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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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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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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).

Table 1
Demographic, clinical characteristics and Doppler markers (mean ± SD) of the studied pregnancies and their newborns.

Characteristic	Normal pregnancies	Pregnancies complicated with preeclampsia
N	47	12
Maternal age at 1st trimester (y)	32.2 ± 4.1	34.3 ± 3.7
Parity (% nulliparus)	47%	67%
Smokers (%)	17%	25%
Maternal BMI at 1st trimester (Kg/m^2)	24.3 ± 4.1	28.1 ± 5.0*
Doppler PI of uterine artery		
1st trimester	1.65 ± 0.43	1.68 ± 0.40
2nd trimester	0.97 ± 0.28	1.24 ± 0.42**
Doppler PI of Ductus Venosus 1st trimester	0.93 ± 0.17	0.95 ± 0.1
Mean blood pressure at 3rd trimester		
Systolic (mm Hg)	109 ± 9	144 ± 11
Diastolic (mm Hg)	67 ± 7	87 ± 8#
Gestational age at delivery (weeks)	39.2 ± 0.9	35.7 ± 3.0#
Birth weight (g)	3167 ± 428	2196 ± 917#

BMI, body mass index, PI: pulsatility index.
* $p = 0.007$.
** $p = 0.008$.
\# $p < 0.001$.

Summary statistics of the studied biochemical markers are shown in Table 2. A significantly higher level of serum NGAL ($p = 0.005$) was detected in preeclamptic pregnancies in the first trimester, whereas in the second trimester NGAL ($p = 0.012$), sFlt-1/PlGF ratio ($p = 0.006$) and ADMA ($p = 0.043$) showed significantly higher levels in preeclamptic group while PlGF ($p = 0.05$) was significantly lower. It is worth mentioning that PAPP-A concentration in the first trimester was lower in preeclamptic pregnancies and that difference marginally did not reach statistical significance between the two groups ($p = 0.06$).

We evaluated a logistic regression model to predict the probability of preeclampsia using combination of maternal characteristics, biochemical markers and Doppler parameters in the first and/or second trimester of pregnancy. Table 3 presents the statistical parameters, p value, sensitivity, specificity, positive and negative predictive value of the most effective models.

In the first trimester by using the logistic regression output the combination of NGAL and maternal BMI yielded the best results showing sensitivity 70% and specificity 93.5% ($p = 0.001$). Interestingly, even if UtA-PI and DV-PI did not reach significance, inclusion of those two Doppler variables in the equation increased the sensitivity to 77.8% and the specificity of 96.6% ($p = 0.004$) (results not shown).

Table 2
Mean ± SD concentrations of Neutrophil Gelatinase-Associated Lipocalin (NGAL; ng/ml), Matrix Metalloproteinase-9 (MMP-9; ng/ml), Matrix Metalloproteinase-9/Neutrophil Gelatinase-Associated Lipocalin complex (MMP-9/NGAL; ng/ml), Pregnancy-Associated Plasma Protein-A (PAPP-A; mIU/ml), Placental Growth Factor (PLGF; pg/ml), soluble Fms-like tyrosine kinase-1 (sFlt-1; pg/ml), PLGF/s-Flt-1 ratio, Asymmetric Dimethylarginine (ADMA; μmol/L) in studied groups, in the first and second trimester of pregnancy.

	Normal pregnancies	Pregnancies complicated with preeclampsia	*p*
N	47	12	
1st trimester			
GA at sampling (weeks)	12.6 ± 0.5	12.4 ± 0.3	
NGAL	20.5 ± 19.5	35.3 ± 18.2	0.005
MMP-9	696.5 ± 455.6	726.2 ± 309.8	0.898
MMP-9/NGAL	87.9 ± 82.7	133.3 ± 70.5	0.218
sFlt-1	1420.0 ± 526.5	1328.8 ± 407.1	0.388
PlGF	59.5 ± 20.7	56.2 ± 26.1	0.733
Ratio	26.6 ± 10.7	27.2 ± 11.1	0.99
PAPP-A	3.3 ± 1.97	2.21 ± 1.13	0.06
ADMA	95.6 ± 27.2	112.3 ± 23.8	0.217
2nd trimester			
GA at sampling (weeks)	21.6 ± 0.7	21.6 ± 1.1	
NGAL	31.0 ± 22.8	51.8 ± 28.1	0.012
MMP-9	771.1 ± 515.6	781.6 ± 379.0	0.759
MMP-9/NGAL	122.9 ± 121.8	154.6 ± 65.4	0.167
sFlt-1	1399.9 ± 767.9	1612.1 ± 817.6	0.625
PLGF	328.7 ± 156.3	224.9 ± 152.6	0.05
Ratio	4.7 ± 2.5	20.0 ± 34.3	0.006
ADMA	99.2 ± 25.5	122.8 ± 27.9	0.043

GA, gestational age (mean ± SD).

In the second trimester by using the logistic regression output the combination of NGAL and sFlt-1/PLGF ratio yielded the best results showing sensitivity 33.3% and specificity 100% ($p = 0.001$). Interestingly, even if UtA-PI was statistically different between the two groups, it did not add any consistent discriminant power between cases and controls and was therefore excluded from logistic model.

Regarding the combination of first and second trimester biochemical/ultrasound markers and maternal characteristics a combined model including maternal BMI, age and NGAL in the first trimester along with UtA-PI in the second trimester reached a sensitivity of 80.0% and specificity of 91.9% ($p = 0.001$). Almost the same sensitivity (77.8%) and specificity (96.2%) was shown by the combination of maternal BMI, NGAL and DV-PI in the first trimester along with UtA-PI in the second trimester ($p = 0.005$) (results not shown).

Table 3
Statistical parameters, *p*-value, sensitivity, specificity, positive and negative predictive value of the logistic regression analysis model for the prediction of preeclampsia.

Predictors	β	SE	p-value	Sensitivity (%)	Specificity (%)	PPV (95% CI)	NPV (95% CI)
1st trimester							
NGAL	0.069	0.031	0.028				
BMI	0.303	0.123	0.014				
Constant	-8.844	2.944	0.003				
Model			0.001	70.0	93.5	78 (50-100)	91 (80-100)
2nd trimester							
NGAL	0.036	0.019	0.06				
Ratio	0.348	0.179	0.05				
Constant	−4.928	1.565	0.002				
Model			0.001	33.3	100	100	88 (78-97)
Combination							
NGAL 1st trimester	0.033	0.022	0.128				
BMI 1st trimester	0.292	0.115	0.011				
Age 1st trimester	0.365	0.167	0.028				
UtA-PI 2nd trimester	1.955	1.235	0.113				
Constant	−24.510	8.571	0.004				
Model			0.001	80.0	91.9	73 (47-99)	94 (87-100)

SE: Standard Error, PPV: Positive Predictive Value, NPV: Negative Predictive Value, CI: Confidence Interval

Discussion

PE remains a leading cause of maternal and neonatal mortality and morbidity. Effective prediction of pregnancies in high risk to develop preeclampsia resulting to early diagnosis and treatment may decrease severity of complications or ideally occurrence of the disease [17–19]. This reinforces the need for early identification of high risk women with the objective of implementing targeted interventions for improving perinatal and maternal outcomes [20,21].

According to our results, it is clear that NGAL already from the first trimester seems to be a potent biomarker for the prediction of preeclampsia. This finding is reinforced by the fact that in a statistical analysis with matched (n = 12) groups between normal and preeclamptic pregnancies regarding parity, smoking, maternal BMI and age at the first trimester, NGAL was the only biochemical marker witch showed statistically significant difference between the two groups (p = 0.024 in the first trimester; results not shown).

NGAL is a 25 kDa protein of the lipocalin superfamily [22–24]. NGAL mRNA is normally expressed in a variety of human tissues [24]. Consequently, NGAL seems to have a crucial role in numerous physiological and pathological roles including epithelium function [24]. NGAL is considered as a marker of endothelial damage and it is not surprising that higher serum NGAL values are associated with PE [25–28]. Interestingly, NGAL in the first trimester seems to be an effective biomarker for the prediction of late-onset preeclampsia together with PlGF and sFlt-1 (DR 77% at a 10% false-positive rate) [29]. Moreover, D'Anna et al. [30], reported at DR using first trimester serum NGAL was 33.3% at a false-positive rate of 6.7% (likelihood ratio of 5.5). Our results, indicate also that NGAL is a very potent biomarker for the prediction of preeclampsia already from the first trimester as, when used alone, can detect 30.0% of pregnancies in high risk to develop preeclampsia and in combination with maternal BMI yielded a sensitivity of 70% and specificity of 93.5% (p = 0.001).

PlGF is an angiogenic growth factor involved in the normal implantation of the placenta. Already from the first trimester concentration of PlGF in women at risk of developing PE is lower than in women with normal pregnancy [31]. sFlt-1 is an anti-angiogenic protein that inhibits vascular endothelial growth factor and PlGF signaling. It is well established that serum sFlt-1 is higher in preeclamptic pregnancies [32]. In screening for preterm-PE (requiring delivery before 37 weeks), maternal serum PlGF is a useful marker from the first trimester, while sFlt-1 is likely to have a predictive value from the second trimester [32]. On the contrary, sFlt-1/PLGF ratio seems to be a more sensitive predictive marker already from the late first trimester [33,34]. Our results about PlGF, sFlt-1 and their ratio are in accordance with those observations and as showed sFlt-1/PlGF ratio in combination with NGAL in the second trimester yield a specificity of 100%.

ADMA is an L-arginine analog, which inhibits nitric oxide (NO) formation and potentially can lead to endothelial dysfunction [35]. Many studies have showed increased levels of serum ADMA in preeclamptic pregnancies, compared to normal ones, but this difference seems to become significant only after the early second trimester [36–38], something that can explain the fact that in our study ADMA did not add any power to the logistic regression model in the first trimester (sample collection 11 + 0 to 13 + 6 week).

Many studies have shown that increased impedance of blood flow in uterine arteries is associated with the development of PE [39–41]. A meta-analysis of 74 studies of PE by Cnossen et al. [42] (total of 79,547 singleton pregnancies) showed that uterine artery Doppler ultrasonography has a better performance in the second trimester than in the first trimester, and is useful for identifying severe or early-onset PE. Although in our study we confirmed that preeclamptic pregnancies had higher levels of UtA-PI in the second trimester compared to normal ones (p = 0.008), UtA-PI did not add any consistent discriminant power between cases and controls and was therefore excluded from logistic model. A possible explanation can be the low number of cases in the preeclamptic group (n = 12) or the fact that the combination of second trimester serum NGAL and sFlt-1/PLGF ratio represents actually a more potent model for the prediction of preeclampsia. On the contrary, when UtA-PI was combined with first trimester BMI, NGAL and age yield a sensitivity of 80.0% and specificity of 91.9% (p = 0.001).

Maternal BMI in the first trimester is a well-known risk factor for adverse pregnancy outcome, especially hypertensive disorders of pregnancy including preeclampsia and gestational hypertension [43]. In our study, we confirmed that pregnancies complicated with preeclampsia had significantly higher BMI (p = 0.007) compared to normal ones in the first trimester.

On a logistic regression based analysis, our model in the first trimester combining maternal BMI and serum NGAL yielded a similar DR (70%) and specificity as those reported in the literature for late PE screening, when NGAL is combined with other biochemical markers [29]. When we included in the model UtA-PI and DV-PI of the first trimester, DR reached 77.8%. This observation may indicate that those two Doppler variables can improve prediction of preeclampsia early from the first trimester. However, logistic regression is very model-dependent and any prospective evaluation must be carefully confirmed by several independent observations.

Our study has a number of limitations. These include (a) the small number of preeclamptic pregnancies (n = 12) and (b) the preeclamptic group consists of both early and late onset preeclampsia cases. It is well known that overall DR and specificity are very much influenced by the time of onset of preeclampsia (early or late), with early onset preeclampsia having higher DR, according to the majority of relative studies and meta-analysis [44].

Conclusion

As a conclusion, according to our results, a predictive model in the first trimester including maternal BMI, NGAL and Doppler markers UtA-PI/DV-PI is significant and can potentially aid patient counseling with regard to early screening for PE. NGAL seems to be a very potent biomarker already from the first trimester and improves effectiveness of predictive models.

Conflict of interest

The authors declare no conflict of interest.

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