Placental Protein 13 as an Early Predictor in Egyptian Patients With Preeclampsia, Correlation to Risk, and Association With Outcome

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Introduction: Placental protein 13 (PP13) is a protein expressed only in the placenta. It is involved in gluing the placenta to the uterus and remodeling the maternal arteries to expand them. Women who subsequently develop preterm preeclampsia have low first trimester maternal serum.

Aim of Work: The aim of this work was to assess the value of PP13 as an early marker for screening of preeclampsia and to correlate it with the PP13 messenger RNA (mRNA).

Patients and Methods: As a part of the Antenatal Screening Project, 100 women in the first trimester of pregnancy were selected and subdivided into 2 groups: 50 women who developed preeclampsia in their third trimester (patient group) and 50 women who completed normal uncomplicated pregnancy until full term (control group). Placental protein 13 level was measured using the commercially available enzyme-linked immunosorbent assay kit and PP13 mRNA was tested using reverse transcription polymerase chain reaction.

Results: The maternal serum PP13 level in the preeclamptic group was (157.9 ± 45.5 pg/mL), which is significantly lower than that of the control group (225.3 ± 67.3 pg/mL), with highly statistically significant difference ($P < 0.0001$). The frequency of maternal PP13 mRNA expression was lower in the preeclamptic group (28%) compared to that in the control group (76%), with highly statistically significant difference ($P < 0.0001$).

Conclusion: Combined serum PP13 level assay and PP13 mRNA expression are reliable markers for early detection of preeclampsia, and we recommend doing it as a routine investigation during the first trimester.

Key Words: preeclampsia, PP13, enzyme-linked immunosorbent assay, mRNA, RT-PCR

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Preeclampsia is a medical condition where hypertension arises in pregnancy (pregnancy-induced hypertension) in association with significant protein in the urine. Its cause remains unclear, although the principal cause seems to be a substance or substances from the placenta causing endothelial dysfunction in the maternal blood vessels.[1]

Whereas blood pressure elevation is the most visible sign of the disease, it involves generalized damage to the maternal endothelium and kidneys and liver, with the release of vasopressive factors only secondary to the original damage.[1]

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Preeclampsia may develop at varying times within pregnancy, and its progress differs among patients; most cases are diagnosed preterm. Preeclampsia occurs in 6% of pregnancies, usually in the second or third trimester and after the 32nd week. Some women will experience preeclampsia as early as 20 weeks, although this is rare. It is much more common in women who are pregnant for the first time.[2]

Although much research into the etiology and mechanism of preeclampsia has taken place, its exact pathogenesis remains uncertain. Most studies support the notion of inadequate blood supply to the placenta, making it release particular hormones or chemical agents that, in mothers predisposed to the condition, leads to damage of the endothelium (lining of blood vessels), alterations in metabolism, and inflammation.[1]

Studies suggest that hypoxia resulting from inadequate perfusion up-regulates soluble fms-like tyrosine kinase receptor-1 (sFlt-1), a vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF) antagonist, leading to a damaged maternal endothelium and restriction of placental growth.[3] In addition, endoglin, a transforming growth factor β antagonist, is elevated in pregnant women who develop preeclampsia.[4] Soluble endoglin (sEng) is likely up-regulated by the placenta in response to an up-regulation of cell-surface endoglin produced by the maternal immune system, although there is also the potential that sEng is produced by the maternal endothelium. Levels of both sFlt-1 and sEng increase as severity of disease increases, with levels of sEng surpassing the levels of sFlt-1 in hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome cases.

Placental protein 13 (PP13), a homodimer galectin made of two 16-kd monomers (molecular weight, 32 kd), is predominantly produced in the placenta, rare malignant tumors, and juvenile glands. It is thought to be involved in normal placentation and maternal artery remodeling.[5–9]

Nicolaides et al.[10] found a significant reduction of serum PP13 levels at 11 to 13 gestational weeks in women who subsequently developed early preeclampsia. This was subsequently confirmed by Spencer et al.[11] and also by Chefetz et al.[12]

Considering the putative role of PP13 in placentation,[5,6] we hypothesized that testing PP13 levels at 6 to 10 gestational weeks could predict preeclampsia better than later testing.

The aim of the study was to assess maternal PP13 early in the first trimester and to correlate this with the maternal PP13 messenger RNA (mRNA) expression in the first trimester.

STUDY DESIGN

This is a comparative, prospective case-control study. Placental protein 13 was measured by solid-phase sandwich enzyme-linked immunosorbent assay in serum samples that were collected at the first prenatal visit between 9 to 12 weeks of gestation (as a part of antenatal screening project), from women who subsequently experienced preeclampsia (n = 50), Women with uncomplicated term deliveries served as control subjects (n = 50) and were matched to cases by gestational age.

Patients and Methods

This comparative, prospective case-control study was carried out on 100 women in the first trimester of pregnancy (as a part of antenatal screening project) at the Department of Obstetrics and Gynecology, Kasr El-Eini Teaching Hospital, Faculty of Medicine, Cairo University, during the period from December 2010 to July 2011, and was approved by the local ethics committee. The subjects were further subdivided later in pregnancy into 2 groups: 50 women who developed preeclampsia in the third trimester (patient group) and 50 women who completed normal pregnancy until full term (control group). They were enrolled in this study after giving their informed consent. All were nearly of the same maternal and gestational ages. Preeclampsia was defined as blood pressure (BP) of 140/90 mm Hg or higher—on at least 2 occasions separated by at least 6 hours—after the 20th week of gestation, with proteinuria of 0.3 g or more in a 24-hour urine collection. The control group comprised healthy normotensive pregnant women, with full-term deliveries of a healthy neonate. All women with hypertension before pregnancy, pregestational diabetes mellitus, history of recurrent pregnancy loss, history of hepatic or renal disease, severe infection (viral or bacterial), administration of oxidants (conditions that are external triggers for apoptosis), or smoking were excluded. Patients and controls were subjected to (1) history taking, including maternal age, previous hypertension, family history of hypertension, and the onset of hypertensive problem in relation to the duration of pregnancy; (2) general examination, including examination of pulse, BP, pallor, purpuric eruptions, jaundice, stigmata of end organ damage due to hypertension, and stigmata of liver cell failure (vascular spiders, jaundice, palmar erythema, and so on); (3) abdominal examination to determine the fundal level, fundal grip, umbilical grip, first and second pelvic grips, and auscultation of fetal heart sounds; (4) pelvic examination to detect whether the patient is in labor and to detect the stage of labor; (5) laboratory investigations, including serum PP13 assay , maternal PP13 mRNA expression, total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), creatinine, uric acid, hematocrit value, platelet count, and 24 urinary proteins; (6) obstetric ultrasound to determine presentation, position, gestational age, placental insertion, and biophysical profile (BPP) for the assessment of fetal well-being (including nonstress test, fetal movement, fetal breathing, fetal tone, and amniotic fluid volume).

Laboratory Methods

Nine milliliters of venous blood samples were withdrawn from controls and patients and were collected in 3 tubes: 3 mL was collected in a plain tube for assay of total bilirubin, AST, ALT, creatinine, and uric acid; 3 mL was collected in an ethylenediaminetetraacetic acid tube for assay of hematocrit value, platelet count, and PP13 mRNA using reverse transcription polymerase chain reaction (RT-PCR); 3 mL of blood was collected on a plain tube with separator for serum PP13 assay. Placental protein 13 level was measured using the human PP13 enzyme-linked immunosorbent assay kit (catalog number CSB-E12733h). The detection range varied between 25 and 1000 pg/mL.

Placental protein 13 mRNA was detected using RT-PCR following the method used by Sammar et al.[13] Placental protein 13 RNA was determined by RT-PCR. A portion of RNA solution (obtained from mononuclear cell separation) was reverse-transcribed and amplified by the single-step RT-PCR (QIAGEN one-step RT-PCR kit, catalogue no. 210212); to amplify PP13, we used the sense (5′-TATTGCCTTCCGTTTCCGAGT-3′) and anti-sense (5′-GCTCAAATTGT TTGCCATCCTCA-3′) oligonucleotide pair. The primers used for BCL-2 (as a housekeeping gene) were as follows; 5′-GCAATTCCGCATT TAATTCAT GG-3′ and 5′-GAAACAGGCCACGTAAAGCAAC-3′.

For each transcript, PCR was performed with 0.5 mL of complementary DNA, 25 mL of 2 ready master mix and 0.4 mmol/L of sense and antisense primers. Polymerase chain reaction was initialized by one cycle of 2 minutes at 95°C followed by 30 cycles of 3 steps of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension for 1 minute at 72°C. Polymerase chain reaction products were analyzed using 2% agarose gels and visualized with ethidium bromide. Placental protein 13 mRNA was expressed in the placenta of all women (control and cases) as indicated by a single band of PCR products. This was corresponding to 120 base pairs but with varying intensity with a relatively lower intensity were found in preeclampsia compared to control. The quantity of the housekeeping gene BCL-2 was similar in all groups.

Statistical Analysis

A predesigned Statistical Package for Social Science version 17 file was used for data entry and analysis. Results were reported as mean ± SD or frequency (%) when appropriate. The following tests were used: unpaired t test, Pearson correlation for comparison of quantitative measurement data, and Z test for comparison of proportion. P value was calculated using MedCalc. $P < 0.05$ was considered statistically significant.

RESULTS

The clinical and laboratory data of the studied groups are shown in Table 1. We did not find any statistically significant differences between the 2 studied groups regarding maternal age, parity, or gestational age ($P = 0.0586$; 0.3655, and 0.8034, respectively). However, systolic and diastolic blood pressures were significantly higher in preeclamptic women compared with the control group ($P < 0.0001$ for both; Table 1).

There were no statistically significant differences between the 2 groups regarding hematocrit value ($P = 0.0723$), platelet count ($P = 0.6459$), and ALT ($P = 0.0914$); there was statistically significant difference regarding creatinine ($P = 0.0120$), but there were highly statistically significant differences between the 2 studied groups regarding total bilirubin, AST, serum PP13 assay, and PP13 mRNA ($P < 0.0001$; Table 1).

Concerning the patients' group, it was found that there were positive correlation between serum PP13 level and both systolic blood pressure ($r = 0.366$, $P < 0.0001$), diastolic blood pressure ($r = 0.345$, $P < 0.0001$), urinary proteins per 24 hours ($r = 0.234$, $P < 0.0001$). There was correlation between serum PP13 level and platelet count ($r = 0.435$, $P = 0.045$). In addition, there were negative correlation between serum PP13 level and both AST ($r = 0.465$, $P < 0.0001$) and ALT (r = j0.634, $P < 0.0001$). However, other clinical and laboratory data did not show any statistically significant correlation with serum PP13 level. In addition, the control group did not reveal any statistically significant correlation between serum PP13 level and any of the studied clinical and laboratory parameters (Table 2).

Concerning the patients' group, it was found that there were positive correlation between PP13 mRNA and both systolic blood pressure ($r = 0.347$, $P < 0.0001$), diastolic blood pressure ($r = 0.384$, P G 0.0001), urinary proteins per 24 hours ($r = 0.249$, $P < 0.0001$). In addition, there were negative correlation between PP13 mRNA and both AST ($r = -0.545$, $P < 0.0001$) and ALT ($r = -0.453$, $P < 0.0001$). However, other clinical and laboratory data did not show any statistically significant correlation with PP13 mRNA (Table 3). In addition, the control group

TABLE 1. Comparison of Clinical and Laboratory Data of the Studied Groups

	Preeclampsia (n = 50)	Controls (n = 50)	*P*
Maternal age, yrs	26.3 ± 6.12	28.6 ± 5.9	0.0586 (NS)
Parity	1.7 ± 1.1	1.5 ± 1.1	0.3655 (NS)
Gestational age at antenatal screening, wks	11.8 ± 2.1	11.7 ± 1.9	0.8034 (NS)
Blood pressure (third trimester), mm Hg			
Systolic blood pressure	153.8 ± 14.4	114.6 ± 6.13	<0.0001 (HS)
Diastolic blood pressure	99.4 ± 11.8	74.3 ± 6.38	<0.0001 (HS)
Hematocrit value	33.1 ± 5.5	34.9 ± 4.34	0.0723 (NS)
Platelets count, $10^3/mm^3$	218.3 ± 68.06	224.4 ± 64.39	0.6459 (NS)
Total bilirubin, mg/dL	1.33 ± 0.4	1.03 ± 0.35	<0.0001 (HS)
AST	31.7 ± 14.4	21.2 ± 9.56	<0.0001 (HS)
ALT	26.3 ± 10.2	23.2 ± 7.83	0.0914 (NS)
Creatinine	1.15 ± 0.45	0.95 ± 0.32	0.0120 (S)
Urinary proteins, g/24-h urine	1.4 ± 0.5	Nil	
Serum PP13 assay, pg/mL	157.9 ± 45.5	225.3 ± 67.3	<0.0001 (HS)
PP13 mRNA	14 (28%)	38 (76%)	<0.0001 (HS)

did not reveal any statistically significant correlation between PP13 mRNA and any of the studied clinical and laboratory parameters (Table 3).

DISCUSSION

Placental protein 13 is one of the proteins specifically synthesized by the placenta. Although the function of PP13 is not clear, PP13 is associated with implantation and maternal artery remodeling.[8] Placental protein 13 prevents erythrocyte adhesion in areas with reduced blood flow such as intervillous space. Furthermore, PP13 is suggested to have special immune functions at the feto-maternal interface.[9,14]

The present study assessed the both maternal serum PP13 and PP13 mRNA expression in pregnant women with and without preeclampsia to determine the valuable role of these markers for early prediction of preeclampsia. Concerning the maternal serum PP13, it was found that its mean level in the preeclamptic group (157.9 ± 45.5 pg/mL) was significantly lower than that of the control group (225.3 ± 67.3) with highly statistically significant difference ($P < 0.0001$). These results were in agreement with the study of Chafetz et al.[12] who reported that the median first-trimester PP13 level was 132.5 pg/mL in the control subjects. Median PP13 levels were significantly lower among women who had preeclampsia (27.2 pg/mL; $P < 0.001$). When PP13 was expressed as multiples of the gestational age-specific medians among the control subjects, the multiples of the medians were 0.2 for preeclampsia ($P < 0.001$ compared with the control subjects).

In addition, our results were in agreement with the study of Huppertz et al.[15] who reported that in normal pregnant women delivering at term, median maternal serum PP13 levels were growing from 166 to 202 pg/mL and 382 pg/mL in the first, second, and third trimester, respectively. Preeclamptic women had significantly reduced PP13 levels in the first trimester (multiples of median of 0.14 at 7–8 weeks; $P = 0.005$ compared to normal). Placental protein 13 in the third trimester was significantly higher compared to normal at 35 to 36 weeks, with PP13 multiples of median of 1.79. From these results,

TABLE 2. Correlation Between Serum PP13 and Clinical and Laboratory Parameters in Preeclampsia and Control Groups

	Preeclampsia Group (n = 50)		Control Group (n = 50)	
	r	*P*	*r*	*P*
Maternal age, yrs	0.186	0.065 (NS)	0.278	0.067 (NS)
Parity	0.072	0.254 (NS)	0.178	0.342 (NS)
Gestational age at antenatal screening, wks	0.175	0.076 (NS)	0.257	0.072 (NS)
Blood pressure (third trimester), mm Hg				
Systolic blood pressure	0.366	<0.0001 (HS)	0.236	0.345 (NS)
Diastolic blood pressure	0.345	<0.0001 (HS)	0.236	0.087 (NS)
Hematocrit value	0.245	0.073 (NS)	0.161	0.079 (NS)
Platelets count, $10^3/mm^3$	0.435	0.045 (S)	0.189	0.067 (NS)
Total bilirubin, mg/dL	0.164	0.082 (NS)	0.165	0.084 (NS)
AST	0.465	<0.0001 (HS)	0.244	0.056 (NS)
ALT	−0.634	<0.0001 (HS)	0.178	0.074 (NS)
Creatinine	0.178	0.346 (NS)	0.287	0.065 (NS)
Urinary proteins, g/24-h urine	0.234	<0.0001 (HS)	0.210	0.097 (NS)

TABLE 3. Correlation Between PP13 mRNA and Clinical and Laboratory Parameters in Preeclampsia and Control Groups

	Preeclampsia Group (n = 50)		Control Group (n = 50)	
	r	*P*	*r*	*P*
Maternal age, yrs	0.173	0.074 (NS)	0.168	0.076 (NS)
Parity	0.0522	0.324 (NS)	0.288	0.453 (NS)
Gestational age at antenatal screening, wks	0.265	0.083 (NS)	0.157	0.065 (NS)
Blood pressure (third trimester), mm Hg				
Systolic blood pressure	0.347	<0.0001 (HS)	0.225	0.438 (NS)
Diastolic blood pressure	0.384	<0.0001 (HS)	0.245	0.078 (NS)
Hematocrit value	0.387	0.065 (NS)	0.174	0.074 (NS)
Platelets count, $10^3/mm^3$	0.334	0.072 (NS)	0.248	0.052 (NS)
Total bilirubin, mg/dL	0.245	0.065 (NS)	0.187	0.079 (NS)
AST	−0.0.545	<0.0001 (HS)	0.256	0.069 (NS)
ALT	−0.453	<0.0001 (HS)	0.145	0.074 (NS)
Creatinine	0.158	0.476 (NS)	0.376	0.068 (NS)
Urinary proteins, g/24-h urine	0.249	<0.0001 (HS)	0.267	0.085 (NS)

they concluded that low levels of PP13 in early pregnancy identify at-risk pregnancies, whereas high levels precede the syndrome in late pregnancy and suggest syncytiotrophoblast necrosis.

Than et al.[16] reported that third trimester maternal serum PP13 concentration increased with gestational age in normal pregnancies ($P < 0.0001$), and it was significantly higher in women presenting with preterm preeclampsia ($P = 0.02$) and hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome ($P = 0.01$) than in preterm controls.

Wortelboer et al.[17] reported that lower maternal serum first-trimester PP13 and PlGF levels are associated with preeclampsia/HELLP syndrome if compared with women with normal pregnancies with median 0.68 and $P < 0.0001$. It is possible that if combined with other variables such as blood pressure, uterine artery Doppler flow velocity waveforms and maternal history, they may have some value in the prediction of early preeclampsia in a low-risk population.

In addition, Gonen et al.[18] reported that at 6 to 10 gestational weeks, PP13 levels were significantly lower among the preeclampsia group with a median 0.28 MoM (95% CI, 0.15–0.39; $P < 0.004$). Using a cutoff of 0.40 MoM, the sensitivity was 80%, false-positive rate was 20% and odds ratio was 16.0 (95% CI, 5.3–48.4). Combining MoM of 6 to 10 weeks and slope between 6 and 10 and 16 and 20 weeks, the sensitivity was 78%, the false-positive rate was 6%, and odds ratio was 55.5 (95% CI, 18.2–169.2). The gestational hypertension group was not different from the normal group. They concluded that PP13 in the first trimester alone or in combination with the slope between the first and the second trimesters may be a promising marker for assessing the risk of preeclampsia.

According to our study, the maternal PP13 mRNA expression was lower in the preeclamptic group (28%) if compared with the control group with normal pregnancies (76%) with highly statistically significant difference between the 2 groups ($P < 0.0001$).

Shimizu et al.[19] reported that PP13 RNA levels were lower in the preeclampsia cases than those in the controls ($P < 0.001$). After MoM conversion, PP13 RNA was 1.00 ± 0.34 for the controls and 0.33 ± 0.19 for the preeclampsia cases ($P < 0.001$). They concluded that a decrease in the PP13 mRNA expression was observed in the cellular component of blood from both preeclamptic patients during the third trimester and asymptomatic pregnant women during the early second trimester who develop preeclampsia during later gestation. These findings indicate that an alteration in the PP13 mRNA expression in the placenta may therefore be associated with the pathogenesis of preeclampsia, and that this marker could potentially be one of the key markers to predict the clinical onset of preeclampsia.

Sammar et al.[13] reported that PP13 mRNA levels in term control and preterm were similar, whereas PP13 mRNA levels in preeclampsia and HELLP were significantly lower compared to term controls or preterm delivery or the two combined. They concluded that there is a reduced expression of PP13 mRNA and an increased protein level in the placenta and body fluids in preeclampsia and the HELLP syndrome at the time of disease.

Finally, Than et al.[16] reported that placental PP13 mRNA ($P = 0.03$) and protein, as well as cytoplasmic PP13 staining of the syncytiotrophoblast ($P < 0.05$) was decreased in preeclamptic patients compared to controls. No differences in placental expression and serum concentrations of PP13 were found at term between the patients with preeclampsia and the control women. They concluded that parallel to its decreased placental expression, an augmented membrane shedding of PP13 contributes to the increased third trimester maternal serum PP13 concentrations in women with preterm preeclampsia and HELLP syndrome.

In conclusion, combined serum PP13 level assay and PP13 mRNA expression are reliable markers for early detection of preeclampsia, and we recommend to add them to the routine antenatal investigations during the first trimester.

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