (5.8%) women reported pre-eclampsia or intrauterine growth restriction (IUGR) in a previous pregnancy. Tobacco use was reported in 9.4% of patients. At first-trimester enrollment, 30% of women met criteria for obesity and 36 women (4%) had a mean arterial pressure above 100 mm Hg. The mean uterine artery PI z-score was 0.16 (IQR −0.60, 0.88) and bilateral notching was observed in 30% of women. Umbilical artery end-diastolic velocity was present in the majority of fetuses (619, 68.2%).

The median concentration of PP-13 was 73.7 pg/mL (IQR: 47.8–111.1 pg/mL). The median levels for each gestational week are reported in Table 2. The levels showed a weak correlation with gestational age (Spearman's rho −0.07, P=0.04).

Table 1: Characteristics of the study population at enrollment.

Demographic and obstetric characteristics	n=908
Maternal characteristics	
Age	31 (25, 36)
Advanced maternal age(>35 year)	308 (33.9%)
Ethnicity	
African American	404 (44.5%)
Caucasian	432 (47.6%)
Hispanic	53 (5.8%)
Other	19 (2.1%)
Pre-existing conditions	
Tobacco use	85 (9.4%)
Diabetes mellitus	42 (4.6%)
Hypertension	79 (8.7%)
Obstetrical history	
Nulliparity	370 (40.7%)
Prior pre-eclampsia	49 (5.4%)
Prior IUGR	4 (0.4%)
Gestational age at examination (weeks)	12.6 (12.1, 13.0)
First trimester maternal physical examination	
Body mass index (kg/m^2)	26.2 (22.7, 31.4)
Body mass index ≥30 kg/m^2	275 (30.3%)
Systolic blood pressure (mm Hg)	114 (106, 123)
Diastolic blood pressure (mm Hg)	67 (63, 72)
Mean arterial blood pressure (mm Hg)	82.7 (78.0, 88.3)
Mean arterial blood pressure >100 mm Hg	36 (4.0%)
First trimester ultrasound examination	
Mean uterine artery PI z-score	0.16 (−0.60, 0.88)
Bilateral notching	274 (30.2%)
Umbilical artery PI z-score	−0.14 (−0.71, 0.42)
Umbilical artery end-diastolic velocity[a]	
Present	619 (68.2%)
Absent	238 (26.2%)

Data are given as n (%) or median and interquartile range (IQR, 25th–75th percentile).
IUGR=Intrauterine growth restriction.
[a]Missing value in 51 patients.

Relationship between PP-13 levels and first-trimester parameters

No association was found between first-trimester PP-13 concentrations and maternal ethnicity, parity or mean arterial blood pressure. PP-13 levels showed a significant negative correlation with BMI (Spearman's rho −0.20, P<0.0001) and a weaker positive correlation with maternal age (Spearman's rho 0.11, P=0.001). Similarly, women with a BMI greater 30 kg/m^2 had significantly lower PP-13 levels, and advanced maternal age was associated with higher values (P<0.01) (Table 3). The correlation with BMI was stronger in Caucasian than in African-American

Table 2: Distribution of placental protein 13 levels (pg/mL) throughout gestational age.

Gestational week	n	Median PP-13 level (pg/mL)	Interquartile range (25th–75th percentiles)
11 weeks and 0 day to 11 weeks and 6 days	137	72.2	52.9, 110.6
12 weeks and 0 day to 12 weeks and 6 days	531	76.9	48.1, 112.7
13 weeks and 0 day to 13 weeks and 6 days	240	69.5	43.7, 105.9

Table 3: Distribution of placental protein 13 levels (pg/mL) in relation to maternal characteristics, uterine and umbilical artery Doppler parameters.

Variable	No	Yes	P-value
Advanced maternal age (>35 years)	68.6 (44.1, 107.4)	83.5 (53.0, 120.4)	<0.01
African-American ethnicity	77.6 (48.1, 116.5)	70.9 (47.4, 105.4)	0.07
Nulliparity	74.9 (44.7, 109.8)	72.4 (49.4–111.8)	0.37
Tobacco use	76.9 (49.9, 113.4)	49.9 (31.7,88.2)	<0.01
BMI >30 Kg/m^2	80.7 (51.6, 120.1)	61.7 (38.1, 92.9)	<0.01
First trimester MAP>100 mm Hg	74.0 (47.9, 111.1)	66.2 (35.0–115.2)	0.49
Bilateral notching	73.4 (47.8, 110.3)	74.2 (47.4, 111.8)	0.77
Umbilical artery absent end-diastolic flow	73.2 (44.8, 110.7)	78.8 (53.8, 115.0)	0.10

PP-13 levels are given as median and interquartile range (IQR, 25th–75th percentile).
All tests are Mann-Whitney-U.
BMI=Body mass index, MAP=mean arterial blood pressure.

women (Spearman's rho −0.23 and −0.14, respectively). Cigarette smoking was associated with a profound decrease in PP-13 levels (P<0.01). First-trimester placental blood flow studies did not show any significant relationship with PP-13 values.

A multivariate linear regression analysis was then performed to determine the independence and relative contribution of variables identified as significant in the correlation analyses. After linear regression analysis, only BMI and smoking were found to be independent predictors of PP-13 concentration, while maternal age and gestational age showed no effect.

Relationship between PP-13 levels and outcome

After exclusion of 240 women that received first-trimester aspirin, the association between first-trimester PP-13 concentrations and pre-eclampsia or SGA was evaluated in 668 women considered at low-risk for placenta-related diseases. The incidence of pre-eclampsia and small for gestational age in this group was 3.3% and 6.4%, respectively. Maternal characteristics, delivery outcome and PP-13 levels are described in Table 4. In order to evaluate the correlation with pregnancy outcomes, the measured PP-13 concentrations were converted into multiples of the median (MoM) corrected for maternal BMI. No significant association was found between first-trimester PP-13 MoM and development of pre-eclampsia, SGA or a composite outcome of pre-eclampsia or SGA (P=0.99, P=0.72, P=0.46) (Figure 1). Even when the analysis was limited to cases of severe SGA with a birth-weight <5th percentile, no difference was found in the levels of PP-13 compared to controls.

Discussion

The aims of this study were to determine the relationship between maternal and placental characteristics and serum PP13 levels in the first trimester and further to test the relationship between PP13 levels and adverse outcome in low-risk women. First-trimester PP-13 correlated negatively with maternal BMI and was profoundly decreased by maternal smoking. In contrast, no relationship was observed with maternal blood pressure or Doppler parameters of the uterine and umbilical arteries. In low-risk patients PP-13 did not predict placenta-related disease at birth.

Recognition of interactions between first-trimester biomarkers and patient characteristics is important in the construction of effective risk assessment strategies.

In most studies, absolute PP-13 levels were converted to gestational week- specific multiples of the median (MoM), which in some cases were further adjusted for maternal age, weight, smoking or ethnicity [3, 11, 16]. These adjustments, however, are not uniform between studies. In the study by Nicolaides et al. [10], PP-13 was not significantly associated with maternal age, parity or weight. Similarly, Chafetz et al. [6] found no association between PP-13 levels and maternal age, ethnicity, arterial blood pressure or maternal BMI among controls. Consequently, no adjustments for these variables were made. On the other hand, and in accordance to our results, other studies found a significant inverse correlation between PP-13 and a) maternal BMI or weight [3, 11, 16, 24] and b) tobacco use in unaffected controls [16, 24]. These findings suggest that there is heterogeneity in the relationship between PP-13 and maternal characteristics across different populations and that the adjustment of MoM levels may not be uniformly applicable.

Table 4: Maternal characteristics, delivery outcomes and first-trimester PP-13 MoM in low-risk patients grouped by pregnancy outcome.

Characteristics of the study population	All (n=668)	Unaffected (n=609)	PE (n=22, 3.3%)	Early-PE (n=2, 0.3%)	SGA (n=43, 6.4%)	SGA<5th percentile (n=21, 3.1%)	SGA or PE (n=59, 8.8%)
Gestational age at enrollment (weeks)	12.4 (12.1, 13.0)	12.6 (12.1, 13.0)	12.3 (12.1, 13.1)	12.9 (12.2, 13.5)	12.6 (12.3, 13.2)	12.6 (12.4, 13.3)	12.4 (12.1, 13.1)
Maternal age (years)	31 (26, 36)	31 (26, 36)	29 (25, 37)	30 (25, 35)	33 (24, 36)	33 (23, 38)	32 (25, 36)
BMI (kg/m^2)	26.3 (23.1, 31.3)	26.3 (23.1, 31.3)	29.3 (25.3, 35.1)	36.4 (23.3, 49.6)	25.5 (21.5, 28.4)	25.9 (21.7, 28.8)	26.2 (22.9, 31.1)
Nulliparity	270 (40.4%)	244 (40%)	12 (54.5%)	1 (50%)	18 (41.9%)	9 (42.9%)	26 (44.1%)
Smoking	63 (9.4%)	54 (8.9%)	3 (13.6%)	0	7 (16.3%)	3 (14.3%)	9 (15.3%)
MAP (mm Hg)	82 (77, 88)	82 (77, 88)	91 (82, 102)	103	81 (76, 87)	81 (77, 90)	83 (78, 91)
Gestational age at delivery (w)	39.1 (38.2, 40.0)	39.3 (38.3, 40)	37.8 (34.8, 39.3)	31.0 (29.9, 32.1)	39 (37.7, 39.4)	38.7 (36.8, 39.3)	38.9 (37.3, 39.4)
Infant BW (g)	3291 (2980, 3579)	3330 (3065, 3600)	2916 (2293, 3346)	1187 (725, 1650)	2560 (2285, 2699)	2304 (2030, 2515)	2619 (2365, 2860)
PP-13 (pg/mL)	74.9 (47.9, 109.8)	74.9 (48.1, 110.3)	72.7 (40.7, 103.8)	88.7 (78.3, 99.2)	78.1 (55.2, 98.1)	80.7 (59.9, 111.3)	74.8 (45.1, 97.2)
PP-13 MoM	1.03 (0.65, 1.50)	1.03 (0.65, 1.52)	1.16 (0.62, 1.6)	1.6 (1.2, 2.0)	0.99 (0.75, 1.45)	1.13 (0.79, 1.5)	0.96 (0.66, 1.36)

PE=Preeclampsia, SGA=small-for-gestational age, BW=birth-weight, MAP=mean arterial blood pressure.
Data are presented as median and interquartile range (IQR, 25th–75th percentile) or n (%).

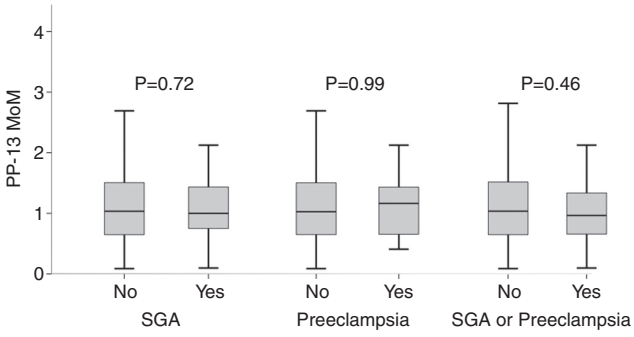

Figure 1: Box plots display of PP-13 MoM as a function of preeclampsia and SGA. (SGA=Small for gestational age.)

In the absence of a large multimarker cohort study, it is difficult to ascertain the reason for the inverse correlation between PP-13 and BMI. A possible explanation is to consider the placenta as the single source of PP13, with the BMI having a dilutional effect. However, other mechanisms are possible. Body mass index elevation is one of the components of the metabolic syndrome that is associated with placental disfunction. It is possible that lower PP-13 levels in women with increasing BMI could be a reflection of early placental dysfunction produced by metabolic risk factors [25]. Clarification of the underlying mechanism is important to determine if BMI adjustement is appropriate.

The lack of a correlation between PP-13 levels and uterine artery Doppler characteristics in our population indicates an independent role of these two markers. This can be explained by the fact that uterine artery velocity waveform and serum biomarkers such as PP-13 and PlGF reflect different vascular areas, and therefore are independent of each other [26]. Their use in combination in a contingent screening for pre-eclampsia has been suggested [10]. In a large study by Odibo et al. [13], however, combinations of two or more of the first-trimester markers (PP-13, PAPP-A and uterine artery Doppler) failed to yield an improvement above single marker sensitivity for the prediction of pre-eclampsia [13]. This likely reflects that some of the parameters studied (e.g. PP-13 and PAPP-A) are involved in the same pathway of maternal decidual modification in early pregnancy [13]. Similarly, measurement of serum PP-13 in the first trimester appeared not to improve the performance of screening for early pre-eclampsia achieved by a combination of maternal factors, uterine artery PI and serum PAPP-A in a case control study including 208 cases that developed pre-eclampsia [24]. However, there are also studies in which PP13 and mean arterial pressure [27], PP13 and PlGF [14] and PP13 with Doppler [28] showed improved prediction.

When we looked at the ability of PP-13 to predict pre-eclampsia or SGA in a population considered at low risk for placental-related disease, we were unable to find any significant correlation between first-trimester levels of this marker and the outcomes investigated. Previous studies on the predictive properties of PP-13 for pre-eclampsia and SGA yielded conflicting results. While some studies found significantly lower first-trimester levels of PP-13 in pregnancy complicated by pre-eclampsia [3, 6, 10, 12–14, 29] or SGA [6, 15, 29] suggesting a potential incorporation of this biomarker in the risk assessment for pre-eclampsia, especially for the early-onset form [3, 10], other cohorts and case-control studies failed to find such associations [16–19]. Moreover, even when a significant association is found, the predictive accuracy varies, with some studies showing promising results [3, 6, 10–12] and others showing less remarkable predictive properties [13–15, 24, 29]. In their systematic review and meta-analysis of 8 studies, Schneuer et al. reported sensitivity rates of 24% and 45% for pre-eclampsia and early-onset pre-eclampsia, respectively, at 5% false positive rate [29]. In a more recent meta-analysis of 19 studies, the detection rate ranged from 47% for all cases of pre-eclampsia to 83% for early cases alone, at a 10% false positive rate [30]. A possible explanation of the difference among studies is the variation in study design, numbers of cases, employed assay, and the characteristics of the study population [29]. Our study differs from previous reports because we excluded patients who received aspirin, who were considered at high risk for pre-eclampsia based on history or first-trimester screening. For this reason, our results suggest that while PP13 may be a statistically valid cofactor in an unselected population, it does not have sufficient independent discriminatory power in women at low risk for pre-eclampsia. It is unclear if this is due to the heterogeneity of first-trimester associations between first-trimester PP-13 and maternal variables in different study populations or due to variability in the association with outcome. Recently, single nucleotide polymorphism of the PP-13 sequence was determined between White and Black British women in the United Kingdom and also between South African and British Black women, providing additional explanation for study result diversity (Meiri et al., 14th World Congress in Fetal Medicine, 2015).

The median PP-13 concentrations found in our cohort are consistent with those reported in previous studies, where PP-13 median levels range from 53 to 66 pg/mL in the control group [16, 24, 29, 31]. The wide range of variation observed in PP13 levels probably reflects the diversity of this protein release from the placenta to the maternal blood and the potential various factors influencing this release.

One possible limitation of our study is that we used the automated ELISA method for the quantification of PP-13, while the newest AutoDELFIA technique has been recently reported to be a more robust and reproducible assay than the ELISA [32]. However, ELISA technique has been demonstrated to have a better clinical discrimination between PP-13 levels in cases of pre-eclampsia and in controls [32], and it has been employed in numerous previous studies. Further studies are needed to compare the performance the two assays.

In conclusion, first-trimester PP-13 levels show significant correlation with BMI, a maternal risk factor for subsequent placental dysfunction, and with tobacco use. These relationships appear independent of uterine or umbilical artery Doppler. In a population at low risk for pre-eclampsia by first-trimester screening, PP-13 levels failed to independently identify women at risk for placental dysfunction.

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