Original Article

JHSMR Journal of Health Science and Medical Research

Serum Angiopoietin-1/Angiopoietin-2 at 16-18 Weeks of Gestation to Predict Preeclampsia

Vorapong Phupong, M.D., Patau Tanbirojn, M.D., Ruangsak Lertkhachonsuk, M.D.

Placental Related Diseases Research Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand.

Received 22 September 2020 ● Revised 20 October 2020 ● Accepted 21 October 2020 ● Published online 26 March 2021

Abstract:

Objective: To determine whether serum angiopoietin-1/angiopoietin-2 ratio can predict preeclampsia in women at 16–18 weeks of gestation, or not.

Material and Methods: This was a prospective observational study that was conducted in pregnant women with gestational age of 16-18 weeks. Serum angiopoietin-1 and angiopoietin-2 levels were acquired. The predictive values of these tests were calculated.

Results: Data from 269 pregnant women were analyzed. Twenty-two cases developed preeclampsia, and five of these cases had early onset preeclampsia. When the angiopoietin-1/angiopoietin-2 ratio was above 6.2, the sensitivity, specificity, positive predictive value and negative predictive values to predict preeclampsia were 50.0%, 72.9%, 14.1% and 94.2%, respectively. When angiopoietin-1 was used to predict preeclampsia, the sensitivity, specificity, positive predictive value and negative predictive values were 59.1%, 65.2%, 13.1% and 94.7%, respectively. When angiopoietin-2 was used to predict preeclampsia, the sensitivity, specificity, positive predictive value and negative predictive values were 63.6%, 50.2%, 10.2% and 93.9%, respectively.

Conclusion: This study demonstrated that serum angiopoietin-1/angiopoietin-2 ratio at 16-18 weeks of gestation was not effective in predicting preeclampsia. However, angiopoietin-2 may be used to predict preeclampsia.

Keywords: angiogenesis, angiopoietin, prediction, preeclampsia, ratio

Contact: Prof. Vorapong Phupong, M.D.
Placental Related Diseases Research Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand.
E-mail: vorapong.p@chula.ac.th

J Health Sci Med Res .....
doi: 10.31584/jhsmr.2021793
www.jhsmr.org

Introduction

Preeclampsia (PE) is one of the most common obstetric complications that can result in perinatal morbidity and maternal mortality. The etiology of PE is still unknown. It has been proposed that having a defective angiogenesis may be one of the etiologies of PE. The imbalance between pro-angiogenic and anti-angiogenic factors can lead to impaired placentation, causing PE.[1]

The angiopoietin (Ang)/Tie signaling system is a second vascular endothelium-specific receptor tyrosine kinase pathway, apart from the vascular endothelial growth factor system, that is involved in the regulation of angiogenesis.[2] The Ang system has four ligands: Ang-1, Ang-2, Ang-3 and Ang-4. Ang-1 and Ang-2 have been well characterized, additionally they have two corresponding tyrosine kinase receptors (Tie-1 and Tie-2).[3-5] Ang-1 and Ang-2 are both expressed in the placenta and are involved in placental development.[3,6] The placental expression of Ang-1 normally increases; whereas, that of Ang-2 and Tie-2 decrease throughout gestation.[3,6] In PE, circulating concentrations of Ang-1 are elevated[7], while Ang-2 are lowered.[8-10]

Leinonen et al found that serum Ang-2 concentrations were elevated in the early midtrimester (16-20 weeks of gestation) in women that subsequently developed PE.[11] These findings support the hypothesis that an excess of anti-angiogenic factors may be a predisposing factor for PE, and may be apparent before the clinical onset of the disease. Bolin et al demonstrated that Ang-1/Ang-2 ratio increased during pregnancy in low-risk women, but the ratios were significantly lower in women who later developed preeclampsia at gestational age 25 and 28 weeks. They concluded that the plasma Ang-1/Ang-2 ratio may be a possible predictive biomarker for women who later developed preeclampsia.[12] One previous study found that Ang-2 levels were not significantly elevated in women with preeclampsia.[13]

A recent study assessed Ang-1, Ang-2 and the Ang-1/Ang-2 ratio levels in the first trimester of pregnancy and the association with adverse pregnancy outcomes; such as, small for gestational age, preterm birth, PE, miscarriage after 10 weeks of gestation and stillbirth. According to the findings of a former study, low Ang-2 levels and a high Ang-1/Ang-2 ratio were related to an increased risk for most adverse pregnancy outcomes, but did not improve the prediction of PE when used alone.[14] Thus, the objective of this study was to determine the value of the serum Ang-1/Ang-2 ratio to predict PE in women at 16–18 weeks of gestation.

Material and Methods

This study was a prospective observational study of pregnant women who attended the Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; between July, 2016 and July, 2017. This study was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from all participants.

Singletons, pregnant women with gestational age at 16-18 weeks who visited the antenatal clinic were invited and enrolled into the study. Gestational age was calculated by the last menstrual period, and confirmed by first trimester ultrasound. Blood samples were collected from the participants and centrifuged at 2,500 rounds per minute, for 10 minutes, and stored at -80 °C until assayed. Women who had fetal anomalies, medical diseases (chronic hypertension, renal disease), history of aspirin use and who later developed gestational hypertension were excluded from this study. Maternal and neonatal outcomes were extracted from the medical and delivery records.

Sample size calculations were based upon the expected sensitivity of Ang-1/Ang-2 ratio in predicting PE. The expected sensitivity was 70.0%. In order to have a 90.0% power and 20.0% allowable error, we required 20

cases with PE for the study. The incidence of PE at our institute was 7.0%. Hence, for this study, we would need a total of 286 women.

Angiopoietin-1 and angiopoietin-2 immunoassay

Serum Ang-1 and Ang-2 levels were measured by an enzyme-linked immunosorbent assay [R&D Systems, Minneapolis, Minnesota, The United States of America (USA)], according to the manufacturer's recommendations. The enzyme-linked immunosorbent assay kit is an enzymatically amplified two-step sandwich-type immunoassay. The minimal detectable concentration of the assays for Ang-1 and Ang-2, as reported by the manufacturer, were 0.0625 and 0.012 ng/mL, respectively. The inter-assay and intra-assay coefficients of variation were <10.0%.

Study outcome measurement

The study outcome was the diagnosis of PE. PE is defined as having a new onset of hypertension (systolic blood pressure 140 mmHg or higher, or diastolic blood pressure 90 mmHg or higher) and proteinuria (300 mg or higher in a 24-hour urine collection or 1+ or higher on dipstick testing) after 20 weeks of gestation.[15]

Statistic analysis

Data were analyzed with the Statistical Package for the Social Science for Windows software package version 17.0 (SPSS, Chicago, Illinois, USA) and are expressed as means, standard deviation, sensitivities, specificities, positive predictive value and negative predictive value; with 95% confidence intervals. The optimal cut-off value for Ang-1/Ang-2 ratio was 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. 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 286 pregnant women were enrolled into this study. Seventeen cases were excluded, due to lost to follow-up. Data from 269 pregnant women were analyzed. Twenty-two cases developed PE, and five of these cases had early onset PE.

Basic characteristics of the participants and pregnancy outcomes are shown in Table 1. There were no statistically significant differences in; age, parity, total weight gain, total time of antenatal care and gestational age at blood collection, between pregnant women with PE and the controls. Pregnant women with PE had a higher prepregnancy body mass index than the controls. Pregnant women with PE had significantly lesser gestational age at delivery and lower birth weight than the controls. Pregnant women with PE had higher rates of preterm delivery, fetal growth restriction, and neonatal respiratory distress syndrome (RDS) than the controls.

Ang-1 and Ang-2 levels were not different between pregnant women with PE and the controls (Table 2). Ang-1/Ang-2 ratio in pregnant women with PE was not significantly higher than the controls (4.9 vs 3.8, p-value=0.183).

The cut-off values for Ang-1, Ang-2, and Ang-1/Ang-2 ratio were established by using receiver operator characteristic curve, and the values were 80 ng/ml, 17 ng/ml and 6.2, respectively (Figure 1). When Ang-1 above 80 ng/ml was used, the sensitivity, specificity, positive predictive value and negative predictive values to predict PE were 59.1%, 65.2%, 13.1% and 94.7%, respectively. When Ang-2 below 17 ng/ml was used, the sensitivity, specificity, positive predictive value and negative predictive values to predict PE were 63.6%, 50.2%, 10.2% and 93.9%, respectively. When Ang-1/Ang-2 ratio above 6.2 was used, the sensitivity, specificity, positive predictive value and negative predictive values to predict the overall PE were 50.0%, 72.9%, 14.1% and 94.2%, respectively (Table 3).

Table 1 Baseline characteristics and pregnancy outcomes of women with and without preeclampsia

Variable	Control (n=247)	Preeclampsia (n= 22)	p-value
Maternal age (years)	36.7±2.9	37.3±4.9	0.375
Advanced maternal age (≥35 years old)	222 (89.9)	20 (90.9)	1.000
Primigravida	74 (30)	6 (27.3)	0.792
Parity			0.264
0	109 (44.1)	7 (31.8)	
≥1	138 (55.9)	15 (68.2)	
Prepregnancy BMI (kg/m^2)	22.4±3.4	24.9±5.3	0.004
Obesity (BMI ≥30 kg/m^2)	14 (5.7)	3 (16.7)	0.099
Total weight gain (kg)	14.0±5.0	15.1±5.7	0.373
Total time of ANC	9.7±2.2	9.6 ±2.7	0.702
GA at blood collection (weeks)	17.6±0.5	17.7±0.5	0.308
Pregnancy outcomes			
Gestational diabetes	32 (13)	0 (0.0)	0.087
Fetal growth restriction	2 (0.8)	4 (18.2)	<0.001
GA at delivery (weeks)	38.0±1.4	36.4±2.8	<0.001
Delivery at GA <37 weeks	20 (8.1)	8 (36.4)	<0.001
Delivery at GA <34 weeks	4 (1.6)	4 (18.1)	<0.001
Mode of delivery			0.286
Vaginal delivery	78 (31.6)	4 (18.2)	
Cesarean section	169 (68.4)	18 (81.8)	
Birth weight (grams)	3,129.6±446.7	2,559±783.9	<0.001
Low birth weight (<2,500 grams)	18 (7.3)	10 (45.5)	<0.001
Apgar scores			
1 minute	8.9±0.9	8.0±1.9	<0.001
5 minutes	9.9±0.9	9.4±1.9	0.022
Neonatal respiratory distress syndrome	2 (0.8)	4 (18.2)	<0.001
Perinatal death	3 (1.2)	1 (4.5)	0.291
Length of hospital stay	4.3±1.5	7.5±10.8	<0.001

Data are presented as mean±S.D. or n (%).
BMI=body mass index, GA=gestational age

Table 2 Serum angiopoietin-1 level, angiopoietin-2 level and angiopoietin-1/angiopoietin-2 ratio in women with preeclampsia compared with healthy control women

Test	Control (n=247)	Preeclampsia (n=22)	p-value
Ang-1 (ng/ml)	67.4 (41, 91)	84.3 (49.4, 115.7)	0.083
Ang-2 (ng/ml)	17.4 (12.1, 24.2)	14.9 (12.2, 23.6)	0.694
Ang-1/Ang-2 ratio	3.8 (2.1, 6.4)	4.9 (2.4, 9.3)	0.183

Data are presented as median (interquartile range)
Ang=angiopoietin

Table 3 Predictive value of serum angiopoietin-1 level, angiopoietin-2 level, and angiopoietin-1/angiopoietin-2 ratio for preeclampsia

Test	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Ang-1/Ang-2 ratio>6.2	50.0 (28.2, 71.8)	72.9 (66.9, 78.3)	14.1 (9.4, 20.7)	94.2 (91.5, 96.2)	1.8 (1.2, 2.9)	0.7 (0.5, 1.1)
Ang-1>80 ng/mL	59.1 (36.4, 79.3)	65.2 (58.9, 71.1)	13.1 (9.3, 18.2)	94.7 (91.5, 96.8)	1.7 (1.2, 2.5)	0.6 (0.4, 1.1)
Ang-2<17 ng/mL	63.6 (40.7, 82.8)	50.2 (43.8, 56.6)	10.2 (7.5, 13.8)	93.9 (89.8, 96.5)	1.3 (0.9, 1.8)	0.7 (0.4, 1.3)

Ang=angiopoietin, PPV=positive predictive value, NPV=negative predictive value, LR=likelihood ratio

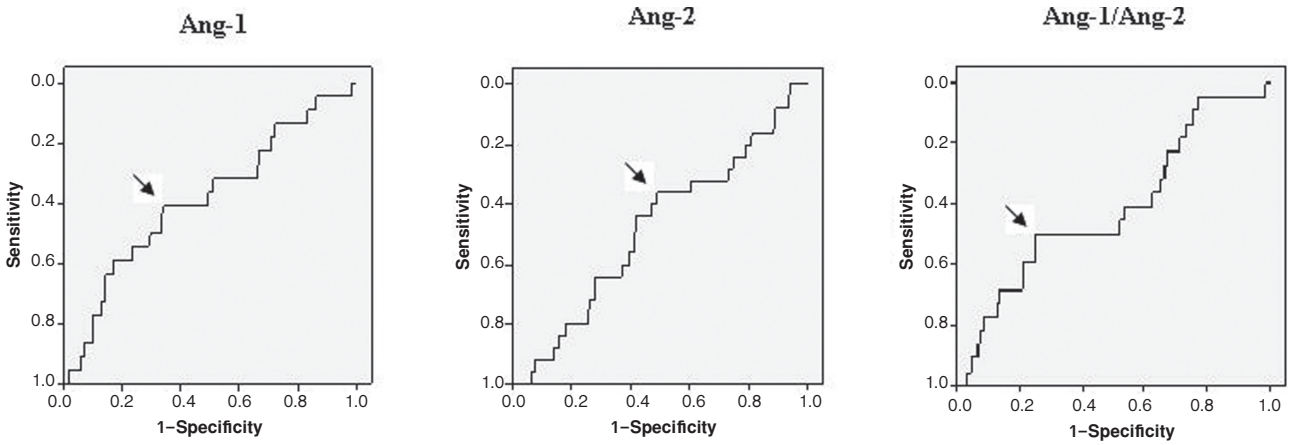

Figure 1 Receiver operator characteristic curve of angiopoietin-1, angiopoietin-2 and angiopoietin-1/angiopoietin-2 ratio

Discussion

This study demonstrated that serum Ang-1/Ang-2 ratio at 16-18 weeks of gestation was not effective in predicting PE. The result of this study was inconsistent with a previous study by Bolin et al.[12], who showed that the serum Ang-1/Ang-2 ratio at 25 weeks of gestation was effective in predicting PE. However, the sensitivity was only 47.0% and had a small sample size (19 preeclampsia cases and 43 controls). The difference between the studies may be due to different ethnicity and the gestational age at measurement. On the other hand, the result of this study was in agreement with Machado et al's study.[16] They performed a case-control study of pregnant women with gestational age of 20-25 weeks. They found that Ang-1 level, Ang-2 level and Ang-1/Ang-2 ratio were not different between pregnant women with PE and the healthy controls. They concluded that Ang-1 and Ang-2 levels were not good predictors of PE.

The sensitivity and specificity of serum Ang-1/Ang-2 ratio at 16-18 weeks of gestation to predict PE in this study was 50.0% and 72.9%, respectively. Bolin et al used a cut-off value of 1.41 for the Ang-1/Ang-2 ratio at

gestational age of 25 weeks. They found a sensitivity of 47.0% and a specificity of 87.0% to predict PE later in pregnancy.[12] Schneuer et al found that a high serum Ang-1/Ang-2 ratio in the first trimester was associated with most of the adverse pregnancy outcomes; such as, small for gestational age, preterm birth, preeclampsia and miscarriage, but could not predict outcomes any better than clinical and maternal risk factors.[14]

In contrast, other studies have shown that angiopoietin was somehow related to PE. Hirokoshi et al found that serum Ang-2 was low and sFlt-1 level was elevated among women with PE compared to healthy, pregnant women.[9] Another study compared the levels of Ang-1 and Ang-2 in normotensive pregnant women with pregnant women with severe PE in the third trimester. They found that the Ang-2 level was higher in pregnant women with severe PE, than in normotensive pregnant women. However, there was no difference in Ang-1 levels between women with severe PE and normotensive pregnant women.[17]

Regarding prepregnancy body mass index, pregnant women with PE had a higher prepregnancy body mass index than the controls in this study. This may be explained by being overweight and having obesity increased the risk of PE.[18]

The strength of this study was its prospective design, which allowed us to be able to ascertain whether the serum Ang-1/Ang-2 ratio could predict PE during the second trimester, or not. The limitation of this study was that there were a few cases of early-onset PE, and the gestational age of the study might be too early to show the differences in Ang-1/Ang-2 ratios.

Conclusion

This study demonstrated that the serum Ang-1/Ang-2 ratio at 16-18 weeks of gestation was not effective in predicting PE. However, angiopoietin-2 may be used to predict preeclampsia. Further studies, using a combination of Ang-2 with other markers or other measurements, should be conducted.

Acknowledgement

The authors would like to thank the staff and nurses at the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, for their kind suggestions and assistance. The authors would also like to thank Ms. Natnicha Huangham and Ms. Walailak Thongthab for their technical assistance.

Funding sources

This work was supported by a Grant for International Research Integration: Research Pyramid, Ratchadaphiseksomphot Endowment Fund, Chulalongkorn University and Placental Related Diseases Research Unit, Chulalongkorn University.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Cunningham FG, Lenovo KJ, Bloom SL, Dashe JS, Hoffman BL, Casey BM, et al. Williams obstetrics. 25th ed. New York: McGraw Hill; 2018.
2. Charnock-Jones DS. Soluble flt-1 and the angiopoietins in the development and regulation of placental vasculature. J Anat 2002;200:607-15.
3. Kappou D, Sifakis S, Konstantinidou A, Papantoniou N, Spandidos DA. Role of the angiopoietin/Tie system in pregnancy (Review). Exp Ther Med 2015;9:1091-6.
4. Thurston G. Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis. Cell Tissue Re 2003;314:61-8.
5. Thomas M, Augustin HG. The role of the Angiopoietins in vascular morphogenesis. Angiogenesis 2009;12:125-37.

6. Geva E, Ginzinger DG, Zaloudek CJ, Moore DH, Byrne A, Jaffe RB. Human placental vascular development: vasculogenic and angiogenic (branching and nonbranching) transformation is regulated by vascular endothelial growth factor-A, angiopoietin-1, and angiopoietin-2. J Clin Endocrinol Metab 2002; 87:4213-24.
7. Nadar SK, Karalis I, Al Yemeni E, Blann AD, Lip GY. Plasma markers of angiogenesis in pregnancy induced hypertension. Thromb Haemost 2005;94:1071-6.
8. Hirokoshi K, Maeshima Y, Kobayashi K, Matsuura E, Sugiyama H, Yamasaki Y, et al. Increase of serum angiopoietin-2 during pregnancy is suppressed in women with preeclampsia. Am J Hypertens 2005;18:1181-8.
9. Hirokoshi K, Maeshima Y, Kobayashi K, Matsuura E, Sugiyama H, Yamasaki Y, et al. Elevated serum sFlt-1/Ang-2 ratio in women with preeclampsia. Nephron Clin Pract 2007;106: c43-50.
10. Vuorela P, Matikainen MT, Kuusela P, Ylikorkala O, Alitalo K, Halmesmaki E. Endothelial tie receptor antigen in maternal and cord blood of healthy and preeclamptic subjects. Obstet Gynecol 1998;92:179-83.
11. Leinonen E, Wathen KA, Alfthan H, Ylikorkala O, Andersson S, Stenman U, et al. Maternal serum angiopoietin-1 and -2 and tie-2 in early pregnancy ending in preeclampsia or intrauterine growth retardation. J Clin Endocrinol Metab 2010;95:126-33.
12. Bolin M, Wiberg-Itzel E, Wikstrom AK, Goop M, Larsson A, Olovsson M, et al. Angiopoietin-1/angiopoietin-2 ratio for prediction of preeclampsia. Am J Hypertens 2009;22:891-5.
13. Puttapitakpong P, Phupong V. Combination of serum angiopoietin-2 and uterine artery Doppler for prediction of preeclampsia. Hypertens Res 2016;39:95-9.
14. Schneuer FJ, Roberts CL, Ashton AW, Guilbert C, Tasevski V, Morris JM, et al. Angiopoietin 1 and 2 serum concentrations in first trimester of pregnancy as biomarkers of adverse pregnancy outcomes. Am J Obstet Gynecol 2014;210:345e1-9.
15. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183:S1-22.
16. Machado JSR, Machado MSR, Bertagnolli TV, Martins LA, Freitas SF, Ovidio PP, et al. Role of plasma PlGF, PDGF-AA, ANG-1, ANG-2, and the ANG-1/ANG-2 ratio as predictors of preeclampsia in a cohort of pregnant women. Pregnancy Hypertens 2019;16:105-11.
17. Han SY, Jun JK, Lee CH, Park JS, Syn HC. Angiopoietin-2: a promising indicator for the occurrence of severe preeclampsia. Hypertens Pregnancy 2012;31:189-99.
18. Aksornphusitaphong A, Phupong V. Risk factors of early and late onset pre-eclampsia. J Obstet Gynaecol Res 2013;39:627-31.