Thai Journal of Obstetrics and Gynaecology
January 2021, Vol. 29, No. 1, pp. 26-39

## OBSTETRICS

# First Trimester Risk Factors of Pregnancy Associated Hypertension at Sanpasitthiprasong Hospital

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## ABSTRACT

**Objectives:** To determine risk factors in first trimester for development of pregnancy associated hypertension (PAH) at Sanpasitthiprasong Hospital.

**Materials and Methods:** A retrospective cohort study was conducted in pregnant women who attended antenatal care at Sanpasitthiprasong Hospital at $10^{+0}$ - $13^{+6}$ weeks of gestation and had first trimester Down syndrome screening test from October 2015 to November 2018. A total of 230 medical records of pregnant women were reviewed. Maternal baseline characteristics, ultrasonographic findings, serum biochemical levels, maternal and fetal outcomes were recorded. The association of these factors with development of PAH was examined using logistic regression.

**Results:** Among 230 pregnant women, 26 (11.3%) developed PAH. Factors significantly associated with developing PAH included pregestational diabetes mellitus (PGDM) (odds ratio (OR) 15.02, 95%CI 1.199-188.107; p = 0.036), pre-pregnancy body mass index (pBMI) ≥ 25 kg/m$^2$ (OR 4.06, 95% CI 1.443-11.442; p = 0.008), and mean arterial pressure (MAP) (mmHg) (OR 1.11, 95% CI 1.038-1.178; p = 0.002). Pregnant women with PAH developed more preterm delivery (23.1%vs 8.8%; p = 0.025) and neonatal hypoglycemia than those without (11.5% vs 2.5%; p = 0.017). An equation to predict risk of PAH is shown as follows: PAH score = -12.571 + (1.51 x if pBMI ≥ 25 kg/m$^2$ = 1, pBMI < 25 kg/m$^2$ = 0) + (0.122 x mean MAP mmHg) + (2.616 x if PGDM=1, no PGDM = 0). The probability of developing PAH was 1- (0.88 $^{\text{Exp(PAHscore+2.7734)}}$)).

**Conclusion:** Pregestational diabetes mellitus, pBMI ≥ 25 kg/m2, and MAP were independent first trimester risk factors for developing PAH.

**Keywords:** pregnancy associated hypertension (PAH), risk factors, first trimester screening.

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**Received: 30** September 2019, **Revised:** 27 November 2019, **Accepted:** 11 December 2019

# ปัจจัยเสี่ยงในไตรมาสแรกที่ก่อให้เกิดภาวะความดันโลหิตสูงขณะตั้งครรภ์ในโรงพยาบาลสรรพสิทธิประสงค์

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## บทคัดย่อ

***วัตถุประสงค์:*** *เพื่อศึกษาปัจจัยเสี่ยงในไตรมาสแรกที่ก่อให้เกิดภาวะความดันโลหิตสูงขณะตั้งครรภ์ในสตรีที่มาฝากครรภ์ที่โรงพยาบาลสรรพสิทธิประสงค์*

***วัสดุและวิธีการ:*** *ทำการศึกษาเชิงวิเคราะห์แบบย้อนหลังโดยทบทวนเวชระเบียน ของสตรีตั้งครรภ์ที่มาฝากครรภ์ที่โรงพยาบาลสรรพสิทธิประสงค์อายุครรภ์ตั้งแต่ $10^{+0}$ - $13^{+6}$ สัปดาห์ และได้รับการตรวจเลือดคัดกรองความเสี่ยงต่อทารกดาวน์ซินโดรม ระหว่างเดือน ตุลาคม พ.ศ.2558 ถึง พฤศจิกายน พ.ศ.2561 จำนวน 230 คน โดยเก็บข้อมูลเกี่ยวกับลักษณะพื้นฐานของสตรีตั้งครรภ์ และผลกระทบต่อการตั้งครรภ์และทารกแรกเกิด การตรวจคลื่นความถี่สูง และ ค่าสารชีวเคมีในเลือด เพื่อนำมาวิเคราะห์หาความสัมพันธ์กับการเกิดภาวะความดันโลหิตสูงในสตรีตั้งครรภ์*

***ผลการศึกษา:*** *ผลการศึกษาสตรีตั้งครรภ์ 230 คนพบภาวะความดันโลหิตสูงขณะตั้งครรภ์ 26 ราย คิดเป็นร้อยละ 11.3 จากการวิเคราะห์พบว่าปัจจัยที่มีผลทำให้เกิดภาวะความดันโลหิตสูงในการขณะตั้งครรภ์คือ ภาวะเบาหวานก่อนตั้งครรภ์ (odds ratio (OR) 15.02, 95%CI 1.199-188.107, p = 0.036), ดัชนีมวลกายก่อนตั้งครรภ์ (pBMI) มากกว่าเท่ากับ 25 kg/$m^2$ (OR 4.06 95% CI 1.443-11.442, p = 0.008) และค่าเฉลี่ยความดันเลือดแดง (OR 1.11 95% CI 1.038-1.178, p = 0.002) พบว่า ในสตรีที่มีความดันโลหิตสูงขณะตั้งครรภ์ มีการคลอดก่อนกำหนดมากกว่า (23.1%vs 8.8%; p = 0.025) และพบภาวะน้ำตาลในเลือดต่ำในทารกแรกเกิดได้มากกว่าสตรีที่ไม่มีความดันโลหิตสูงขณะตั้งครรภ์ (11.5% vs 2.5%; p = 0.017) เมื่อนำปัจจัยเสี่ยงมาคำนวณความเสี่ยงในการเกิดความดันโลหิตสูงในขณะตั้งครรภ์ และแทนค่า PAH score = -12.571 + (1.51 x ถ้า pBMI ≥ 25 kg/$m^2$ = 1, pBMI < 25 kg/$m^2$ =0) + (0.122 x mean MAP mmHg) + (2.616 x ถ้ามี pregestational DM=1, ถ้าไม่มี pregestational DM = 0) แล้วความเสี่ยงในการเกิดความดันโลหิตสูงในขณะตั้งครรภ์ = 1 - (0.88 $^{Exp(PAHscore+2.7734)}$))*

***สรุป:*** *ปัจจัยเสี่ยงในไตรมาสแรกต่อการเกิดภาวะความดันโลหิตสูงในขณะตั้งครรภ์ได้แก่ ภาวะเบาหวานก่อนตั้งครรภ์ ดัชนีมวลกายก่อนตั้งครรภ์มากกว่าเท่ากับ 25 kg/$m^2$ และค่าเฉลี่ยความดันเลือดแดง*

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## Introduction

Pregnancy associated hypertension (PAH) was one of the common serious problems during pregnancy, with a worldwide prevalence of approximately 10%[1]. There is evidence that the burden of the disease increased over time; for example, PAH prevalence increased by 20% over 20 years from 1987 to 2004[2]. In Thailand, preeclampsia occurred in 19.2 per 1,000 deliveries in 2012-2016[3]. PAH contributes to 50,000-60,000 deaths annually worldwide[1] and PAH was the second most common cause of maternal mortality in Thailand[4]. PAH has been reported to cause eclampsia, cerebral hemorrhage, abnormal renal and liver functions and a number of deleterious pregnancy outcomes, with some evidence suggesting that the disease may be associated with long term consequences involving cardiovascular, neurovascular, metabolic, renal and central nervous systems. PAH also resulted in poor fetal outcomes such as preterm delivery and intrauterine growth retardation[5]. In 2012, the estimated healthcare cost of PAH within the first 12 months after delivery in the United States was reported to be $2.18 billion[6].

Although causes of PAH were not clearly understood, a number of pathogenesis has been described, such as abnormal of placenta implantation, abnormal immune adaptation of mother to father and fetal tissue, abnormal cardiovascular adaptation and genetic aspects[5]. Many previous studies were conducted to find out risk factors associated with PAH development. Myatt et al[7] studied in nulliparous low risk women found that African American race, systolic blood pressure, body mass index (BMI), educational level, a disintegrin and metalloproteinase-12 (ADAM-12), pregnancy associated protein-A (PAPP-A) and placental growth factor (PlGF) levels in first trimester were significantly different in women who developed preeclampsia compared with controls. A meta-analysis of studies examining risk factors for PAH[8] found that women with antiphospholipid syndrome, prior preeclampsia, chronic hypertension, pre-pregnancy BMI of > 30 kg/m$^2$, and use of assisted reproductive technology (ART) were risk factors for developing PAH. In addition to these clinical risk factors and biomarkers, uterine artery (UtA) Doppler may also be useful to evaluate uteroplacental circulation and hence predict preterm preeclampsia[9].

Due to a poor predictive ability of a single risk factor, the multi-factorial models for prediction of preeclampsia were developed with varying sensitivity and specificity[10-12]. In Thailand, only a few studies[13, 14] investigated the accuracy of an approach using combined information from multiple risk factors especially in the first trimester to predict preeclampsia. Thus, this study aimed to examine the association of first trimester factors, including clinical, biochemical, and ultrasonographic factors, with risk of developing PAH. The secondary objective was to compare maternal and neonatal outcomes in PAH with non-PAH group and to develop a risk score combining information from multiple factors to predict early and late PAH in Thai women comparing western preexisting criteria[11,15] when applied to our population. This may help identify high risk groups to whom interventions to prevent PAH could be targeted.

## Materials and Methods

This retrospective cohort study was conducted after the approval by the Sanpasitthiprasong Hospital Ethic Committee (IRB No.049/2561). A total of 243 pregnant women who attended antenatal care at $10^{+0}$ - $13^{+6}$ weeks of gestation to perform first trimester Down syndrome screening and PlGF test at Sanpasitthiprasong hospital, Ubon Ratchathani, Thailand during October 2015 - November 2018 were eligible. By medical record review, data on risk factors were obtained. These included participant's age, gravidity, parity, gestational age, pre-pregnancy BMI (pBMI), mean arterial pressure (MAP), and a history of previous preeclampsia, chronic hypertension, systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), pregestational diabetes (PGDM), and previous gestational diabetes (GDM), previous Cesarean section, pregnancy by assisted reproductive technology, smoking, first degree relatives of hypertensive disorder in pregnancy. Data on blood levels of free beta-human chorionic gonadotropin (β-hCG), Pregnancy-associated plasma

protein A (PAPP-A), Placental growth factor (PlGF), and ultrasonographic findings such as crown-rump length (CRL), nuchal translucency (NT), UtA Doppler pulsatility index (PI) and notch, ductus venosus (DV) PI were also collected. Additionally, data on pregnancy outcomes, such as gestational age at delivery, route of delivery, obstetric complications, and neonatal outcomes namely birth weight, Apgar score, neonatal complications were obtained. The participants with missing data on prenatal data and delivery outcomes and those who aborted before 26 weeks of gestation were excluded. The exclusion of those who aborted before 26 weeks of gestation was owing to pre-specified within-institution agreement between obstetricians and pediatricians that < 26 weeks of gestation was considered abortion.

The MAP, UtA-PI, DV-PI were measured according to standardized protocols[16-18], A serum sample of 70 microliter was used to measure PlGF concentration by B-R-A-H-M-S PlGF plus KRYPTOR based on TRACETM Technology (Time-Resolved Amplified Cryptate Emission). The detection range of the assay was 3.6-7,000 pg/mL. Maternal serum 50 microliter was used to measure PAPP-A by the B-R-A-H-M-S PAPP-A KRYPTOR based on TRACETM technology. The detection range of the assay was 0.004-90 IU/L.

Pregnancy associated hypertension (PAH) was defined as gestational hypertension (GH) and/or preeclampsia (PE)[1]. GH was defined as an occurrence of new-onset hypertension after 20 weeks gestation (1. a systolic blood pressure (BP) of 140 mmHg or greater, a diastolic BP of 90 mmHg or greater at two separate measurements at least 4 hours apart or 2. systolic BP of 160 mmHg or more or diastolic BP of 110 mmHg or more confirmed at a short interval) in the absence of significant proteinuria. PE was defined as GH plus new-onset of proteinuria (24-hour excretion equals or exceeds 300 mg or protein/creatinine ratio of 0.3 mg/dL or more or dipstick reading of 2+) or as in the absence of proteinuria, new-onset hypertension with the new onset of any of the followings: thrombocytopenia (platelet count < 100,000 x 109/L) or renal insufficiency (serum creatinine (Cr) > 1.1 mg/dL or a doubling of the serum Cr in the absence of other renal disease) or impaired liver function (elevated blood concentrations of liver transaminases to twice normal concentration) or pulmonary edema or new-onset headache (unresponsive to medication and not accounted for alternative diagnoses) or visual symptom. Early PAH was defined as the occurrence of PAH before 34 weeks, and late PAH occurred at 34 weeks and beyond.

Sample size was calculated based on a research question "what is the risk factor for PAH?" According to data from a study by Goetzinger et al[11], 34% of pregnant women with chronic hypertension developed preeclampsia (P1 = 0.34), while 6% of those without chronic hypertension developed preeclampsia (P2 = 0.06) and the ratio of without to with chronic hypertension (non-exposed to exposed ratio) was approximately 10%. At 80% power and 95% confidence level, a sample size of 200 was required. Assuming 10% missing data on main outcomes, the final sample size was approximately 220.

***Statistical analyses***

Statistical analyses were performed using IBM SPSS Statistics Version 21. Baseline maternal and obstetric characteristics were described as number (%), mean (standard deviation (SD)) and median (interquartile range (QR)) for categorical, normally and non-normally distributed continuous variables, respectively. Distribution of data was tested by Kolmogorov-Smirnov test. Continuous variables were compared between two groups using student t-test. Categorical variables were compared between groups using chi-square or Fisher's exact test. Maternal and neonatal outcomes were compared between those with and without PAH. Risk factors for PAH were examined using univariate and multivariate logistic regression, with crude and adjusted odds ratio (OR) and 95% confidence interval (CI) reported. Variables which were associated with PAH at p value of less than 0.05 in univariate analyses were included in multivariable logistic regression. A p value of < 0.05 was considered statistically significant. The beta-coefficients for factors independently associated

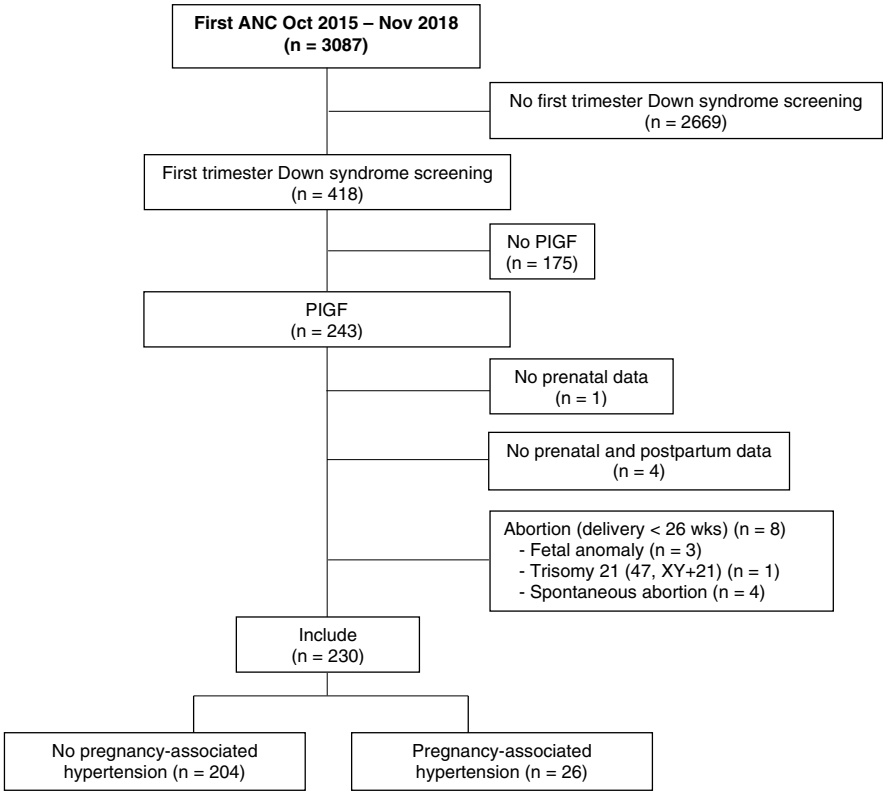
with PAH were used to construct PAH risk score: Risk score = (X1x $\beta$1) +(X2x $\beta$2)… + (Xpx $\beta$p). The *probability of developing PAH was calculated by the following equation:*

$$\text{Probability of PAH} = 1 - S(j)^{\text{EXP(Risk Score – Mean of the Risk score)}}$$

To identify the optimal cut-off points of PAH score and PAH probability that signified high risk groups, we computed sensitivity/specificity, positive predictive value/ negative predictive value (PPV/NPV) and the area under the receiver operating characteristic curves (AUC) for each particular cut-off points (10%, 20%, 30%, 40% and 50% probability of PAH). Additionally, the sensitivity, specificity, PPV/NPV and AUC of newly developed PAH score and three other existing criteria (NICE and Thermo Fisher (TF) software and Goetzinger criteria (K score)) in prediction of all PAH, early PAH and late PAH in these 230 pregnant women were compared using nonparametric methods. AUC curves for the above scoring criteria were also plotted.

## Results

A total of 3,087 pregnant women attended antenatal care in their first trimester in Sanpasitthiprasong Hospital during December 2015-November 2018. Only 418 (13.5%) had first trimester Down syndrome screening test and among these participants, 175 women had no PlGF results. After review of medical records, 13 pregnant women were excluded (three cases of multiple fetal abnormalities, one case of termination of pregnancy due to fetal Down syndrome, four cases of spontaneous abortion before 26 weeks of gestation, one case of no prenatal data, and six cases of no perinatal outcomes) leaving a final study sample of 230 pregnant women (Fig. 1).




ANC: Antenatal care, PIGF: Placental growth factor

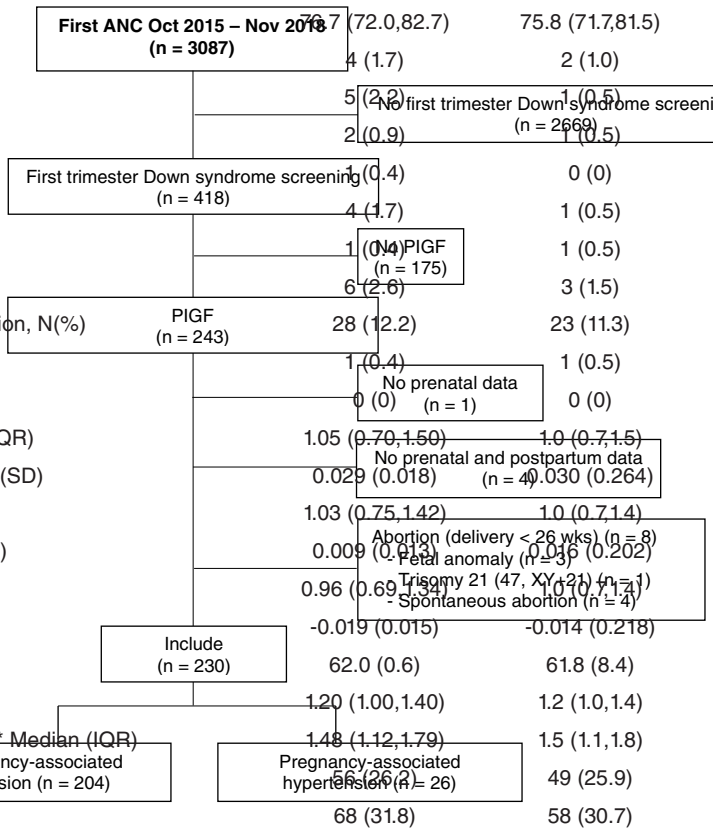
**Fig. 1.** Flow diagram of recruitment into the cohort study.

Pregnancy associated hypertension (PAH) developed in 26 (11.3%) women. These included 11 (4.8%) cases of gestational hypertension, 2 (0.9%) cases of preeclampsia without severe features, 8 (3.5%) cases of preeclampsia with severe features, 4 (1.7%) cases of chronic hypertension superimposed preeclampsia 4 (1.7%), and 1 (0.4%) case of eclampsia.

Table 1 shows characteristics of 230 pregnant women participating in the study, overall and by PAH status. Those with PAH had higher pBMI, and MAP (75.8 vs 85.1 mmHg, p < 0.001) than those without. A higher proportion of PAH group reported a history of previous preeclampsia, chronic hypertension, APS, PGDM, and hyperthyroidism than non-PAH group. Other maternal characteristics, Doppler ultrasound findings, and serum biochemical level were not significantly different between the two groups.

**Table 1.** Maternal characteristics, ultrasound findings, and serum biochemical test results, overall and by PAH group (n=230).

| Characteristics | Total (n=230) | Non-PAH (n=204) | PAH (n=26) | p value |
|---|---|---|---|---|
| Maternal age, years, Mean (SD) | 32.4 (0.3) | 32.4 (4.5) | 32.7 (5.7) | 0.738 |
| Nulliparous, N(%) | 86 (37.4) | 80 (39.2) | 6 (23.1) | 0.109 |
| History of abortion, N(%) | 52 (22.6) | 42 (20.6) | 10 (38.5) | 0.040 |
| Gestational age at first ANC, weeks, Median (IQR) | 12.6 (12.0,12.9) | 12.4 (12.1,12.9) | 12.6 (12.0,13.4) | 0.354 |
| pBMI, kg/m$^2$, Median (IQR) | 21.7 (19.7,24.1) | 21.4 (19.6,23.6) | 25.4 (22.6,29.0) | < 0.001 |
| pBMI ≥ 25 kg/m$^2$, Median (IQR) | 45 (19.6) | 30 (14.7) | 15 (57.7) | < 0.001 |
| MAP, mmHg, Median (IQR) | 76.7 (72.0,82.7) | 75.8 (71.7,81.5) | 85.1 (77.6,96.1) | < 0.001 |
| Previous preeclampsia, N(%) | 4 (1.7) | 2 (1.0) | 2 (7.7) | 0.014 |
| Chronic hypertension, N(%) | 5 (2.2) | 1 (0.5) | 4 (15.4) | < 0.001 |
| SLE, N(%) | 2 (0.9) | 1 (0.5) | 1 (3.8) | 0.083 |
| APS, N(%) | 1 (0.4) | 0 (0) | 1 (3.8) | 0.005 |
| PGDM, N(%) | 4 (1.7) | 1 (0.5) | 3 (11.5) | < 0.001 |
| Previous GDM, N(%) | 1 (0.4) | 1 (0.5) | 0 (0) | 0.721 |
| Hyperthyroidism, N(%) | 6 (2.6) | 3 (1.5) | 3 (11.5) | 0.002 |
| 1st degree relative hypertension, N(%) | 28 (12.2) | 23 (11.3) | 5 (17.9) | 0.243 |
| ART, N(%) | 1 (0.4) | 1 (0.5) | 0 (0) | 0.721 |
| Smoking, N(%) | 0 (0) | 0 (0) | 0 (0) | - |
| Free β-hCG, MoM, Median (IQR) | 1.05 (0.70,1.50) | 1.0 (0.7,1.5) | 1.4 (0.7,1.6) | 0.922 |
| Free β-hCG, log MoM, Mean (SD) | 0.029 (0.018) | 0.030 (0.264) | 0.004 (0.272) | 0.650 |
| PAPP-A, MoM, Median (IQR) | 1.03 (0.75,1.42) | 1.0 (0.7,1.4) | 0.9 (0.8,1.3) | 0.392 |
| PAPP-A, log MoM, Mean (SD) | 0.009 (0.013) | 0.016 (0.202) | -0.017 (0.187) | 0.425 |
| PIGF, MoM, Median (IQR) | 0.96 (0.69,1.34) | 1.010 (7.14) | 0.8 (0.7,1.3) | 0.389 |
| PIGF, log MoM, Mean (SD) | -0.019 (0.015) | -0.014 (0.218) | -0.057 (0.205) | 0.352 |
| CRL, mm, Mean (SD) | 62.0 (0.6) | 61.8 (8.4) | 63.5 (8.7) | 0.330 |
| NT, mm, Median (IQR) | 1.20 (1.00,1.40) | 1.2 (1.0,1.4) | 1.2 (0.9,1.4) | 0.415 |
| Mean UtA PI Doppler, MoM, * Median (IQR) | 1.48 (1.12,1.79) | 1.5 (1.1,1.8) | 1.5 (1.1,1.9) | 0.837 |
| Bilateral UtA notch*, N(%) | 56 (26.2) | 49 (25.9) | 7 (28.0) | 0.825 |
| Unilateral UtA notch*, N(%) | 68 (31.8) | 58 (30.7) | 10 (40.0) | 0.347 |
| DVPI (MoM)**, Median (IQR) | 0.86 (0.75,1.00) | 0.9 (0.8,1.0) | 0.9 (0.7,0.9) | 0.430 |




* Data available in 219 cases ** Data available in 178 cases

PAH: pregnancy associated hypertension, MAP: mean arterial pressure, SLE: systemic lupus erythematosus, APS: antiphospholipid syndrome, PGDM: pregestational diabetes, GDM: previous gestational diabetes, PAPP-A: placental growth factor, PIGF: placental growth factor, CRL: crown-rump length, NT: nuchal translucency, UtA: uterine artery.

Table 2 demonstrates maternal and neonatal outcomes by PAH status. Although gestational age at delivery was comparable between PAH and non-PAH groups, higher proportion of preterm delivery was in PAH than non-PAH groups (23.1% vs 8.8%, p = 0.025). The MAP at delivery was 22 mmHg higher in PAH than non-PAH groups. There was no difference in other maternal complications such as GDM, anemia, premature ruptured of membrane (PROM), oligohydramnios. Neonatal hypoglycemia occurred more frequently in PAH than non-PAH groups (11.5% vs 2.5%, p = 0.017). Neonatal birth weight and birth asphyxia were comparable between the two groups. There was no difference in other neonatal complications such as jaundice, respiratory distress, and low birthweight.

**Table 2.** Maternal and neonatal outcomes, overall and by PAH status (n=230).

| Outcomes | Total | Non-PAH | PAH | p value |
|---|---|---|---|---|
| GA at delivery, week, Median (IQR) | 38.3 (37.6,38.9) | 38.3 (37.7,38.9) | 38.1 (37.0,39.2) | 0.456 |
| BP at delivery, mmHg, Median(IQR) | | | | |
| - SBP | 122.5 (113.0,132.8) | 120.0 (112.0,130.0) | 150.0 (140.0,169.25) | < 0.001 |
| - DBP | 75.0 (68.0,83.0) | 73.5 (67.8,80.0) | 91.5 (87.8,100.0) | < 0.001 |
| - MAP | 91.5 (83.0,98.8) | 90.0 (82.0,96.0) | 112.0 (107.0,121.0) | < 0.001 |
| Body weight gain, kg, Median(IQR) | 12.20 (9.00,15.35) | 12.2 (9.8,15.5) | 12.9 (6.5,15.1) | 0.285 |
| Route of delivery, N(%) | | | | |
| Normal delivery | 48 (20.9) | 42 (20.6) | 6 (23.1) | 0.769 |
| Vacuum extraction | 10 (4.3) | 9 (4.4) | 1 (3.8) | 0.894 |
| Forceps extraction | 6 (2.6) | 5 (2.5) | 1 (3.8) | 0.674 |
| Cesarean section | 166 (72.2) | 148 (72.5) | 18 (69.2) | 0.722 |
| GDMA1, N(%) | 18 (7.8) | 14 (6.9) | 4 (15.4) | 0.128 |
| GDMA2, N(%) | 2 (0.9) | 1 (0.5) | 1 (3.8) | 0.083 |
| Anemia, N(%) | 54 (23.5) | 47 (23.0) | 7 (26.9) | 0.660 |
| Preterm delivery, N(%) | 24 (10.4) | 18 (8.8) | 6 (23.1) | 0.025 |
| PROM, N(%) | 20 (8.7) | 18 (8.8) | 2 (7.7) | 0.847 |
| Postpartum hemorrhage, N(%) | 28 (12.2) | 24 (11.8) | 4 (15.4) | 0.595 |
| Placental abruption, N(%) | 1 (0.4) | 1 (0.5) | 0 (0) | 0.721 |
| Oligohydramnios, N(%) | 7 (3.0) | 7 (3.4) | 0 (0) | 0.337 |
| Maternal length of stay, day, Median(IQR) | 3.0 (3.0,3.0) | 3.0 (3.0,3.0) | 3.0 (3.0,4.0) | 0.052 |
| Neonatal birth weight, gm, Mean(SD) | 3040.19 (30.647) | 3048.0 (441.5) | 2979.2 (626.0) | 0.591 |
| Apgar score at 1 minute, Median(IQR) | 9 (9,9) | 9 (9,9) | 9 (9,9) | 0.339 |
| Apgar score at 5 minutes, Median(IQR) | 10 (10,10) | 10 (10,10) | 10 (10,10) | 0.339 |
| Newborn length of stay, day, Median(IQR) | 3.0 (3.0,4.0) | 3.0 (3.0,3.0) | 3.0 (3.0,5.3) | 0.097 |
| NICU admission: N(%) | 11 (4.8) | 10 (4.9) | 1 (3.8) | 0.812 |
| Neonatal complication, N(%) | | | | |
| - Jaundice | 39 (17.0) | 33 (16.2) | 6 (23.1) | 0.377 |
| - Respiratory distress | 30 (13) | 24 (11.8) | 6 (23.1) | 0.107 |
| - Low birth weight | 18 (7.8) | 14 (6.9) | 4 (15.4) | 0.128 |
| - Hypoglycemia | 8 (3.5) | 5 (2.5) | 3 (11.5) | 0.017 |

PAH: pregnancy associated hypertension, GA: gestational age, BP: blood pressure, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, GDM: previous gestational diabetes, NICU: neonatal care unit, PROM: premature ruptured of membranes

***Factors associated with the risk of developing PAH and Development of PAH risk score***

Factors that were associated with PAH in univariate and multivariate logistic regression were shown in Table 3. In multivariate logistic regression, factors independently associated with the risk of PAH included being overweight, MAP and pregestational diabetes mellitus. Pregnant women with pBMI ≥ 25 kg/m$^2$ were at 4-fold high risk of PAH than those with pBMI < 25kg/m$^2$ (adjusted OR 4.06; 95% CI = 1.443-11.442, p = 0.008). Risk of PAH increased 11% for every mmHg increase in MAP (adjusted OR 1.11; 95% CI = 1.038-1.178, p = 0.002). Pregestational diabetes mellitus was associated with a 15-times higher risk of PAH (adjusted OR 15.02 with 95% CI = 1.199-188.107, p = 0.036). These associations remained statistically significant even after adjusting for a history of abortion, previous preeclampsia, chronic hypertension, PGDM and hyperthyroidism.

**Table 3.** Crude odds ratios and Adjusted odd ratios for the risk of PAH derived from logistic regression analysis (n=230).

| Characteristics | PAH, n (%) | Non PAH, n (%) | Crude OR (95%CI) | p value | Model 1 | | Model 2 | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Adjusted OR (95%CI)* | p value | Adjusted OR (95%CI)* | p value |
| History of abortion | | | | | | | | |
| Yes | 10(19.2) | 42(80.8) | 2.41 (1.020-5.696) | 0.045 | 1.46 (0.472-4.522) | 0.511 | - | - |
| No | 16(9.0) | 162(91.0) | | | | | | |
| Pregnancy BMI | | | | | | | | |
| ≥ 25 kg/m$^2$ | 15(33.3) | 30(66.7) | 7.91 (3.316-18.863) | <0.001 | 4.06 (1.443-11.442) | 0.008 | 4.53 (1.693-12.100) | 0.003 |
| < 25 kg/m$^2$ | 11(5.9) | 174(94.1) | | | | | | |
| MAP (mmHg) | - | - | 1.15 (1.087-1.215) | <0.001 | 1.11 (1.038-1.178) | 0.002 | 1.13 (1.068-1.194) | <0.001 |
| Previous preeclampsia | | | | | | | | |
| Yes | 2(50.0) | 2(50.0) | 8.42 (1.133-62.511) | 0.037 | 3.38 (0.147-77.786) | 0.446 | - | - |
| No | 241(0.6) | 202(89.4) | | | | | | |
| Chronic hypertension | | | | | | | | |
| Yes | 4(80) | 1(20) | 36.91 (3.949-344.980) | 0.002 | 2.41 (0.155-37.321) | 0.530 | - | - |
| No | 22(9.8) | 203(90.2) | | | | | | |
| PGDM | | | | | | | | |
| Yes | 3(75) | 1(25) | 26.48 (2.644-265.127) | 0.005 | 15.02 (1.199-188.107) | 0.036 | 13.68 (1.108-168.883) | 0.041 |
| No | 23(10.2) | 203(89.8) | | | | | | |
| Hyperthyroidism | | | | | | | | |
| Yes | 3(50) | 3(50) | 8.74 (1.666-45.844) | 0.010 | 3.06 (0.326-28.745) | 0.328 | - | - |
| No | 23(10.3) | 201(89.7) | | | | | | |

* Adjusted for all variables in the table.

PAH = pregnancy associated hypertension, OR: odds ratio, BMI: body mass index MAP: mean arterial pressure, PGDM: pregestational diabetes.

β coefficients from the multivariate logistic regression model for pBMI ≥ 25 kg/m$^2$, MAP, and the presence of PGDM were used to develop risk score to predict PAH as the equation shown below.

PAH score = -12.571 + 1.51 (pBMI ≥ 25 kg/m$^2$) + 0.122 (MAP; mmHg) + 2.616 (PGDM)

The probability of developing PAH was computed from the equation below.

Probability of PAH = 1- 0.88 $^{EXP(PAH\ Score\ +2.7734)}$

Measures of predictive ability for different cut-off points of PAH risk score are shown in Table 4. Different cut-off points of PAH scores represent 10%, 20%, 30%, 40%, 50% risk of PAH had different sensitivity, specificity, PPV/NPV and AUC. PAH score of -2.87 showed the highest sensitivity and lowest specificity and the sensitivity increased and specificity decreased as the PAH risk scores decreased. The cut-off PAH score -1.68 representing a PAH probability of 30% yielded the highest discriminatory ability with the AUC of 0.787.

**Table 4.** Predictive ability for different cut-off points of PAH risk score.

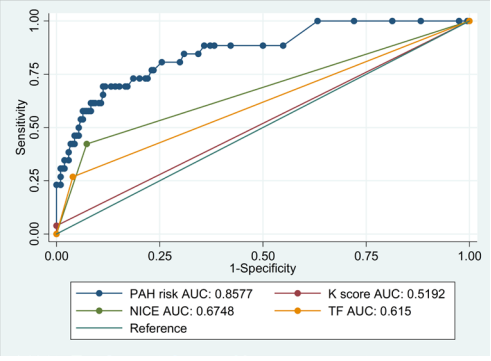
| Probability of PAH (number of women with risk higher than the value) | PAH Score | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|
| 10% (n=99) | -2.87 | 88.46% (69.85-97.55) | 62.75% (55.72-69.40) |
| 20% (n=60) | -2.16 | 73.08% (52.21-88.43) | 79.90% (73.74-85.17) |
| 30% (n=42) | -1.68 | 69.23% (48.21-85.67) | 88.24% (83.00-92.31) |
| 40% (n=33) | -1.36 | 61.54% (40.57-79.77) | 91.67% (86.99-95.07) |
| 50% (n=28) | -1.02 | 57.69% (36.92-76.65) | 93.63% (89.35-96.56) |
| | **PPV (95% CI)** | **NPV (95% CI)** | **AUC (95% CI)*** |
| 10% (n=99) | 23.23% (19.45-27.50) | 97.71% (93.61- 99.20) | 0.756 (0.670-0.842) |
| 20% (n=60) | 31.67% (24.44-39.90) | 95.88% (92.49-97.78) | 0.765 (0.661-0.869) |
| 30% (n=42) | 42.86% (32.24-54.17) | 95.74% (92.65-97.57) | 0.787 (0.679-0.895) |
| 40% (n=33) | 48.48% (35.25-61.93) | 94.92% (91.99-96.82) | 0.766 (0.650-0.882) |
| 50% (n=28) | 53.57% (38.29-68.21) | 94.55% (91.71-96.46) | 0.757 (0.637-0.876) |

Note: * According to nonparametric methods, no difference between AUC of different cut-off points of PAH score.
PAH: pregnancy associated hypertension, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the receiver operating characteristic curve, C: confidence interval

***Comparison of different approaches to risk prediction***

Comparison of different approaches to risk prediction Fig. 2. shows the AUC curves for prediction of PAH for different scoring methods. PAH risk performed better than NICE, TF and K score (p value < 0.001 for each pairwise comparison). Table 5. shows comparison of predictive ability for different approaches: PAH score of -1.68, NICE[15], TF) and K score[11]. The PAH score cut-off point of

-1.68 was as good as NICE, but performed better than TF and K score in predicting all PAH, with considerably higher sensitivity and AUC (p = 0.064, p = 0.028 and p < 0.001 respectively). Similar findings were observed for prediction of early and late PAH.




* p value < 0.001 for pairwise comparison of NICE, TF and K score with PAH risk score
PAH: antenatal care, AUC: area under the receiver operating characteristic curves.

**Fig. 2.** Receiver-operating characteristic (ROC) curves for prediction of PAH for different scoring methods.

**Table 5.** Comparison of sensitivity, specificity, positive predictive value, negative predictive value and area under the receiver operating characteristic curves (AUC) of PAH score and other existing criteria (NICE, Thermo Fisher (TF) software and K score) in prediction of all PAH, early PAH and late PAH.

| | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **PPV (95% CI)** | NPV (95% CI) | AUC (95% CI) |
|---|---|---|---|---|---|
| All PAH | | | | | |
| PAH score of -1.68 | 69.23% (48.21-85.67) | 88.24% (83.00-92.31) | 42.86% (32.24-54.17) | 95.74% (92.65-97.57) | 0.787 (0.679-0.895) |
| NICE | 42.31% (23.35-63.08) | 92.65% (88.16-95.83) | 42.31% (27.44-58.72) | 92.65% (90.05-94.61) | 0.675 (0.548-0.801) |
| TF | 26.92% (11.57-47.79) | 96.08% (92.42-98.29) | 46.67% (25.69-68.89) | 91.16 % (89.08-92.88) | 0.615 (0.486-0.744)* |
| K score | 4.00% (0.10-20.35) | 100% (98.07-100.00) | 100% | 88.73% (87.91-89.51) | 0.519 (0.398-0.641)** |
| **Early PAH** | | | | | |
| PAH score of -1.68 | 100.0% (29.24-100.0) | 82.82% (77.27-87.49) | 7.14% (5.47-9.28) | 100.0% | 0.914 (0.850-0.978) |
| NICE | 66.67% (9.43-99.16) | 89.43% (84.68-93.11) | 7.69% (3.32-16.80) | 99.51% (97.62-99.90) | 0.780 (0.462-1.000) |
| TF | 0.00% (0.00-70.76) | 97.80% (94.93-99.28) | 0% | 98.67% (98.64-98.69) | 0.467 (0.159-0.775)** |
| K score | 33.33% (0.84-90.57) | 100.0% (98.27-100) | 100% | 99.06% (97.93-99.58) | 0.667 (0.288-1.000) |
| **Late PAH** | | | | | |
| PAH score of -1.68 | 65.22% (42.73-83.62) | 86.96 (81.59-91.23) | 35.71 (25.94-46.84) | 95.74 (92.77-97.53) | 0.761(0.642-0.880) |
| NICE | 39.13% (19.71-61.46) | 91.79% (87.18-95.14) | 34.62% (21.09-51.19) | 93.14 % (90.70-94.97) | 0.655 (0.520-0.789) |
| TF | 30.43% (13.21-52.92) | 96.14% (92.53-98.32) | 46.67% (25.89-68.67) | 92.56 % (90.46-94.23) | 0.633 (0.496-0.770)* |
| K score | 0.00% (0.00-15.44) | 99.48% (97.13-99.99) | 0% | 89.67% (89.58-89.77) | 0.498 (0.373-0.622)** |

* p value < 0.05, ** p value < 0.001
PAH: pregnancy associated hypertension, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the receiver operating characteristic curve, CI: confidence interval.

## Discussion

In this retrospective cohort study, the prevalence of PAH was modest. Factors independently associated with risk of developing PAH were being overweight, MAP, and pregestational diabetes mellitus. By combining information from the three risk factors, we developed PAH risk score which performed better than NICE[15] and K score[11] in predicting PAH.

PAH remains one of the most common complications during pregnancy and results in poor maternal and fetal outcomes. In our study the PAH prevalence of 11% was comparable with that observed worldwide[1]. Our study confirmed previous study by Premkumar A (19) that PAH was associated with an increased risk of preterm delivery. Of note, the risk of preterm delivery reported in a previous study in a Thai population by Kampruan, et al was considerably high (20) with the occurrence rate of preterm delivery of 45% and 17% in severe preeclampsia and normotensive women. These almost doubled that observed in our study. The difference may be explained by different criteria of participant recruitment and diagnosis as well as discrepancy in study designs between the previous study and our study.

Our study suggested that BMI values lower than previously reported may be considered a cut-off point to identify high risk groups. A previous study in Thailand by Fang et al[21] found that BMI of ≥ 30 kg/m$^2$ significantly increased the risk of preeclampsia by almost 5 folds (unadjusted OR 4.76, 95%CI 1.73-13.12). Similarly, a study by Aksornphusitaphong et al[22], reported that a BMI of ≥ 30 kg/m$^2$ increased the risk of late onset preeclampsia by 6 folds (adjusted OR 5.8, 95% CI 2.8-11.9). Our study further suggested that a lower prepregnancy BMI (≥ 25 kg/m$^2$) was already associated with a considerably increased risk of PAH (adjusted OR 4.06, 95% CI 11.44-11.44). This underlines the need for strategies to identify those with overweight, not only obesity, early in their pregnancy for interventions to prevent PAH.

With a similarly increasing trend in the prevalence of both obesity and diabetes mellitus, the latter has increasingly been recognized as one of the risk factors for PAH. Our study showed that a history of diabetes mellitus before pregnancy was associated with a substantially increased risk of PAH, with those with pregestational diabetes having 15-fold higher PAH risk than those without. Our findings were consistent with previous studies suggesting strong association between pre-existing diabetes and PAH risk[23]. Further, a previous study found that even among women with pregestational diabetes mellitus, the risk of preeclampsia rose with increasing severity of diabetes[24]. Interestingly, our study observed the association of PAH with pregestational but not gestational diabetes mellitus. This highlights the close relationship between long-term exposure to hyperglycemic state, preeclampsia and cardiovascular risk, the issue needed to be further explored.

Our study underlines that the risk of PAH with regard to blood pressure is a continuum and early detection of elevated blood pressure during pregnancy may play an important role in prevention of PAH. In a previous study by Techawathakul[14], MAP in the second trimester was significantly associated with the risk of developing preeclampsia. In our study, MAP measured during the first trimester was also associated with an increased risk of PAH with PAH risk increased by 11% for every 1 mmHg increase in first trimester MAP. This finding suggests that raised blood pressure, even only minimally, during the first antenatal care visit should be concerned and pregnant women with raised blood pressure should be monitored more closely and preventive interventions such as aspirin or another lifestyle modification may be given.

The history of preexisting or chronic hypertension was one of the major risk factors for preeclampsia. A previous study in United States found that chronic hypertension was associated with a 13-fold increased risk of preeclampsia after adjusting for maternal age, race/ethnicity, insurance type at delivery, education level, parity, number of prenatal visits, obesity, and renal disease[25]. Data from a study in Thai pregnant women[13] showed that pre-existing hypertension was associated with an almost 20-time higher risk of PAH. In contrast, our study showed that a history of hypertension was not associated with the risk of PAH. This may be explained by that risk of PAH may be

moderated through other strong risk factors such as MAP in the first trimester and pregestational diabetes, regardless of the history of hypertension. Also, those with preexisting hypertension may have already been prescribed with antihypertensive drugs and having well disease control. This in turns might counteract the risk of PAH from pre-existing hypertension.

Our new PAH risk prediction model appeared to perform better than previously established risk assessment methods in predicting PAH in Thai pregnant women. While NICE criteria requiring the presence or absence of clinical risk factors may be seen as a simple and practical way to identify individuals/ groups at high risk of developing PAH, this method performed rather poor in predicting PAH in the Thai population. By combining clinical, biochemical and ultrasonographic information for risk prediction, Poon, et al[10] developed risk equations to predict PAH and they found that the risk equations were superior to traditional methods using the presence maternal risk factors in predicting PAH. However, this approach which requires sophisticated testing and procedures (calculated using Thermo Fisher software) was not superior to our PAH prediction model including solely a small number of clinical risk factors. In fact, our PAH prediction model performed better than Poon's equations in predicting PAH in the Thai pregnant women. The poor performance of existing methods may be due to the difference in background risk of PAH in difference populations and this suggests that recalibration of risk equations should be done when applying the risk models to a new population.

The present study underlines the importance of primary prevention of PAH using a high-risk approach. Our study suggests that it is feasible to identify pregnant women who are at high risk of develop PAH, to whom preventive intervention could be given. In addition to prescription of prophylactic aspirin, these high-risk individuals or groups should be given preventive interventions addressing overweight and obesity as well as early blood pressure control. However, further randomized control trials are needed to investigate the effectiveness of intervention to control body weight and blood pressure in preventing PAH.

This was the first study to develop risk score to predict risk of PAH and found that our new PAH score performed better than previous approaches in predicting PAH. This PAH risk score included solely clinical factors, did not require biochemical or other complicated/ invasive variables. Hence, it is feasible to use the PAH score in normal practice. However, our study had a number of limitations. First, this study was performed in the pregnant women who underwent first trimester Down syndrome screening test. Although screening for Down's syndrome was offered to all pregnant women, it was not reimbursed by the National Health Security Office and only a small number of pregnant women undertook the screening test. They might represent those who were very health conscious or those with government official's health insurance. This made our study sample a very specific group of pregnant women. Therefore, generalizability of our findings to general population of pregnant women at all age groups and types of health insurance may be limited. Secondly, as sample size of the present study was computed using data on association between chronic hypertension and PAH, it was possible that our study might not have adequate power to detect the association of PAH with other risk factors, particularly biochemical and ultrasonographic factors. Thirdly, a retrospective study design may not allow investigation into the association of PAH with factors or variables not collected in routine practice. Larger and prospective studies may be needed to be able to demonstrate such the associations. Fourthly, the gestational age of abortion was defined as < 26 weeks according to the agreement of obstetricians and pediatricians in Sanpasitthiprasong hospital, this is one of the limitations of this study in the aspect of generalizability.

## Conclusions

Pregestational diabetes mellitus, high mean arterial pressure and pre-pregnancy BMI ≥ 25 kg/m$^2$ were significant risk factors for developing PAH. Preterm birth and neonatal hypoglycemia were unfavorable outcomes related to PAH. The new Thai PAH risk model using data on a few risk factors was simple and effective at predicting PAH in Thai pregnant

women. This approach may represent an effective strategy to identify high risk groups and help prevent PAH, which in turns might help reduce disease burden and its complications.


## Acknowledgement

We thank all the contributors of Sanpasitthiprasong hospital for funding resource and Malee Kasiprong (head of clinical pathology department, Sanpasitthiprasong hospital) for accommodation of this study.


## Potential conflicts of interest

Material in PIGF test and preeclampsia risk calculation software were supported by Thermo Fisher, Global Biotechnology Co., LTD.

**References**


1. The American College of Obstetricians Gynecologists. Task Force on Hypertension in Pregnancy: Hypertension in pregnancy. Obstet Gynecol 2013;122:1122-312.
2. Wallis AB, Saftlas AF, Hsia J, Atrash HK. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987-2004. Am J Hypertens 2008;21:521-6.
3. Kongwattanakul K, Saksiriwuttho P, Chaiyarach S, Thepsuthammarat K. Incidence, characteristics, maternal complications, and perinatal outcomes associated with preeclampsia with severe features and HELLP syndrome. Int J Womens Health 2018;10:371-7.
4. Institute for Population and Social Research; Mahidol University: Thai Health 2017: Empowering Vulnerable Populations Creating an Inclusive Society, 1st : Institute for Population and Social Research, Mahidol University, Nakhon Pathom 2017:89.
5. Cunningham FG, Leveno KJ, Bloom LS, Dashe JS, Hoffman BL, Casey BM, et al. Hypertensive disorder. In: Willams Obstetrics, 25th ed. New York: McGraw-Hill Education 2018:709-54.
6. Stevens W, Shih T, Incerti D, Ton TGN, Lee HC, Peneva D, et al. Short-term costs of preeclampsia to the United States health care system. Am J Obstet Gynecol 2017; 217:237-48.
7. Myatt L, Clifton RG, Roberts JM, Spong CY, Hauth JC, Varner MW, et al. First-trimester prediction of preeclampsia in low-risk nulliparous women. Obstet Gynecol 2012;119:1234-42.
8 Bartsch E, Medcalf KE, Park AL, Ray JG. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. BMJ 2016;353:i1753.
9. Melchiorre K, Wormald B, Leslie K, Bhide A, Thilaganathan B. First-trimester uterine artery Doppler indices in term and preterm preeclampsia. Ultrasound Obstet Gynecol 2008;32:133-7.
10. Poon LC, Kamatas NA,Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension 2009;53:812-8
11. Goetzinger KR, Tuuli MG, Cahill AG, Macones GA, Odibo AO. Development and validation of a risk factor scoring system for first-trimester prediction of preeclampsia. Am J Perinatol 2014;31;1049-56.
12. Giguère Y, Charland M, Bujold E, Bernard N, Grenier S, Rousseau F, et al. Combining biochemical and ultrasonographic markers in predicting preeclampsia: a systematic review. Clin Chem 2010;56:361-75.
13. Luealon P, Phupong V. Risk factors of preeclampsia in Thai women. J Med Assoc Thai 2010;93:661-6.
14. Techawathakul S, Ratinthorn A, Yusamran C, Boriboonhirunsarn D. Risk Factors Related to Preeclampsia. Nursing Sci J Thai 2014;32:61-70.
15. National Collaborating Centre for women's and Chidren's Health. Reducing the risk of hypertensive disorders in pregnancy. Hypertension in pregnancy: diagnosis and management: RCOG Press 2019.
16. Poon LC, Zymeri NA, Zamprakou A, Syngelaki A, Nicolaides KH. Protocol for measurement of mean arterial pressure at 11-13 weeks' gestation. Fetal Diagn Ther 2012;31:42–8.
17. Rattanapuntamanee O, Uerpairojkit B. Reference range and characteristic of uterine artery Doppler in pregnant Thai women at 11-13(+6) gestational weeks. J Med Assoc Thai 2011;94:644-8.
18. Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. Screening for chromosomal abnormalities at 10-14 weeks: the role of ductus venosus blood flow. Ultrasound Obstet Gynecol 1998;12:380-4.
19. Premkumar A, Baer RJ, Jelliffe-Pawlowski LL, Norton ME. Hypertensive disorders of pregnancy and prterm birth rates among black women. Am J Perinatol 2019; 36: 148-54.
20. Kampruan R, Sukonpan K, Wasinghon P. Pregnancy outcomes amongst normotensive and severe preeclampsia with or without underlying chronic hypertension pregnancy. Thai J Obstet Gynaecol 2016; 24:202-8.
21. Fang R, Dawsona A, Lohsoonthorna V, Williams MA. Risk factors of early and late onset preeclampsia among Thai women. Asian Biomed 2009;3:477-86.
22. Aksornphusitaphong A, Phupong V. Risk factors of early and late onset pre-eclampsia. J Obstet Gynaecol Res 2013;39:627-31.
23. Weissgerber TL, Mudd LM. Preeclampsia and diabetes.

Curr Diab Rep 2015;15:9.
24. Sibai BM, Caritis S, Hauth J, Lindheimer M, VanDorsten JP, MacPherson C, et al. Risks of preeclampsia and adverse neonatal outcomes among women with pregestational diabetes mellitus. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Am J Obstet Gynecol 2000;182:364-9.
25. Yanit KE, Snowden JM, Cheng YW, Caughey AB. The impact of chronic hypertension and pregestational diabetes on pregnancy outcomes. Am J Obstet Gynecol 2012;207:333-e1.