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Can first trimester placental biomarkers copeptin and PP13 predict preeclampsia in advanced age nulliparous women?

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ABSTRACT

Preeclampsia (PE) affects approximately 3% of all pregnancies and it is still a major cause of adverse perinatal outcome. PE is a multisystem pregnancy related disorder of unknown aetiology with a progressive course and with no established therapy. In recent times excessive research was conducted on early screening for PE with an aim to reduce the prevalence of the disease with early medical treatment starting from the first trimester of pregnancy in the high-risk group. The aim of this study is to detect if the first trimester serum copeptin and PP13 can predict preeclampsia in advanced age nulliparous women. These are the preliminary results of an ongoing prospective study that will include 400 pregnant women undergoing first trimester aneuploidy screening at the Department for Obstetrics and Gynaecology, University Hospital Centre Zagreb. Maternal risk factors used as inclusion criteria in this study were: nulliparity and age >35. Participants were asked to complete a short questionnaire regarding personal and medical information. Blood samples were collected and maternal serum PP13 and copeptin levels were measured. Following the inclusion criteria, we analysed the records of 40 women who gave birth to this date. Three patients (7.5%) developed preeclampsia and eight (20%) had gestational hypertension (GH). All PE patients had elevated plasma copeptin. Our preliminary data suggests that early screening for PE at 11-13 weeks of gestation using biomarkers copeptin and PP-13, in combination with maternal risk factors, is by far the most promising method for early detection of PE in advanced age nulliparous women.

Keywords: preeclampsia, biomarkers, copeptin, high-risk pregnant women

INTRODUCTION

Preeclampsia (PE) is a placental disease and sa evere multi-system pregnancy related disorder that complicates 2-8% of all pregnancies. (1, 2) It is characterized by a new onset of hypertension and proteinuria after 20 weeks of gestation in previously normotensive women, or in the absence of proteinuria, hypertension with thrombocytopenia, increased levels of liver transaminases, renal failure, pulmonary oedema and visual or cerebral disturbances. (3) Neonatal complications of PE include preterm delivery, fetal growth restriction (IUGR), hypoxia-related neurologic injury, perinatal death and long-term cardiovascular morbidity associated with low birth weight. (4) PE is a leading cause of perinatal deaths (23.6%) and maternal deaths (13%) in developing countries. (5) In developed countries, PE causes a high rate of iatrogenic preterm deliveries and about 20% of neonatal intensive care admissions. (6) There is no effective treatment or optimal prophylaxis for preeclampsia in clinical use, mostly caused by late diagnosis. Delivery and removal of the placenta is the only sufficient treatment. There are some known a priori risk factors for the development of preeclampsia, such as a prior history of preeclampsia, chronic hypertension, nulliparity, chronic kidney disease, pregestational diabetes, multiple gestations, obesity and advanced maternal age. (7 - 9) There are many theories regarding the pathophysiology, mostly a combination of immune, genetic and biological factors. (10, 11) This is the reason why it is so difficult to identify a specific biomarker directly related to this multifactorial disease. The importance of early treatment of PE seems promising. (12, 13) The SCOPE study validated 47 different biomarkers in the early detection of PE according to a priori maternal risk factors, with the aim to develop a personalised risk rating. (14) Copeptin is a 39-amino acid glycopeptide that comprises the C-terminal part of the arginine vasopressin (AVP) precursor and it was found to be a stable and sensitive surrogate marker for AVP release. (15) Copeptin seems useful in various clinical disorders, including diabetes insipidus and the monitoring of sepsis and cardiovascular diseases. In contrast to AVP, copeptin has a longer plasma half-life, is very stable at room temperature and easy and robust to measure. (16) Copeptin has been associated with renal function decline and may possibly serve as a useful early biomarker for PE. Some studies documented elevation in circulating copeptin in preeclampsia. (17 - 19) Placental protein (PP13) is a 32-kDa dimer protein which is highly expressed in the placenta. There are many studies aimed at describing normative levels of PP13 in maternal serum in the first trimester of pregnancy and to determine the value of this biomarker in predicting preeclampsia. PP13 levels gradually increase during normal pregnancy, but abnormally low levels of PP-13 have been

shown in first trimester gestation in women who develop preeclampsia. (20 - 22) This study is evaluating the importance of early screening for PE at 11-13 weeks of gestation using plasma level copeptin and PP13 biomarkers in combination with maternal risk factors in nulliparas. Having a new and useful tool for early prediction of the disease will give the clinicians the opportunity for early treatment avoiding serious and life threating complications with unfavourable perinatal outcome.

MATERIALS AND METHODS

These are preliminary results of an ongoing prospective study that will include 400 pregnant women who will undergo first trimester prenatal aneuploidy screening at the Department for Ob/Gyn, University Hospital Centre Zagreb. All participants signed a written informed consent. Attributed risk factors for preeclampsia are: prior history of preeclampsia, chronic hypertension, nulliparity, chronic kidney disease, pregestational diabetes, multiple gestations, obesity, and age >35 years old. (7 - 9) We used the two most frequent (nulliparity and age >35) as the including criteria in our study. Pregnant women with multiple pregnancies, miscarriages before 24weeks and major fetal chromosomal or structural abnormalities were excluded from the study. We analysed 5 mL of blood samples from pregnant women collected as a part of a first trimester aneuploidy screening during the period from 11+0-13 +6 weeks of gestation. The values of PP13 and copeptin in maternal blood samples were analysed. The concentration of PP13 was measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) with a pair of PP13-specific monoclonal antibodies. Serum copeptin concentrations were measured with an enzyme-linked Immunosorbent assay (ELISA) by using the Human Copeptin ELISA Kit according to the methods recommended by the manufacturer. Participants were asked to complete a questionnaire regarding age, body weight, smoking status, pre-existing chronic diseases, blood pressure, parity, and previous miscarriages. Data regarding ongoing pregnancy and delivery were collected from maternal and neonatal medical records. Gestational age was calculated from the first day of the last menstrual period. The diagnosis and classification of preeclampsia, gestational hypertension and chronic hypertension are based according to the American College of Obstetrics and Gynecology (ACOG) definitions: elevated blood pressures of greater than 140/90 at least two occasions six hours apart and proteinuria or elevated liver enzymes. (3) Severe preeclampsia is defined as gestational hypertension (systolic BP ≥160 mmHg and/or diastolic BP ≥100 mmHg on at least two occasions four hours apart after 20 weeks of gestation, but before the onset of labour with proteinuria (≥500 mg/24 h). Early preeclampsia was defined as preeclampsia resulting in a delivery before 34 weeks of gestation. Late onset preeclampsia was defined as preeclampsia resulting in a delivery after 34 weeks of gestation. Preterm delivery was defined as delivery before 37 weeks of gestation. (3) A descriptive analysis of maternal characteristics was conducted, separating the unaffected group from the women affected by preeclampsia and gestational hypertension. The maternal age, BMI, gestational weeks, birth weight, Copeptin and PP13 levels were expressed as medians. The statistics computed included mean, standard deviation and number of available observations of continuous variables which are presented as median±SD. Frequencies are presented for the categorical variables between the groups. Comparisons between the groups were by chi-square or Fisher exact test for categoric variables and Mann-Whitney U test for continuous variables. Logistic regression analysis was used to determine which of the maternal characteristics and biomarker levels had a significant contribution in predicting PE and gestational hypertension. The level of significance was $p<0.05$. We used the ROC curves to find a cut-off value for the biomarkers. The statistical software package SPSS 21.0 was used for data analyses.

RESULTS

Following the inclusion criteria, to this date medical records of 40 women who gave birth were analysed. Within this group, three patients (7.5%) developed preeclampsia and eight (20%) had gestational hypertension (GH). All PE cases were late-onset (Table 1). In the PE group copeptin was significantly higher (p=0.012). Caesarean section was carried out in 66.6% of PE compared to 24.14 % of unaffected pregnancies and in 12.5% of GH cases. IUGR was significantly frequented in the PE group (p=0.012). First-trimester systolic blood pressure values were significantly higher in the PE group (p=0.024) and newborns had significantly lower birth weight (p=0.039). The median of diastolic blood pressure was higher in the PE group compared to unaffected pregnancies (80±2.88, 70±5.19). Using the ROC curves, we found a cut-off value for the copeptin 0.43 ng/mL (AUC=0.838, P=0.05), and for the PP13 of 96.7 pg/mL (AUC 0.266, p=0.182).
Analysing maternal factors and biomarkers separately we found that copeptin is statistically significant for PE prediction (p=0.049,OR 2.62, 95% Cl 1.097-6.306). Other factors like BMI, blood pressure or smoking did not show any significance for the early prediction of PE. (Table 2) Our logistic regression model for PE using maternal factors and biomarkers (including all variables in model) confirmed a significant contribution for the combination of two biomarkers for the prediction of PE (p=0.007). At this date the preliminary results are that a decreased level of PP13 and an increased level of copeptin can predict preeclampsia with a sensitivity of 66.7% and a specificity of 97.3%, with a false positive rate of 2.7%.

DISCUSSION

Preeclampsia is still a major cause of maternal mortality, perinatal deaths, preterm birth, and intrauterine growth restriction and many long-term complications. (1, 23) Despite decades of research, predicting the disease before clinical diagnosis remains the leading problem. Many studies relied on maternal risk factors such as pre-existing diseases, increased maternal age, nulliparity, family history and BMI. (7, 9, 24) It is still unknown why millions of women with such risk factors did not develop PE. A number of potential clinical and molecular markers were evaluated as an early predictive test, but none were useful. Combinations of markers are likely to result in clinically useful screening tests. Recent advances in proteomic and metabolomic technologies will allow researchers to discover and map differences in molecules circulating in the blood of women who later develop preeclampsia. This has created the opportunity to develop effective methods of predicting these diseases, with the potential to dramatically improve maternal and neonatal health worldwide. (14) Similar to the SCOPE study, we found no significance in smoking status (p=0.879) or previous miscarriages (p=0.807) between PE and healthy women, but we found a higher rate of first trimester systolic blood pressure (p<0.001) and birth weight (p=0.039) in the PE group. (14) The incidence of PE varies between the countries. In our hospital, the incidence of PE is approximately 3%. This study included

Table 1. Maternal and pregnancy characteristics

Variable	No hypertensive disorders during pregnancy (N=29)	Gestational hypertension (N=8)	Preeclampsia (N=3)	P-value
Maternal age	35.97±0.31	36.25±69	35.33±0.33	0.798
BMI	23.97±0.46 (20-31)	24.75±0.88	23.33±0.88	0.796
Smoking cigarettes	18 (62.07%)	6 (75%)	2 (66.67%)	0.879
Miscarriages in medical hystory	7 (24.14%)	1 (12.5%)	0	0.807
DM in medical history	1 (3.45%)	0	0	0.823
Chronic hypertension	1 (3.45%)	0	0	0.823
Renal disease	1 (3.45%)	1 (12.5%)	0	0.535
Assisted reproductive technology	4 (13.79%)	2 (25%)	1 (33.3%)	0.574
Gestational weeks	39.31±1.49 (36-41)	39.5±0.76	38 ±1.7	0.268
Cesarean section	7 (24.14%)	1 (12.5%)	2 (66.6%)	0.178
Admission to NICU	1(3.45%)	0	1 (33%)	0.06
Birth weight (g)	3306.9 g ±446.36	3618.75±533.79	2733±485	0.039
Gestational diabetes	5 (17.24%)	2(25%)	1 (33.3%)	0.385
IUGR	2 (6.90%)	1 (12.5%)	2 (66.6%)	0.012
PP13 (pg/mL)	114.5±19.02	114.35±20.08	96.8±7.35	0.604
Copeptin (ng/mL)	0.31±0.07	0.38±0.09	0.44±0.08	0.012
Systolic BP (mmHg)	110±11.01	125±8.76	130±2.88	<0.001
Diastolic BP (mmHg)	70±5.19	80±8.42	80±2.88	0.054

Data are median ±SD or numbers (%)
P values are comparison between the groups with $\chi 2$ or Student t-test
BMI- Body Mass Index (kg/m2)
BP-Blood Pressure
DM-Diabetes Mellitus
IUGR-Intrauterine Growth Restriction
NICU-Neonatal Intensive Care Unit
PP13 –Placental Protein 13

Table 2. Logistic regression analysis for prediction of preeclampsia

Variable	Preeclampsia		
	P- value	OR	95% CI
BMI (kg/m2)	0.578	0.850	0.480-1.505
Systolic blood pressure (mmHg)	0.077	1.408	0.963-2.058
Diastolic blood pressure (mmHg)	0.061	1.253	0.989-1.587
Smoking cigarettes (if true)	0.974	1.042	0.86-12.655
PP13 (pg/mL)	0.228	0.939	0.848-1.040
Copeptin (ng/mL)	0.049	2.621	1.097-6.306

BMI- Body Mass Index (kg/m2)
PP13- Placental Protein 13

the two most frequent maternal risk factors (nulliparity and advanced maternal age) evaluating their influence to the appearance of the disease. Prediction of PE in our study with maternal inclusion risk factors in the combination with serum level biomarkers seems to be more successful in early detection of the disease compared to similar studies. (1, 2, 24, 25) All of our patients had late onset PE. One patient had severe type with an emergency caesarean section. Delivery is always indicated in case of imminent eclampsia, IUGR, placental abruption or multiorgan dysfunction. There are many studies evaluating the use of different biomarkers in prediction of preeclampsia. (26 - 28) Nicolaides et al. demonstrated that the combination of PP13 and uterine artery pulsatility index is a sufficient tool for the prediction of PE in the first trimester. (29) Other studies confirmed PP13 as a reliable marker in the prediction of PE with the early onset of the disease. (30, 31) Our PE patients had a median plasma PP13 level lower (96.8±7.35 pg/mL vs. 114.5±19.02) than in unaffected pregnancies and all of the patients had late onset of the disease. Preeclampsia is inconsistently associated with intrauterine growth restriction. (4, 9, 32) We found IUGR in 66% of PE cases. According to Geyl et al. preeclampsia with early onset was more severe in the group with IUGR, whereas Srinivas et al. reported IUGR in 27% of cases. (32, 33) Our study found increased maternal level of serum copeptin in PE cases compared to unaffected pregnancies (p=0.012), especially in patients with IUGR. Ashraf et al. (34) also reported significantly higher maternal copeptin levels in pregnancies complicated with IUGR,

and Benzing et al. found that compromised placental perfusion and chorioamnionitis were associated with significantly elevated cord copeptin levels in preterm infants born vaginally. (35) Copeptin is a biomarker of arginine vasopressin, which acts on multiple systems to increase blood pressure and water retention. Many studies suggest that copeptin levels might be useful in the early prediction of PE, as well as in the evaluation of the severity of the disease. (17, 36 - 38). Preeclampsia conduct serious pregnancy complication with uncertain cause. Identification of potential biomarkers before clinical diagnosis of the disease remains important. According to many studies, preeclampsia has long-life implications with an increased risk of hypertension, coronary artery disease, stroke, and type 2 diabetes mellitus. (39, 40) Copeptin levels are elevated before clinical diagnosis of preeclampsia and is significantly higher among women who develop preeclampsia. These results indicate the importance of copeptin in the pathophysiology of preeclampsia, and that the maternal copeptin level might be a novel marker in preeclamptic patients, especially in those with risk factors such as nulliparity and advanced maternal age.

CONCLUSION

The challenge involved in applying models to clinical prediction for PE is the low prevalence of the disease, which often results in a large number of false-positive tests. Our inclusion factors helped us to involve the higher number of potential PE cases in our project in regard to all pregnant women. The limitation of our preliminary results is that we did not have enough severe and early-onset PE cases which are usually complicated with unfavourable outcomes, and the levels of biomarkers would probably be with more disrupted cut-off values compared to unaffected pregnancies. According to our preliminary results of the ongoing prospective study, a combination of most frequent maternal risk factors and serum biomarkers PP13 and copeptin levels provides effective first trimester screening for preeclampsia before clinical diagnosis.

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