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Association between first trimester plasma htra1 level and subsequent preeclampsia: A possible early marker?

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ABSTRACT

Introduction: Preeclampsia (PE) is associated with risk of maternal and fetal mortality and morbidity. Several promising predictors of PE have been identified, but early pregnancy screening for PE remains insufficient, and randomized controlled trials that used biomarkers to identify high-risk women have been disappointed. Our aim is to identify a possible early marker of PE.

Methods: 158 women attending a routine antenatal care visit were recruited from 2014 to 2016 and prospectively followed until delivery (14 of whom had a diagnosis of PE). We have tested the plasma concentration of High temperature requirement factor A1 (HtrA1) at 12 weeks of gestation by ELISA technique in order to identify women at risk for developing PE. A multiple logistic regression analysis was used to estimate the independent effect of women' characteristics on the probability of developing PE. Likelihood ratio test and Hosmer-Lemeshow test were used to select the most parsimonious model and to evaluate the model's goodness of fit. Predictiveness of preeclampsia was estimated by ROC curve.

Results: PE cases had significantly higher BMI, before and after pregnancy, shorter gestational age at delivery and higher HtrA1values than healthy women. In addition, higher HtrA1 values in the first trimester maternal plasma, BMI before pregnancy and gestational age at delivery are significantly associated with subsequent development of PE. ROC curve showed a good accuracy in predicting preeclampsia, with an AUC of 0.83.

Conclusions: These results suggest the HtrA1 as early predictive marker of PE having a strong clinical relevance for disease prevention.

1. Introduction

Preeclampsia (PE) is clinically defined for new onset hypertension (blood pressure ≥ 140/90 mm Hg) and substantial proteinuria (≥ 300 mg in 24 h) after the 20th week of gestation [1,2]. PE, affecting 2–8% of pregnancies, is associated with risk of maternal and fetal mortality and morbidity [3]. Early prediction of women at risk for PE would make it possible to institute preventative measures and offer appropriate surveillance [4]. Prevention strategies such as aspirin [5] have shown some benefit, but are reserved for those women at highest risk, who developed PE in a previous pregnancy. Several promising predictors of PE have been identified, including uterine artery ultrasonography and maternal serum/urinary levels of human chorionic gonadotropin, inhibin A, activin A, pregnancy associated plasma protein A, sex hormone-binding globulin, placental growth factor, soluble fms-like tyrosine kinase 1 and serum placental protein 13 (PP13) [6–9]. Nonetheless, early pregnancy screening for PE remains insufficient, and randomized controlled trials that used biomarkers to identify high-risk women have been disappointing perhaps because the sensitivity of most of these markers is high in the second trimester, long after the placental dysfunction that culminates in clinical disease.

HtrA1, a member of the family of HtrA1 proteins, is a secreted multi-domain protein with serine protease activity. It is characterized by the presence of a trypsin-like serine protease domain and one PDZ

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domain. It also contains an IGFBP/mac25 like domain and a kazal-type inhibitor domain at the N-terminus, a signal sequence for secretion [10,11] and it is involved in the physiological development of many organs [12–14], as well as in development and progression of several pathologies, including neoplastic and degenerative diseases [15,16]. HtrA1 is expressed in placental tissue during gestation suggesting a role in placental development [17]. In addition, placental HtrA1 expression increases from the first to the third trimester of pregnancy [14] and HtrA1 autocatalytic form is abnormally elevated in preeclamptic placentas [18] as well as HtrA1 mRNA four-fold increases compared to controls [19]. HtrA1 protein has also been reported to be elevated in plasma of patients with PE [20].

The purpose of this study was to determine whether the first-trimester plasma concentration of HtrA1could be used to identify women at risk for developing PE during first trimester gestation.

2. Materials and methods

2.1. Patients

This is an observational prospective cohort study of healthy pregnant women at 11 weeks + 0 days– 13 weeks + 6 days of gestation. Women attending a routine antenatal care visit at Department of Obstetrics and Gynaecology of Hospital 'S. Maria della Misericordia' (Perugia, Italy), were recruited from 2014 to 2016 and prospectively followed until delivery.

Eligible subjects were all pregnant women following the inclusion criteria of the study: maternal age between 18 and 45 years; singleton pregnancy; no genetic diseases (e.g. aneuploidies) and those who signed the informed consent. Exclusion criteria were: multiparity, multiple gestation, a history of hypertension, renal disease, cardiac disease, diabetes mellitus, thyroid and immunologic diseases and congenital or acquired thrombophilia.

At the first visit, the gestational age was calculated from the last menstrual period and confirmed by ultrasound crown-rump-length measurement; a maternal venous blood sample was collected.

Baseline demographics, medical history including obstetric history, current and before pregnancy habits (smoking, eating, physical activity) were collected through an interview and anthropometric characteristics were taken (Body Mass Index – BMI). Anthropometric and clinical features were monitored during gestation.

PE was defined as two readings of blood pressure $\geq$140/90 mmHg at least 4 h apart, and proteinuria $\geq$ 300 mg in 24 h, or two readings of at least + 2 on dipstick analysis of midstream or catheter urine specimens if no 24-h urine collection was available [3].

Data of pregnancy outcome (fetal gender, birth weight and newborn health) were collected after birth. Stillbirths and presence of chromosomal and other fetal anomalies were excluded.

The study was approved by the Institutional Ethics Committee of University of Perugia and informed consent was obtained from each recruited woman.

2.2. Plasma collection and HtrA1 ELISA

Blood samples were obtained by Vacutainer™ venipuncture of the median cubital vein after overnight fasting. Plasma samples were prepared from a fresh EDTA venous blood centrifuged at 1500 × *g* for 15 min at 4 °C. Plasma was then aliquoted and stored at −80 °C until use. We have previously tested a good stability of HtrA1 protein at this temperature in plasma samples [20]. Plasma HtrA1 concentration was measured using commercial ELISA kit (USCN Life Science Inc., Wuhan, P.R. China). The minimum detectable dose of HtrA1 is less than 13.5 pg/ml as described in the instruction manual of over-mentioned ELISA kit. In addition, intra-assay and inter-assay CV% are minor of 10% and 12% respectively. The measurements were conducted in duplicate, according to the manufacturer's recommended protocol. One hundred μl of plasma samples were used for each well. Internal negative and positive quality controls were supplied with the kit. Total proteins were used to normalize the expression data.

2.3. Statistical analysis

A non-parametric approach was used since variables had a no-normal distribution, when evaluated with the Shapiro test. Quantitative variables were summarized using median and interquartile range (IR, 1st-3rd quartiles), respectively as measure of centrality and variability; qualitative variables were expressed as absolute and percent frequencies. Comparisons between groups were evaluated using Wilcoxon rank sum test and Fisher exact test. Htra1 levels were analyzed after log transformation.

A multiple logistic regression analysis was used to estimate the independent effect of women' characteristics on the probability of developing PE. All estimates were obtained calculating 95% Confidence Intervals (95%CI). Likelihood ratio (LR) test and Hosmer-Lemeshow test were used to select the most parsimonious model and to evaluate the model's goodness of fit. The accuracy of the model in predicting preeclampsia was analyzed using the ROC curve, the Area Under Curve and 95% CIs. ROC curve was estimated including the variables having a p-value lower than 0.1 at the logistic regression analysis.

The R statistical program was used for the analyses and a probability of 0.05 was set as the threshold for statistical significance.

3. Results

Overall, 158 women were recruited in this study, 14 (8.9%) of whom had a diagnosis of PE at delivery. Women's characteristics according to health status are shown in Fig. 1 and Table 1. Healthy women and PE cases were found not significantly different with regards to age at pregnancy, BMI variation after pregnancy (Fig. 1), smoking, nutritional habits, physical activity and caesarian delivery (Table 1). PE cases had significantly higher BMI, before and after pregnancy, shorter gestational age at delivery and higher log HtrA1 than healthy women (Fig. 1). No significant difference was found in newborns' birth weight and Apgar Score (Fig. 1). Among women with preeclampsia only a newborn suffered from jaundice, no other immediate neonatal complications were observed. Fourteen newborns affected of immediate neonatal complications were observed among healthy women, i.e. 7 jaundices, 3 sepsis, 3 congenital organ defects (2 kidneys, 1 genital), 1 hyperglycemia.

Table 2 shows the results of the multiple logistic regression analysis. PE was significantly associated with log HtrA1 values, BMI before pregnancy and gestational age at delivery. In particular, the probability of developing PE increased about 90% for every added unit of log HtrA1 and about 10% for every added unit of BMI, while decreased of about 37% for every week added of gestational age at delivery. The ROC curve (Fig. 2) showed that the model had a good accuracy in predicting preeclampsia, with an AUC equal to 0.83 (95%CI: 0.74–0.92).

4. Discussion

HtrA1 is a secreted protein [3,21] and placental secretion of HtrA1 is expected to be present in the blood vessel of pregnant women. As previously demonstrated, HtrA1 is localized mainly in the villous cytotrophoblast in the first trimester of gestation, in the entire trophoblast (syncytium and villous cytotrophoblast) in the second trimester and mainly in the syncytium at term [17]. HtrA1 levels increase, from first to third trimester of gestation, in maternal plasma of normal gestation [20], and abnormal elevation of HtrA1 has been repeatedly observed in the serum of patients with PE at delivery [18,21].

The results of our study show that higher HtrA1 values in the first trimester maternal plasma are significantly associated with subsequent development of PE. Our model has a good accuracy in predicting

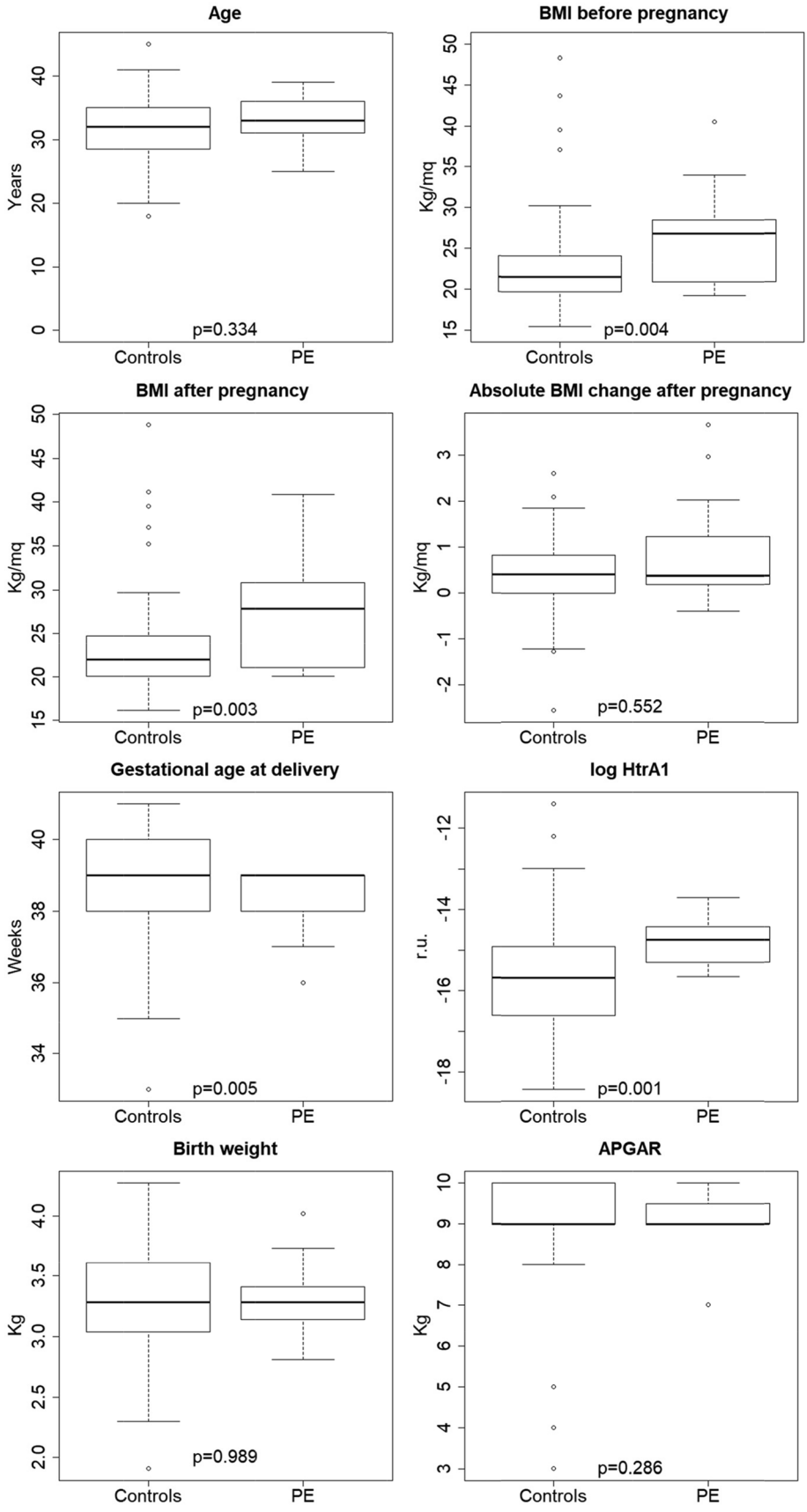

Fig. 1. Demographic and clinical characteristics of the population considered in the study according to its health status. The box blots show the results concerning maternal age, BMI before and after pregnancy, absolute BMI change after pregnancy, maternal gestational age at delivery, logHtrA1, birth weight and APGAR index. PE cases showed significantly higher BMI, before and after pregnancy, shorter gestational age at delivery and higher log HtrA1 than healthy women. No significant difference was found in newborns' birth weight and Apgar Score. p-values refer to Wilcoxon rank sum test.

Table 1
Women's habits and clinical characteristics according to their health status.

	Healthy women	Women with Preeclampsia	
Variables	n = 144	n = 14	p
Smoking (yes)	27 (18.8)	0 (0)	0.130
Low/poor nutrition	32 (22.2)	5 (35.7)	0.319
Physical activity (yes)	97 (67.4)	9 (64.9)	0.775
Caesarean delivery	34 (23.6)	5 (35.7)	0.336

Values are absolute and percent frequencies; p-values Fisher exact test

Table 2
Effect estimate of factors associated to Preeclampsia. Results of the multiple logistic regression analysis.

	OR	95% CI	P
log HtrA1	1.90	1.12; 3.40	0.021
Woman's age (years)	1.05	0.92; 1.22	0.462
BMI before pregnancy (kg/m^2)	1.10	1.00; 1.20	0.044
Gestational age at delivery (weeks)	0.67	0.45; 1.01	0.049
Nutrition (good vs poor/low)	0.84	0.23; 3.40	0.794
Physical activity (yes vs no)	1.54	0.44; 6.10	0.514

Hosmer and Lemeshow goodness of fit test: Chi-square with 8 df, $\chi^2 = 6.04$, p = 0.642
LR test: Chi-square with 6df, $\chi^2 = 17.55$, $p = 0.007$.

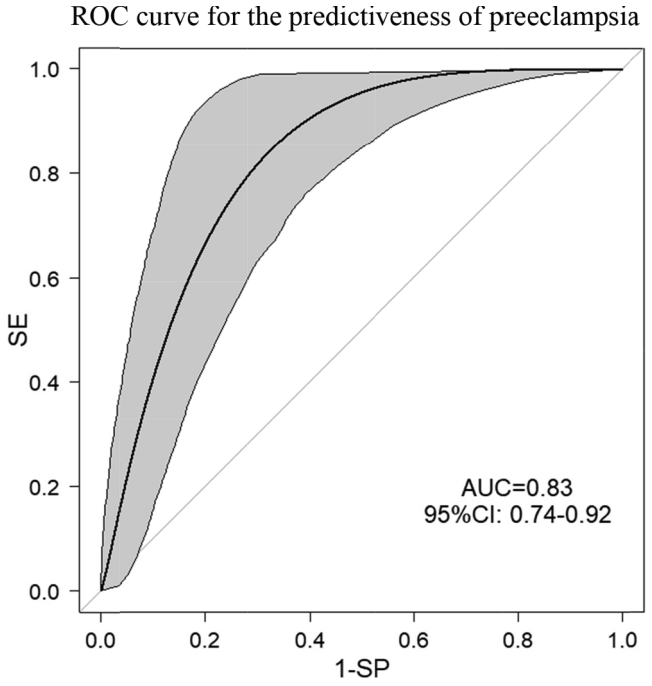

Fig. 2. ROC curve for the predictiveness of preeclampsia. ROC curve was estimated including in the model logHtrA1, BMI before pregnancy, Gestation at delivery (variables with p value < 0.10). SE: Sensitivity; SP: Specificity; Grey area identifies 95% confidence interval of ROC. AUC: Area Under Curve; 95%CI: 95% Confidence Interval.

preeclampsia showing a ROC curve with an AUC equal to 0.83. In the mouse HtrA1 is localized in the endometrium and placenta from the first trimester of pregnancy [22] and high levels are present in decidua capsularis, compared to ectoplacental cone, suggesting a role in the regulation of trophoblast migration and invasion [23]. In humans, it has been demonstrated that HtrA1 is localized in the extravillous cytotrophoblastic cells of cell columns of first trimester placenta that are the structures involved in the invasion of the uterine wall during the placental development and remodeling [17].

The physiopathological bases of PE originate during the first trimester of pregnancy when extravillous cytotrophoblast fails to adopt an invasive phenotype [24] causing an inadequate invasion and adaptation of uterine spiral arteries leading finally to an ischemia–reperfusion injury in the placenta [25]. In this view it has been demonstrated that, in pathological conditions, less invasive cytotrophoblast expresses highest levels of HtrA1, whereas more invasive extravillous cytotrophoblast expresses lower levels of HtrA1 [17].

Although at the moment, we cannot determine if the abnormal elevation of HtrA1 in first-trimester of pregnancies may be the cause or the outcome of a stress response imposed by PE, our hypothesis is that the damaged placental tissues could release this protein in maternal blood during the development of the placenta itself.

The main strengths of our study are the prospective design and the enrolment of healthy women at their first pregnancy. In this way, we could control the effects of those biases related to preexisting diseases and conditions known to be risk factors for PE.

A limitation of the study may be the small number of women with PE, that nevertheless respect the incidence of PE reported in scientific literature [26]. PE is a not common condition that requires a very long study period for enrolling and following a higher number of women involving healthcare and technical professionals.

In conclusion, we showed an independent significant effect of HtrA1 maternal plasma evaluated in the first-trimester of pregnancy on the risk of developing PE. These results may have a strong clinical relevance, also in terms of disease prevention and save resources in public health, since HtrA1 could be tested by ELISA that is a simple and low-cost essay, which could be easily included in clinical practice. Moreover, as recently reported, low-dose aspirin treatment before the 16th weeks of gestation reduces the risk of developing PE [27]. In addition, early detection and treatment of PE could avoid the worst maternal and fetal outcomes, reducing the social, economic and healthcare impacts caused by this pathology [28].

A multicenter larger study should be performed to analyze the predictive ability of plasma HtrA1, also in combination with other clinical characteristics, biochemical or biophysical markers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2019.08.005.

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