ORIGINAL ARTICLE

Combination of serum angiopoietin-2 and uterine artery Doppler for prediction of preeclampsia

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The aim of this study was to determine the predictive value of the combination of serum angiopoietin-2 (Ang-2) levels and uterine artery Doppler for the detection of preeclampsia in women at 16–18 weeks of gestation and to identify other pregnancy complications that could be predicted with these combined tests. Maternal serum Ang-2 levels were measured, and uterine artery Doppler was performed in 400 pregnant women. The main outcome was preeclampsia. The predictive values of this combination were calculated. Twenty-five women (6.3%) developed preeclampsia. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of uterine artery Doppler combined with serum Ang-2 levels for the prediction of preeclampsia were 24.0%, 94.4%, 22.2% and 94.9%, respectively. For the prediction of early-onset preeclampsia, the sensitivity, specificity, PPV and NPV were 57.1%, 94.1%, 14.8% and 99.2%, respectively. Patients with abnormal uterine artery Doppler and abnormal serum Ang-2 levels (above 19.5 ng ml^{-1}) were at higher risk for preterm delivery (relative risk = 2.7, 95% confidence interval 1.2–5.8). Our findings revealed that the combination of uterine artery Doppler and serum Ang-2 levels at 16–18 weeks of gestation can be used to predict early-onset preeclampsia but not overall preeclampsia. Thus, this combination may be a useful early second trimester screening test for the prediction of early-onset preeclampsia.

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INTRODUCTION

Preeclampsia, a syndrome characterized by hypertension and proteinuria, affects approximately 5–10% of all pregnancies. It is a major obstetric complication and is the most common cause of fetal and maternal morbidity and mortality.^{1,2} Early detection of preeclampsia and the identification of pregnant woman at high risk of developing preeclampsia may reduce maternal and neonatal morbidity and mortality.

Although the etiology of preeclampsia is still unknown, a two-stage model has been proposed,³ in which impairment of trophoblastic invasion in the spiral arteries causes them to have small lumina and high resistance. This model can be evaluated by uterine artery Doppler.⁴ Many uterine artery Doppler studies have shown that preeclamptic patients have abnormal uterine artery Doppler results more frequently than normal patients. Systematic reviews and meta-analyses have suggested that the presence of a uterine artery notch or increased uterine artery pulsatility index (PI) in the second trimester is better at predicting preeclampsia than in the first trimester (sensitivity 77–96%).^{5,6} Previous studies in our population have shown that uterine artery PIs above the 95th percentile or bilateral diastolic notches had a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the prediction of preeclampsia of 20–75%, 52.5–95.8%, 9.1–14.3% and 97.1–97.2%, respectively.^{7,8}

Another model proposes angiogenic imbalance. The process of placental development, the coordination of vascular changes and the regulation of trophoblast growth are mediated by several locally acting angiogenic and anti-angiogenic factors and their receptors.⁹ When these processes are suboptimal, placental dysfunction can occur, causing preeclampsia. This can be evaluated by the measurement of angiogenic factors. In the maternal circulation of women with preeclampsia, Sahay *et al.*¹⁰ have found low levels of proangiogenic factors (vascular endothelial growth factor and placental growth factor) and high levels of angiotensin II type 1 receptor autoantibodies and anti-angiogenic factors (soluble fms-like tyrosine kinase-1 (sFlt-1) and the sFlt-1/placental growth factor ratio) during early gestation. One study has also demonstrated that enhanced oxidative stress in preeclamptic women during pregnancy may impair endothelial function, which then improves after delivery.¹¹

Angiopoietin-2 (Ang-2) is produced by endothelial cells. It has been postulated that Ang-2 has a role in angiogenesis in abnormal pregnancy.¹² Ang-2 promotes placental remodeling. Normal pregnancy is characterized by an increase in Ang-2 levels, with significantly lower levels found in pregnancies affected by placental dysfunction, leading to preeclampsia and intrauterine growth retardation.^{13–15} The predictive value of serum Ang-2 in preeclampsia is dependent on gestational age and the serum Ang-2 cutoff value. At 25–40 weeks of gestation, a serum Ang-2 cutoff value below 6.5 ng ml^{-1} showed an

82.8% sensitivity and 71.8% specificity for differentiating preeclamptic women from healthy pregnant women.¹⁴ In a previous study, maternal serum Ang-2 levels were similar between preeclamptic women and healthy pregnant women at 12–15 weeks of gestation; however, at 16–18 weeks of gestation, serum Ang-2 levels were higher in women who later developed preeclampsia using a cutoff level above 17.7 ng ml⁻¹ with an odds ratio of 4.2 (95% confidence interval 1.4–12.6).¹⁶

Combinations of uterine artery Doppler with biochemical markers have higher predictive values for preeclampsia compared with single tests. Because of limitations related to the heterogeneity of the study population and the fact that most tests can better detect early-onset preeclampsia, the best predictive test for preeclampsia is still questionable.^{17–19} No studies have examined the combination of uterine artery Doppler and serum Ang-2 levels for the prediction of preeclampsia in pregnant women in the early second trimester.

The objective of this study was to determine the predictive value of the combination of serum Ang-2 levels and uterine artery Doppler for the detection of preeclampsia in women at 16–18 weeks of gestation and to identify other pregnancy complications that can be predicted with these combined tests.

METHODS

This prospective observational study was performed at the Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand from January 2013 to December 2013. This study was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from all subjects.

Pregnant women with a gestational age of 16–18 weeks were invited to participate in this study. Women with fetal anomalies, medical diseases (chronic hypertension and renal disease) or a history of aspirin use, as well as women for whom we were unable to perform uterine artery Doppler, were excluded from this study. Gestational age was calculated from the last menstrual period and was confirmed by first trimester ultrasound.

Sample size calculation was based on the sensitivity for the prediction of preeclampsia reported by Hirokoshi *et al.*¹⁴ Accordingly, 394 women were required for this study.

Uterine artery Doppler evaluation

Uterine artery flow velocity waveforms were obtained by using ultrasonographic machines (GE Voluson 730 Pro or GE Voluson 730 Expert, GE Medical Systems, Zipf, Austria) with a convex abdominal probe AB 2–7 MHz. Each subject was examined once in the semi-recumbent position after 5 min of bed rest. The uterine artery was visualized by placing the end of the transducer on the left or right lower quadrant of the abdominal wall with the intent of identifying the external iliac artery and the uterine artery, which is medial to the external iliac artery. Flow velocity waveforms were obtained from each uterine artery where it crossed the external iliac artery. At least three waveforms from each side were recorded. Mean PI was calculated, and the presence or absence of an early diastolic notch was noted. A uterine notch was defined as a definite upward change in velocity after the deceleration slope of the primary wave. An abnormal uterine artery Doppler pattern was defined as a mean PI > 95th percentile for each gestational age or the presence of bilateral early diastolic notches.¹⁸ One operator (PP) performed the measurements. The intra-operator coefficient of variation was calculated in 20 women, and each of these 20 women was examined three times. The mean intra-observer coefficient of variation for the PI was 0.96 (95% confidence interval = 0.94–0.97).

Sample collection and Ang-2 immunoassay

After Doppler examination, venipuncture was performed, and blood was collected into clotted tubes. Blood samples were centrifuged at 2500 r.p.m. for 10 min and stored at –80 °C until assayed. Maternal serum Ang-2 levels were measured by an enzyme-linked immunosorbent assay (R&D Systems,

Minneapolis, MN, USA) according to the manufacturer's recommendations. The enzyme-linked immunosorbent assay kit is an enzymatically amplified two-step sandwich-type immunoassay. In this assay, the standards, controls and serum samples were incubated in microtitration wells coated with a monoclonal antibody against Ang-2. After incubation and washing, the wells were treated with Ang-2 conjugate. After a second incubation and washing step, the wells were incubated with the substrate solution. An acidic stopping solution was then added, and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurements at 450 and 540 nm using a microplate reader. The minimal detectable concentration in the assays for

Ang-2, as reported by the manufacturer, was 0.012 ng ml⁻¹. The inter-assay and intra-assay coefficients of variation were <10%.

Study outcomes

The primary outcome was the diagnosis of preeclampsia. Secondary outcomes included preterm delivery, intrauterine growth restriction, gestational diabetes, neonatal respiratory distress syndrome and perinatal death.

Statistical analysis

Data were analyzed with the SPSS software package version 17.0 for Windows (SPSS, Chicago, IL, USA) and are expressed as means, s.d.s, sensitivities, specificities, PPVs and NPVs, as well as relative risks with 95% confidence intervals. The optimal cutoff values for Ang-2 were calculated using the receiver operator characteristic curve. A chi-square test and Fisher's exact test were used for categorical variables; the independent *t*-test was used for continuous variables, and the Mann–Whitney *U* test was used for nonparametric variables when appropriate. A *P*-value < 0.05 was considered statistically significant.

RESULTS

A total of 430 pregnant women were enrolled in this study, with 30 patients ultimately being excluded. Data from 400 patients were analyzed, with 25 women developing preeclampsia (6.3%), 16 developing mild preeclampsia (4%), 9 developing severe preeclampsia (2.3%) and 7 developing early-onset preeclampsia (1.7%). Regarding clinical characteristics, pregnant women with preeclampsia had a higher body mass index than normal pregnant women (Table 1). Pregnant women with preeclampsia had higher rates of preterm delivery, intrauterine growth restriction, neonatal respiratory distress syndrome, lower Apgar scores at the 1st minute after birth and lower birth weights than normal pregnant women (Table 2).

Pregnant women with preeclampsia had higher mean PIs of the uterine artery (1.30 ± 0.45 *vs.* 1.05 ± 0.39, *P* = 0.003) and higher detection rates of unilateral (40.0 *vs.* 8.8%, *P* < 0.001) and bilateral diastolic notching (28.0 *vs.* 4.0%, *P* < 0.001) than normal pregnant women. Using uterine artery Doppler (PI above 95th percentile or bilateral diastolic notches) for the prediction of overall preeclampsia (Table 3), the sensitivity, specificity, PPV and NPV were 44%, 88.8%, 20.7% and 95.9%, respectively. For the prediction of early-onset preeclampsia, the sensitivity, specificity, PPV and NPV were 71.4%, 87.7%, 9.4% and 99.4%, respectively (Table 3).

Pregnant women with overall preeclampsia or early-onset preeclampsia did not have significantly higher serum Ang-2 levels than pregnant women without preeclampsia (median 17.7 *vs.* 17.3 ng ml⁻¹, *P* = 0.79 and 22.5 *vs.* 17.7 ng ml⁻¹, *P* = 0.09, respectively). The optimal cutoff value of 19.5 ng ml⁻¹ for serum Ang-2 level was based on the receiver operator characteristic curve. Using a serum Ang-2 cutoff value above 19.5 ng ml⁻¹ for the prediction of overall preeclampsia, the sensitivity, specificity, PPV and NPV were 36%, 60.3%, 5.7% and 93.4%, respectively. For the prediction of early-onset preeclampsia, the sensitivity, specificity, PPV and NPV were 71.4%, 61.1%, 3.2% and 99.2%, respectively (Table 3).

Table 1 Demographic characteristics

Characteristics	Overall (n = 400)	No preeclampsia (n = 375)	Preeclampsia (n = 25)	P-value
Age	36.3 ± 3.7	36.2 ± 3.6	37.1 ± 4.6	0.24
Primigravida (%)	122 (30.5%)	115 (30.7%)	1 (28.0%)	
Parity				0.21
0	176 (44.0%)	168 (44.8%)	8 (32.0%)	
≥ 1	224 (56.0%)	207 (55.2%)	17 (68.0%)	
Number of ANC	9.8 ± 2.1	9.8 ± 2.1	9.7 ± 2.5	0.79
GA at measurement (weeks)	17.5 ± 0.5	17.5 ± 0.5	17.7 ± 0.5	0.06
Prepregnancy BMI (kg m ⁻²)	22.3 ± 3.6	22.7 ± 3.4	24.8 ± 4.8	0.04
Obesity (BMI ≥ 30 kg m ⁻²)	19 (4.8%)	16 (4.3%)	3 (12.0%)	0.06

Abbreviations: ANC, antenatal care; BMI, body mass index; GA, gestational age.
Data presented as mean ± s.d. or n (%).

Table 2 Pregnancy complications and delivery outcomes

Characteristics	Overall (n = 400)	No preeclampsia (n = 375)	Preeclampsia (n = 25)	P-value
GA at delivery (weeks)	37.9 ± 1.7	38.0 ± 1.5	36.3 ± 2.8	<0.001
Preterm delivery (<37 weeks)	37 (9.3%)	29 (7.7%)	8 (32.0%)	<0.001
IUGR	6 (1.5%)	2 (0.5%)	4 (16%)	<0.001
Gestational diabetes	41 (10.3%)	41 (10.9%)	0 (0%)	0.09
Mode of delivery				0.36
Vagina	119 (29.8%)	93 (31.0%)	4 (16.0%)	
F/E	6 (1.5%)	6 (2.0%)	0	
V/E	2 (0.5%)	1 (0.3%)	0	
Cesarean section	273 (68.3%)	200 (66.7%)	21 (84.0%)	
Birth weight (g)	3088.4 ± 495.9	3123.6 ± 453.9	2561.6 ± 754.5	<0.001
Apgar scores				
1 min	8.8 ± 1.1	8.8 ± 1.1	8.1 ± 1.8	0.005
5 min	9.8 ± 1.2	9.8 ± 1.2	9.4 ± 1.8	0.11
RDS	8 (2.0%)	4 (1.1%)	4 (16%)	<0.001
Perinatal mortality rate	7 (1.8%)	6 (1.6%)	1 (4.0%)	0.37

Abbreviations: F/E, forceps extraction; GA, gestational age; IUGR, intrauterine growth restriction; RDS, respiratory distress syndrome; V/E, vacuum extraction.
Data presented as mean ± s.d. or n (%).

Table 3 Predictive value of uterine artery Doppler for preeclampsia

Characteristics	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR
<i>Overall preeclampsia</i>						
Mean PI > 95th percentile ± bilateral notching	44	88.8	20.7	95.9	3.9	0.6
Ang-2 level > 19.5 ng ml ⁻¹	36	60.3	5.7	93.4	1.4	0.7
Mean PI > 95th percentile ± bilateral notching with serum Ang-2 > 19.5 ng ml ⁻¹	24	94.4	22.2	94.9	4.3	0.8
<i>Early-onset preeclampsia</i>						
Mean PI > 95th percentile ± bilateral notching	71.4	87.7	9.4	99.4	5.8	0.3
Ang-2 level > 19.5 ng ml ⁻¹	71.4	61.1	3.2	99.2	1.8	0.5
Mean PI > 95th percentile ± bilateral notching with serum Ang-2 > 19.5 ng ml ⁻¹	57.1	94.1	14.8	99.2	9.7	0.4

Abbreviations: Ang-2, angiotensinogen; LR, likelihood ratio; NPV, negative predictive value; PI, pulsatility index; PPV, positive predictive value.

Using uterine artery PIs above the 95th percentile or bilateral diastolic notches combined with serum Ang-2 levels with a cutoff value of 19.5 ng ml⁻¹ for preeclampsia prediction, the sensitivity, specificity, PPV and NPV were 24%, 94.4%, 22.2% and 94.9%, respectively (Table 3). For early-onset preeclampsia prediction, the

sensitivity, specificity, PPV and NPV were 57.1%, 94.1%, 14.8% and 99.2%, respectively (Table 3).

Patients with an abnormal uterine artery PI (PI above 95th percentile or bilateral diastolic notches) and an abnormal serum Ang-2 level (above 19.5 ng ml⁻¹) were at a higher risk for preterm

Table 4 Abnormal uterine artery Doppler with abnormal Ang-2 level and pregnancy complications

Characteristics	Relative risk (95% confidence interval)
Preterm delivery (GA < 37 weeks)	2.7 (1.2–5.8)
IUGR	2.7 (0.3–22.8)
Gestational diabetes	1.1 (0.3–3.3)
Neonatal RDS	4.6 (0.9–21.7)
Perinatal death	2.3 (0.3–18.4)

Abbreviations: Ang-2, angiopoietin-2; GA, gestational age; IUGR, intrauterine growth restriction; RDS, respiratory distress syndrome.

delivery (relative risk = 2.7, 95% confidence interval 1.2–5.8) (Table 4).

DISCUSSION

This study demonstrates that the combination of uterine artery Doppler and serum Ang-2 levels is not effective as a second trimester screening test for preeclampsia; however, this combination test is effective at predicting early-onset preeclampsia.

In this study, the combination of uterine artery Doppler and serum Ang-2 level was not effective as a second trimester screening test for preeclampsia. This may be because of the several complex mechanisms involved in the development of preeclampsia.²⁰ Uterine artery Doppler and serum Ang-2 level may predict preeclampsia independently and in different manners. Thus, when these tests are used in combination to predict preeclampsia, they may not complement each other, resulting in reduced effectiveness. This finding was consistent with those of previous studies that have combined uterine artery Doppler with angiogenic factors and have found that the combination test did not improve the predictability of preeclampsia compared with single tests^{18,21,22} but contrasted with the findings of other studies.^{23,24}

The combination of uterine artery Doppler and serum Ang-2 levels at 16–18 weeks of gestation for the prediction of preeclampsia had a lower sensitivity and specificity compared with previous studies of the combination of uterine artery Doppler with other angiogenic factors.^{23,24} This difference may be explained by differences in the gestational ages, differences in the study populations and differences in the angiogenic factors examined by our study and previous studies.

However, no studies of the combination of uterine artery Doppler and serum Ang-2 levels for the prediction of preeclampsia in pregnant women between 16 and 18 weeks of gestation have been conducted previously. This study found that the combination of uterine artery Doppler and serum Ang-2 level had good sensitivity and high specificity for the prediction of early-onset preeclampsia but not for the prediction of overall preeclampsia. This finding was similar to those of previous studies that have found that the combination of uterine artery Doppler and biochemical markers predicts early-onset preeclampsia better than overall preeclampsia.^{18,25–27} Specifically, biochemical markers and uterine artery Doppler results were altered in cases of early-onset preeclampsia more than in cases of late onset preeclampsia.^{18,25,26,28} A recent study has also demonstrated that the combination of pulse wave velocity with soluble fms-like tyrosine kinase-1 improves screening efficacy for predicting early-onset preeclampsia.²⁹

In this study, serum Ang-2 levels at 16–18 weeks of gestation were higher in pregnant women with preeclampsia than in normal pregnant women. This finding was inconsistent with previous findings.¹⁶ The mechanism underlying the increased Ang-2 levels before the onset of preeclampsia remains unclear.¹⁶ As a result of abnormal placentation

in early pregnancy, hypoxic conditions in the placentas of preeclamptic patients may activate Ang-2 expression.³⁰ Ang-2 levels increase before the onset of preeclampsia. This finding is consistent with the increases in other angiogenic factors before the onset of preeclampsia.^{31,32}

Our unique study used a combination test of uterine artery Doppler and serum Ang-2 for the prediction of preeclampsia during a second trimester ultrasonographic examination for fetal anomaly screening. A notable limitation of this study was the time of measurement. We decided to conduct our measurements at 16–18 weeks gestation in conjunction with the ultrasonographic examination. This was more convenient for the patient because two screenings were performed in only one visit. It should be noted that if this combination test was performed at an earlier time, aspirin may have been prescribed before 16 weeks of gestation to prevent preeclampsia. Conversely, if these tests were performed later (such as late in the second trimester), they may have predicted preeclampsia more accurately.¹⁸ Further prospective study of this combination test at different time points during pregnancy should be conducted to evaluate the usefulness of these tests. Another limitation of this study was the small number of patients with early-onset preeclampsia. Further prospective study of this combination test should be conducted to confirm the usefulness of these tests for the prediction of early-onset preeclampsia.

The clinical application of this study is the identification of patients at risk for subsequent development of early-onset preeclampsia by using this combination test. This will be important for the timely referral of patients at high risk of developing early preeclampsia and for identification of the need for surveillance and starting antenatal corticosteroids at the appropriate time to induce fetal lung maturity. This approach will help to reduce maternal and neonatal morbidities in early-onset preeclampsia.

A recent publication has revealed that abnormal pressure-wave reflection from 26 to 32 weeks of gestation showed a stronger correlation with birth weight than conventional brachial blood pressure. This finding may provide new insight into the pathophysiology of fetal growth restriction, as well as superimposed preeclampsia, in pregnancies complicated by chronic hypertension.³³

In conclusion, the combination of uterine artery Doppler and serum Ang-2 levels in pregnant women at 16–18 weeks of gestation can be used for the prediction of early-onset preeclampsia but not overall preeclampsia. This combination test may be useful early second trimester screening tests for the prediction of early-onset preeclampsia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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