European Journal of Obstetrics & Gynecology and Reproductive Biology 205 (2016) 153–157

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Prediction of pre-eclampsia combining NGAL and other biochemical markers with Doppler in the first and/or second trimester of pregnancy. A pilot study.

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ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form 10 August 2016
Accepted 13 August 2016

Keywords:
Pre-eclampsia
Biochemical makers
Uterine artery pulsatility index
Ductus venosus pulsatility Index
First and/or second trimester screening
Neutrophil gelatinase-associated lipocalin

ABSTRACT

Objective: To determine the performance of maternal characteristics, Doppler and a set of biochemical markers for pre-eclampsia (PE) screening at 11 + 0 to 13 + 6 and 20 + 1 to 25 + 6 weeks' gestation.
Study design: Prospectively enrolled women at 11 + 0 to 13 + 6 and 20 + 1 to 25 + 6 weeks. Maternal characteristics, uterine artery pulsatility index (UtA-PI), ductus venosus pulsatility index (DV-PI) and serum biomarkers including pregnancy associated plasma protein – A (PAPP-A), placental growth factor (PlGF), soluble fms-like tyrosine kinase 1 (sFlt-1), s-Flt-1/PLGF ratio, asymmetric dimethylarginine (ADMA), matrix metalloproteinase 9 (MMP-9), neutrophil gelatinase-associated lipocalin (NGAL) and MMP-9/NGAL complex were recorded.
Results: Combination of NGAL and BMI in a logistic regression model detected 70% of PE in the first trimester ($p = 0.001$). Including UtA-PI and DV-PI in the model sensitivity reached 77.8% with 96.6% specificity ($p = 0.004$). Combination of second trimester NGAL and s-Flt-1/PLGF ratio yield specificity 100% ($p = 0.001$). Combination of second trimester UtA-PI with first trimester NGAL, BMI and age detected 80% of PE with specificity 91.9% ($p = 0.001$).
Conclusion: Combination of NGAL, maternal characteristics and Doppler parameters in the first and/or second trimester can detect a consistent number of PE pregnancies. NGAL is a potent new biomarker for the prediction of preeclampsia.

Introduction

Preeclampsia (PE) is a systematic disease of pregnancy characterized by hypertension and proteinuria developing after 20th week of pregnancy. It is estimated that preeclampsia affects 3–5% of all pregnancies worldwide [1].

The pathogenesis of preeclampsia is still not fully elucidated despite intense research [2,3]. The most commonly suggested hypotheses strongly rely on abnormal cytotrophoblastic invasion of spiral arterioles and the subsequent reduced or insufficient placental perfusion [4]. Consequently, insufficient placenta produces a variety of soluble biomarkers leading to maternal endothelial dysfunction [4,5].

Until now it is generally accepted that no single effective test can provide a sufficient accuracy for the prediction of preeclampsia [6]. On the contrary, the interest of research is focused on the combination of biochemical markers, Doppler ultrasound parameters and maternal characteristics into multiparametric models [7,8].

When used alone, maternal history and maternal demographic characteristics can detect about one-third of the women destined

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http://dx.doi.org/10.1016/j.ejogrb.2016.08.034
0301-2115/

to develop PE [8]. To improve the detection rate (DR), many authors have combined patients' history with a series of biophysical and biochemical markers [9]. Studied biophysical markers include mean arterial blood pressure [10], uterine artery Doppler [11,12], maternal cardiac output [13] and brain hemodynamic measurements [14]. Biochemical markers that have been tested include products of fetal and placental origin, markers of renal or endothelial damage, angiogenic and antiangiogenic factors, and markers of oxidative stress [15]. A variant of molecular (cell free DNA) [16] and genetic markers have also been widely studied [9].

The aim of our study was to evaluate the screening accuracy of a predictive model for PE using parameters of the first and/or the second trimester of pregnancy. We investigated the combination of some maternal characteristics as age and body mass index (BMI), uterine artery pulsatility index (UtA-PI), ductus venosus pulsatility index (DV-PI) with the biochemical markers: Pregnancy-Associated Plasma Protein A (PAPP-A), Placental Growth Factor (PlGF), soluble Fms-like tyrosine kinase-1 (sFlt-1), s-Flt-1/PLGF ratio, Asymmetric Dimethylarginine (ADMA), Matrix Metalloproteinase 9 (MMP-9), Neutrophil Gelatinase-Associated Lipocalin (NGAL) and MMP-9/NGAL complex.

Materials and methods

The pregnant women included in the present study were selected from a pool of pregnancies that are recruited for a wider investigation project on biochemical and ultrasound parameters for the development of adverse pregnancy outcomes. Pregnancies are recruited in the study in the first trimester as they appear for the routine prenatal screening for chromosomal abnormalities, in the 2nd Department of Obstetrics & Gynecology of Medical School of Athens University in "Aretaieion" Hospital and in a private setting of obstetric care (EmbryoCare, Fetal Medicine Unit, Athens). All women gave their informed consent for their participation in the study and Hospital's ethics committee approved the protocol.

From a total of 541 women with known pregnancy outcome, in this case-control study we included all 12 singleton pregnancies that developed preeclampsia. One preeclamptic twin pregnancy excluded from the study. Preeclampsia was defined as gestational hypertension (defined as systolic blood pressure $\geq$ 140 mmHg and/or a diastolic blood pressure $\geq$ 90 mmHg on at least two occasions with at least 6 h apart, after the 20th week of gestation in women known to be normotensive before pregnancy and before 20 weeks' gestation) plus proteinuria (300 mg or more per 24-h period). If 24-h urine collection was not available, then proteinuria was defined as a concentration $\geq$ 30 mg/dL (at least 1+ on a dipstick) in at least two random urine samples collected minimum 6 h apart. Out of the 12 pregnancies with preeclampsia, 3 delivered before or at 34th week of gestation (early-onset PE) and the remaining 9 after 34th week of gestation (late-onset PE).

From the pool of 541 pregnancies, we randomly selected 47 women with singleton normal pregnancies that did not have any major complication during their pregnancy and delivered healthy full term neonates. Multiple gestations, pregnancies with fetal chromosomal or major structural anomaly, miscarriage before 20 weeks, gestational diabetes or diabetes type 1, chronic hypertension or hypertension before 20 weeks were excluded. Maternal characteristics and detailed medical and obstetrical history were recorded.

An ultrasound examination was carried out at the first trimester (11 + 0 to 13 + 6 week) for diagnosis of major fetal defects and measurement of nuchal translucency thickness (NT). At the same time, both uterine arteries and Ductus Venosus were examined as suggested by the Fetal Medicine Foundation in London, UK (www.fetalmedicine.com/fmf) by a certified operator. UtA-PI and DV-PI was calculated as the mean PI from three similar consecutive waveforms. All examinations were carried out transabdominally. Examination of uterine arteries repeated again at the second trimester (20 + 1 to 25 + 6 week) by the same operator. Blood samples, were also collected prospectively in the first and in the second trimester. Serum samples were aliquoted and stored at −35 °C until analysis.

Measurement of biochemical markers

Maternal serum PAPP-A was measured using TRACE technology with the B·R·A·H·M·S PAPP-A kit (BRAHMS GmbH, Hennigsdorf, Germany). Total precision estimated with the CV (%) was <5%. Maternal serum PlGF and sFlt-1 were determined with the Roche kits on Elecsys 2010 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The method detection limit was 10 pg/mL for PlGF and 15 pg/mL for sFlt-1 while the total CV was 4.1% for PlGF and 4.3% for sFlt-1. Determinations for NGAL, MMP-9 and MMP-9/NGAL complex were performed with the R&D (R&D Systems Inc, MN, USA) kits: Human Lipocalin-2/NGAL Quantikine; Human MMP-9 Quantikine ELISA; Human MMP-9/NGAL Complex Quantikine ELISA. According to the kits' inserts, the detection limit was 0.012 ng/mL for NGAL, 0.156 ng/mL for MMP-9 and 0.058 ng/mL for the MMP-9/NGAL complex while the total CV was 7.9% for NGAL, 7.9% for MMP-9 and 7.6% for the MMP-9/NGAL complex. ADMA was measured using a commercial Elisa Kit: (ADMA-Elisa DLD, Hamburg, Germany) with a CV of 8%.

Statistical analysis

The statistical software IBM SPSS statistics version 20 (IBM Corporation, Somers, NY 10589, USA) was used for data analysis. Comparison between the PE group and normal pregnancies was performed by X^2-test for categorical variables. Distribution of Doppler parameters, quantitative maternal characteristics and biochemical markers in both normal and preeclamptic pregnancies was tested for normality by Kolmogorov–Smirnov test. Comparisons were executed using *t*-test for data with normal distribution and non-parametric tests (Kruskal–Wallis or Mann–Whitney test) for data without normal distribution. Data are presented as mean ± SD (Tables 1–2). Ethnicity was not considered as a confounding factor because all of the patients were Caucasian. Logistic regression analysis was used for the prediction of preeclampsia probability. We used the forward conditional stepwise method for the selection of the most effective predictors (risk factors).

Logistic regression has the following formula

$$\mathrm{Log}\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \cdots + \beta_n\chi_n$$

where p is the probability of woman for having preeclampsia

χ_i is the risk factor; β_0 is the constant of the model; β_i is the coefficient associated with the risk factor χ_i.

Sensitivity and specificity, of the model were calculated at a cut-off probability of 50%. A probability level of less or equal to 0.05 was considered significant.

Results

The characteristics of the preeclamptic and normal pregnancies are shown in Table 1. No differences have been observed except for maternal BMI in the first trimester, which was higher in the preeclamptic group (p = 0.007). In the second trimester, Doppler UtA-PI was significantly higher in preeclamptic pregnancies (p = 0.008).