Maternal Serum Angiogenic Growth Factors in Intrauterine Growth Restriction versus Normal Pregnancies

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Objective: *To assess maternal serum angiogenic growth factors level of soluble fms-like tyrosine kinase-1 (sFlt-1), placenta growth factor (PlGF) and sFlt-1/PlGF ratio among pregnant women with intrauterine growth restriction (IUGR) compared to those with normal pregnancies.*

Study design: *A prospective cross-sectional study conducted at Srinagarind Hospital, Khon Kaen University, Thailand from July 2014 to April 2015.*

Material and Method: *Twenty-one singleton pregnant women of gestational age between 26 to 39 weeks who had IUGR, and 21 normal pregnant women matched for gestational age were recruited. Descriptive statistics were used for demographic characteristics. Student t-test and Wilcoxon rank-sum test was used when appropriated to compare between the groups.*

Main outcome measures: *Levels of sFlt-1 and PIGF and sFlt-1/PlGF ratio.*

Results: *There were no statistical significant differences in gestational age, maternal age, parity status, maternal blood pressure level and hematocrit level between the groups. Median PlGF level among pregnant women with IUGR was significantly lower than that in control group (121 and 834.8 ng/ml respectively, p-value <0.01). The sFlt-1 level in pregnancies complicated by IUGR was slightly higher than that noted among normal pregnancies (2644 ng/ml and 2,136 ng/ml respectively, a p-value 0.105). The sFlt-1/PlGF ratio among pregnant women with IUGR was significant higher than that observed among normal pregnant women (34.1 and 2.6 respectively, p-value <0.01).*

Conclusion: *Pregnancy with IUGR had low level of PlGF and high sFlt-1/PlGF ratio when compare with normal pregnancy.*

Keywords: *Intrauterine growth restriction, Angiogenic growth factors, soluble fms-like tyrosine kinase-1 (sFlt-1), placenta growth factor (PlGF)*

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Infants suffering from intrauterine growth restriction are at high risk of death and infection[1]. It is possible for them to experience hypoglycemia, birth asphyxia, gastric disorders, pneumonia after birth, necrotizing enterocolitis (NEC), direct hyperbilirubinemia, and chronic lung disease[2]. All of these are caused by organs destruction and low blood flow in the uterus[3]. Children with small bodies experienced IUGR in the early stages of pregnancy are at a high risk of slowed development. The development of cognitive abilities and behavior during the first and second year of age are decreased[4], resulting in low competency in performance, learning skills, memory, eyesight, and the use of language[5]. The highest rate of low birth weight (LBW) and IUGR-LBW was found in Central Asia, mostly in Bangladesh, India and Pakistan. The percentage of LBW and IUGR-LBW in Thai infants were 9.6% and 6.9% respectively[3].

Placental dysfunction is involved in the pathophysiology of obstetrical complications including preeclampsia, intrauterine growth restriction and placental abruption[6]. Blood vessel disorders in pregnant women with pre-eclampsia share the same conditions as slow growth fetuses[7]. Normally, vascular endothe-

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lial growth factor(VEGF), a substance created by the placenta[8,9], is an important factor in building new blood vessels, which decreases blood pressure and maintain the performance of kidney filtration by stimulating increases in the numbers of endothelial cells[9-12], increases size and length of the new blood vessels[13]. Moreover, vascular permeability can increase from vasodilatation due to endothelial nitric oxide synthase (eNOS)[14]. Another study of VEGF showed that in pre-eclampsia, VEGF was inhibited due to it adhering to numerous soluble fms-like tyrosine kinase-1 in the blood stream[15].

Placental growth factor (PlGF) is a factor involved in the changing of throphoblast and the creation of blood vessels, which is the same as VEGF. Soluble fms-like tyrosine kinase-1(sFlt-1) inhibit PlGF and VEGF's sticking to the endothelial receptor. Soluble fms-like tyrosine kinase-1 acts as anti-angiogenic factors. PlGF and sFlt-1 lead to pre-eclampsia due to an imbalance of angiogenesis factors. Women with pre-eclampsia have lower levels of PlGF but high sFlt-1 and sFlt-1/PlGF ratios in comparison to women experiencing normal pregnancies[16].

The sFlt-1/PlGF ratio can predict pre-eclampsia better than sFlt-1 alone[13]. Women at 20-34 weeks gestation with a sFlt-1/PlGF ratio more than 85 are likely to have pre-eclampsia (specificity 99.5%). Women at more than 34 weeks of pregnancy and have more than 110 of sFlt-1/PlGF ratio are at risk of pre-eclampsia (specificity 95.5%)[17]. The results of the study conducted by Bjorn Olav Asvold revealed that low levels of sFlt-1 in pregnancies with small for gestational age (SGA) fetuses occurred in weeks 5-12 of the pregnancy. The level remained unchanged until weeks 16-26 of the pregnancy. The level of PlGF was lower in pregnancies with SGA fetuses at more than 26 weeks of pregnancy when compared to normal pregnancies[18]. The purpose of the present study was to determine what changes occurred in maternal serum angiogenic growth factors level in the IUGR group compared with the no IUGR (control) group.

Material and Method

The prospective cross-sectional study was conducted in Srinagarind Hospital, a Tertiary hospital, in Northeast Thailand, Khon Kaen University from July 2014 to April 2015. The present study was approved by The Khon Kaen University Ethics Committee in Human Research (HE571165).

Twenty-one singleton pregnant women of gestational age between 26 to 39 weeks found to have IUGR after 26 weeks of gestation were recruited in the study. IUGR was *defined as fetal weight below* 10^{th} percentile fetal weight for each gestational age of Thai population[19]. Controls were pregnant women with normal pregnancy matched 1:1 pairwise for gestational age (±7 days). Normal pregnancy in the control group was established by Leopold's maneuver with fundal height measurement for estimating fetal growth. Written informed consents were obtained from all participants.

Sample size was calculated by using the two-sided test, published by John Wiley & Sons, on behalf of the World Health Organization, 1990. Reference values were obtained from Wallner et al (2007)[20].

$$n = \frac{2\delta^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

α = 0.05 (Type I error)
β = 0.2 , Power = 80%
$Z_{\alpha/2}$ = 95% CI
Z_{β} = 0.84 (Type II error)
sFlt-1 of IUGR group = 4479
sFlt-1 of contro groupl = 2199
δ = standard deviation of the outcome = 2633
sample size n = 21/group

Abstracted data included maternal age, history of previous menstruation and contraception, maternal underlying disease, and first trimester ultrasound findings. Samples of 3 ml of maternal peripheral blood were obtained in the day of enrollment for determine levels of PlGF and sFlt-1 and sFlt-1/PlGF ratio. The samples were storage at -20 °C less than 6 months. Plasma concentrations of these angiogenic factors were analyzed (Fig 1). We collected the data of birth weight in pregnant women assigned as a control group to exclude late onset IUGR.

Statistical analysis was carried out with SPSS version 16.0 (IBM, Armonk, NY, USA). Descriptive statistics were used for demographic baseline characteristics. The student t test and Wilcoxon rank-sum test were used when appropriated to compare the groups. A *p*-value of less than 0.05 was considered statistically significant.

Table 1. Clinical characteristics of the study population

Characteristic	IUGR group (n=21)	Control group (n=21)	*p*-value
Mean maternal age (years)	27±5	28±4	0.478
Primigravidity	13 (61.9)	9 (45)	0.217
Mean GA (weeks)	33	32	0.569
Caffeine drinking	1 (4.8)	0 (0)	1.000
Mean SBP ± SD (mmHg)	125±23	117±11	0.158
Mean DBP ± SD (mmHg)	77±14	70±9	0.061
Mean Hct level ± SD (volume%)	35±3	34±2	0.211
Associated maternal diseases			
Grave's disease	1 (4.8)	0 (0)	
Hypertension	1 (4.8)	0 (0)	
SLE	2 (9.5)	0 (0)	
HIV infection	1 (4.8)	0 (0)	
Asthma	0 (0)	1 (4.8)	
Allergic rhinitis	0 (0)	1 (4.8)	
Migraine	0 (0)	2 (9.5)	

Abbreviations: IUGR, intrauterine growth restriction; GA, gestational age; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; SLE, systemic lupus erythematosus; HIV, human immunodeficiency virus
Data are present as number (percentage) unless stated otherwise

Table 2. Maternal serum levels of angiogenic growth factors

Parameter	IUGR group (n=21)	Control group (n=21)	*p*-value
PlGF (ng/ml)	121	834.8	<0.01
sFlt-1 (ng/ml)	2,644	2,136	0.105
sFlt-1/PlGF ratio	34.1	2.6	<0.01

Abbreviations: IUGR, intrauterine growth restriction; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1; NS, nonsignificant
Data are present as median

Table 3. Levels of angiogenic growth factors: comparisons between pregnancies who had IUGR without pre-eclampsia and control group

Angiogenic growth factor	IUGR without Pre-eclampsia (n=17)	Control group (n=21)	*p*-value
PlGF (ng/ml)	166.4	834.8	<0.01
sFlt-1 (ng/ml)	2,561	2,136	0.454
sFlt-1/PlGF ratio	17.2	2.7	0.011

Abbreviations: IUGR, intrauterine growth restriction; PlGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase-1
Data are present as median

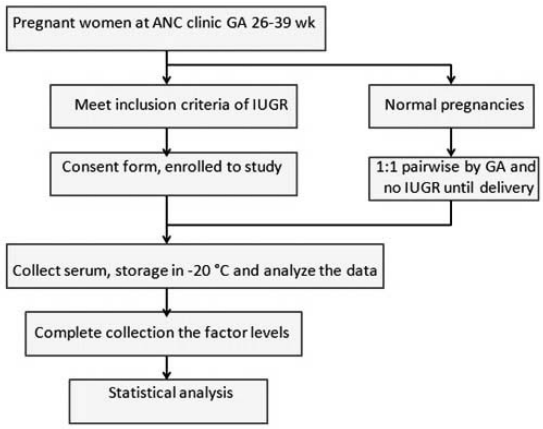

Fig. 1 study flow.

Results

Table 1 showsed the baseline characteristics of participants in the present study. There were no statistical significant differences in gestational age, maternal age, parity status, maternal tobacco and alcohol consumptions, maternal blood pressure level and hematocrit level between the two comparison groups. No pregnant women in the present study declared as having history of tobacco or alcoholic consumptions. In terms of underlying diseases, there were cases of Grave's disease, hypertension, SLE and HIV infection in the IUGR group and asthma, allergic rhinitis and migraine in the control group.

Table 2 displayed the levels of maternal angiogenic growth factors. Median PlGF level among pregnant women with IUGR was significantly lower than that in control group (121 and 834.8 ng/ml, respectively, *p*-value <0.01). The sFlt-1 level in pregnancies complicated by IUGR was slightly higher than among normal pregnancies (2,644 ng/ml and 2,136 ng/ml, respectively, *p*-value 0.105). The sFlt-1/PlGF ratio among pregnant women with IUGR was significantly higher than that observed among normal pregnant women in the control group (34.1 and 2.6 respectively, *p*-value <0.01).

Table 3 showed the levels of maternal angiogenic growth factors among pregnant women who had IUGR after excluding those who had been diagnosed with pre-eclampsia. When compared to control group, pregnant women with IUGR still had significant lower level of PIGF and significant higher sFlt-1/PlGF ratio. The level of sFlt-1 remained similar between these two comparison groups.

Discussion

In everyday practice, we detect IUGR status using fundal height measurements. If the fetus is small for gestational age, an ultrasound is used to diagnose IUGR. IUGR is responsible for many adverse neonatal outcomes and drain national resources. If we can predict IUGR earlier we can then take action in the form of intensive antenatal care, encouraging higher nutrient intake, and observing these pregnancies more closely.

In our study, PlGF in the IUGR group was significantly lower than in the control group (*p*-value <0.01), sFlt-1 in the IUGR group was higher than in the control group – although not enough to be considered significant (*p*-value 0.1047), and sFlt-1/PlGF ratio in the IUGR group was higher than in the control group by a significant amount (*p*-value <0.01). However, in the IUGR group there were 4 cases in which the subjects developed pre-eclampsia, which may have had an effect on the overall result. Comparison of levels of angiogenic growth factors in the IUGR group with pre-eclampsia and without pre-eclampsia yielded results that were similar to those of previous studies in which PlGF was lower but sFlt-1 and sFlt-1/PlGF ratios were significantly higher in the IUGR with pre-eclampsia group. Additionally, we also compared levels of angiogenic growth factors in the IUGR without pre-eclampsia group with the control group (no IUGR). In the IUGR without pre-eclampsia group, PlGF levels were significantly lower, sFlt-1 was not significantly higher and sFlt-1/PlGF ratios were significantly higher than in the control group.

The increased levels of sFlt-1 and reduced levels of PlGF in maternal circulations are commonly used as a predictor of pre-eclampsia[21]. The study conducted by Katja-Anneli Wathén et al found that high levels of sFlt-1 in the blood stream could indicate severe pre-eclampsia in 16-20 weeks of pregnancy with IUGR[22]. The results of the study by Bjorn Olav Asvold revealed that the level of PlGF was lower in pregnancies with SGA fetuses at more than 26 weeks of pregnancy when compared to normal pregnancies[18]. Our hypothesis was the abnormal levels of angiogenic growth factors are associated with impaired uteroplacental blood flow and cause IUGR, similar to pathogenesis of pre-eclampsia.

The present study showed as a preliminary study and may be used to further evaluation of the optimal value for prediction or assess the severity of IUGR in the future. The strength of the present study is that it is one of very few studies on IUGR association with angiogenic growth factors particularly in Asian Ethnic pregnant women. Most other research had predominantly focused on pre-eclampsia status. Limitations of this study were small sample size not sufficient to determine the difference level of sFlt-1, and we did not exclude the pregnancies with other underlying diseases that could be associated with alteration of angiogenic growth factor levels. However, in reality, most of IUGR cases are associated with other underlying diseases, so the results of the present study may be generalizable. Conclusively, in pregnancies with IUGR, there are low levels of PlGF and high sFlt-1/PlGF ratios when compared with normal pregnancies.

What is already known on this topic?

Previous study mostly characterized the values of angiogenic growth factors in pre-eclampsia pregnant in European country.

In ACOG 2014 original research concluded fetal growth restriction is characterized by elevated maternal sFlt-1/PlGF ratio, reaching values as high as those observed in pre-eclampsia or HELLP.

What is study adds?

This is the first study on intrauterine growth restriction (IUGR) association with angiogenic growth factors particularly in Asian ethnic pregnant women, and also determine sFlt-1, PlGF and sFlt-1/PlGF ratio.

What are the implications for public health practice?

This study shows a preliminary study In South East Asian population and may be used to further evaluation of the optimal value for prediction or assess the severity of IUGR in the future.

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Potential conflicts of interest

None.

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การศึกษาเปรียบเทียบระดับ angiogenic growth factors ในเลือดของหญิงตั้งครรภ์ที่ทารกมีภาวะเจริญเติบโตช้าในครรภ์ เทียบกับหญิงตั้งครรภ์ปกติ

รัตนา คำวิลัยศักดิ์, ภัทรพร ตั้งกีรติชัย

วัตถุประสงค์: *เพื่อเปรียบเทียบระดับ angiogenic growth factors ในเลือด ได้แก่ placenta growth factor(PlGF), soluble fms-like tyrosine kinase-1(sFlt-1) และอัตราส่วนระหว่าง sFlt-1/PlGF ของหญิงตั้งครรภ์ที่ทารกมีภาวะเจริญเติบโตช้าในครรภ์ เทียบกับหญิงตั้งครรภ์ปกติ ที่ฝากครรภ์ในโรงพยาบาลศรีนครินทร์*

วัสดุและวิธีการ: *การศึกษาเชิงวิเคราะห์ชนิดแบบตัดขวาง เก็บตัวอย่างเลือดของหญิงตั้งครรภ์อายุครรภ์ระหว่าง 26 ถึง 39 สัปดาห์ที่ทารกมีภาวะเจริญเติบโตช้าในครรภ์ เทียบกับหญิงตั้งครรภ์ปกติตั้งแต่เดือน กรกฎาคม พ.ศ.2557 ถึงเดือนเมษายน พ.ศ.2558*

ผลการศึกษา: *จากสตรีตั้งครรภ์ทั้งหมด 42 คน ในประชากรทั้งสองกลุ่มเทียบกันตามอายุครรภ์พบว่าระดับ PlGF ค่ามัธยฐานในกลุ่มที่ทารกมีภาวะเจริญเติบโตช้าในครรภ์เท่ากับ 121 ng/ml น้อยกว่าในกลุ่มเปรียบเทียบซึ่งมีค่าเท่ากับ 834.8 ng/ml อย่างมีนัยสำคัญทางสถิติ (p-value < 0.01) ระดับ sFlt-1 ค่ามัธยฐานในกลุ่มที่ทารกมีภาวะเจริญเติบโตช้าในครรภ์เท่ากับ 2,644 ng/ml มากกว่าในกลุ่มเปรียบเทียบซึ่งมีค่าเท่ากับ 2,136 ng/ml แต่ไม่มีนัยสำคัญทางสถิติ (ค่า p = 0.105) และอัตราส่วน sFlt-1/PlGF ค่ามัธยฐานของหญิงตั้งครรภ์ที่ทารกมีภาวะเจริญเติบโตช้าในครรภ์เท่ากับ 34.1 มากกว่าในกลุ่มเปรียบเทียบซึ่งมีค่าเท่ากับ 2.6 อย่างมีนัยสำคัญทางสถิติ (p-value <0.01)*

สรุป: *ในหญิงตั้งครรภ์ที่ทารกมีภาวะเจริญเติบโตช้าในครรภ์ มี PlGF น้อยกว่า และอัตราส่วน sFlt-1/PlGF สูงกว่า เมื่อเทียบกับหญิงตั้งครรภ์ปกติ*
