

Prenatal sFlt-1/PlGF value for prediction of adverse neonatal outcome of isolated ultrasonic small-for-gestational-age fetus in normotensive pregnant women: A prospective cohort study

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Abstract

Background: Not only preeclampsia but also small-for-gestational-age (SGA) neonates in the absence of preeclampsia are at increased risk of morbidity and mortality. Early recognition of fetuses at increased risk of being growth-restricted enables more appropriate surveillance and optimization of management for reduced risk of adverse neonatal outcomes. We investigated potential value of soluble fms-like tyrosine kinase-1 (sFlt-1) to placental growth factor (PlGF) ratio, estimated in late-second and early-third trimester respectively, for prediction of SGA neonates with poor neonatal outcome in the absence of preeclampsia.

Methods: Included in this case control study were 530 singleton pregnant women who had attended the prenatal screening program at single institution between January 2011 and March 2012. Demographic and clinical information of maternal and neonatal data were collected. The sFlt-1/PlGF value at 24 to 28+6 weeks and 29 to 36+6 weeks of gestation were analyzed for comparing appropriate for gestational age control group, SGA and SGA with poor neonatal group.

Results: After excluding 22 preeclampsia cases, 47 SGA group and 461 control-group were included. Among SGA group, 17 neonates had adverse neonatal outcome (36.1%, 17/47). Mean gestational age at delivery in SGA group was 37.76 ± 2.05 weeks, which showed no significant difference comparing to control group (38.43 ± 2.1 weeks, $p=0.122$). The sFlt-1/PlGF ratios at late-second trimester were both higher in the SGA group and poor neonatal SGA group than control group (3.74 ± 2.52 vs 6.73 ± 8.22 vs 7.62 ± 15.2 , $p=0.63$) and especially sFlt-1/PlGF ratio at early-third trimester was significantly higher (14.41 ± 12.5 vs 28.62 ± 37.2 vs 109.12 ± 83.96 , $p=0.002$). As gestational age advances, rapid increase in sFlt-1/PlGF ratio detected in poor SGA group comparing to SGA group with no adverse outcome. A cutoff value of 28.15 for the sFlt-1/PlGF ratio at 29 to 36+6 weeks significantly predicted SGA neonates who had adverse outcome, with sensitivity and specificity of 76.9% and 88%, respectively.

Conclusion: In this study, sFlt-1/PlGF ratio of SGA with adverse neonatal outcome group was significantly higher than control group. This study suggests the feasibility of the sFlt-1/PlGF ratio as helpful objective measurement for predicting the adverse SGA neonatal outcome by providing sFlt-1/PlGF cutoff value, besides ultrasound biometry measurement.

Background

Generally, small-for-gestational-age (SGA) fetuses are defined as those having gestational weights below the tenth percentile. During the prenatal period, ultrasound SGA fetuses are detected at a rate of around 5~8% of all late-phase pregnancies(1). When SGA fetuses with combined clinically suspected pre-eclampsia (PE) status are detected on prenatal ultrasonography, they are carefully evaluated for Intrauterine Growth Restriction (IUGR) status, which can lead to fetal death due to pathologic intrauterine fetal asphyxia. With no clinical PE clues or any other abnormal findings of the Doppler index, which is the tool of diagnosis of fetal asphyxia, incidentally detected isolated ultrasonic SGA fetuses have been given normal antenatal care(2-4). Hence these fetuses have long been considered to be constitutionally small babies with a good perinatal outcome. However, as clinical study results have accumulated over the recent decades, it has become clear that SGA fetuses have poorer perinatal outcomes, such as suboptimal neurodevelopment and higher

postnatal cardiovascular risk, than do appropriate for gestational age (AGA) newborns(2–9). One of those studies reported that impaired fetal growth is a serious complication in pregnancy, being a major determinant of perinatal morbidity and mortality(9).

A well-known diagnostic tool of pathologic fetal growth restriction is the uterine artery (UtA) Doppler index. Increased UtA pulsatile index (PI) is considered to be indicative of poorly vascularized placental villi status, though such status is seen only in extremely progressed cases. With earlier detection of pathologic fetal growth restriction, other Doppler ultrasound indices, such as reduced values of middle cerebral artery (MCA) Doppler or cerebroplacental ratio (CPR), have been clinically applied. However, ultrasound surveillance requires trained personnel and equipment, which may not be readily available for all diagnosed SGA pregnancies(1).

Consequently, a clinical need has emerged to develop additional biochemical markers to improve current diagnostic accuracy and reduce the need for ultrasound-based examinations for detection of SGA fetuses. The most widely studied angiogenic markers are placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1)(10). Uteroplacental insufficiency is associated with unbalanced angiogenesis, which results in up-regulation of sFlt-1 and down-regulation of PlGF. So, the sFlt-1/PlGF ratio is significantly higher in cases of uteroplacental insufficiency such as PE originating from placental insufficiency, and has been commercialized and employed in cases of suspected PE to predict poor maternal and perinatal outcomes and help clinicians to determine the most appropriate delivery times.

However, clinicians have to be concerned not only about fetal growth restriction combined with PE but also isolated ultrasonic SGA with no other abnormalities and the optimal management of such cases. Despite the fact that isolated SGA fetuses are also known to be associated with placenta insufficiency, there has been a tendency to focus on mostly SGA with PE and to study only the relevant biomarkers. There has been little research on biomarkers for poor perinatal outcomes in cases of isolated SGA in the absence of PE. As not only PE neonates but also SGA neonates in the absence of PE are at increased risk of morbidity and mortality after birth, a new approach and a different perspective are needed for evaluation of isolated SGA. Early recognition of fetuses at increased risk of being only growth-restricted might enable more appropriate surveillance and (thereby) optimized management, which have been shown to reduce the risk of adverse fetal outcome.

In the present study, we investigated the potential value of maternal serum levels of the sFlt-1/PlGF ratio for prediction of adverse neonatal outcome for isolated SGA fetuses in the absence of PE, and also investigated whether other factors can predict poor perinatal outcomes. Additionally, we endeavored to determine the clinically significant cut-off value of sFlt-1/PlGF for prediction of poor neonatal outcome in cases of isolated ultrasonic SGA fetuses.

Methods

Ours was a prospective cohort study that included 530 singleton pregnant women who had attended a prenatal screening program at CHA Gangnam Medical Center in Seoul, Korea, between January 2011 and March 2012. Written consent was obtained from all of the participants, and the study was approved by the

Institutional Review Board of CHA Gangnam Medical Center, CHA University. In our study, the control group was defined as AGA with no concerns regarding PE before or after delivery. The diagnosis of PE was made according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy criteria. Hypertension was defined as repeated systolic blood pressure measurements of ≥ 140 mmHg (Korotkoff phase 1) and diastolic blood pressure measurements of ≥ 90 mmHg (Korotkoff phase 5). Proteinuria was defined as repeated $\geq 1+$ proteinuria on dipstick urinalysis or ≥ 300 mg of protein in a 24-hour urine collection sample. Our SGA group was defined as infant birth weight less than the tenth percentile of the corresponding curves after adjustment for gestational age. The women were interviewed about their obstetric data and medical history. The gestational age was assessed by embryo fetal crown-rump length in the first trimester. At the gestational ages of 10 weeks and 13 weeks, 6 days, maternal serum levels of pregnancy-associated plasma protein-A (PAPP-A) and fetal nuchal translucency were measured; subsequently, at the gestational ages of 15 weeks and 20 weeks, 6 days, 4 markers (alpha-fetoprotein (AFP), unconjugated estriol (uE3), inhibin-A, and human chorionic gonadotropin (hCG)) were measured as a Down syndrome screening procedure. All of the markers were measured using a UniCel Dxl 800 analyzer (Beckman Coulter Inc., Fullerton, CA, USA), and the values were transformed to multiples of the median (MoM) after adjusting for gestational age and maternal BMI.

We sequentially analyzed 2 periods of gestational age: 24+0 to 28+6 weeks of gestation (early phase) and 29+0 to 36+6 weeks of gestation (late phase).

We also measured, as additional angiogenic biomarkers, the maternal plasma levels of the sFlt-1 and PIGF values at both early-phase gestation and late-phase gestation. After clotting, the samples were centrifuged, and plasma was stored at -80°C . The sFlt-1 and PIGF levels of each of the samples were measured simultaneously using the fully automated Roche Diagnostics Elecsys assay (Roche Diagnostics, Penzberg, Germany), and the sFlt-1/PIGF ratio was calculated.

At each trimester, an ultrasound scan also was performed. Fetal biparietal diameter, femur length, and abdominal and head circumference were measured using the ATL-5000 ultrasound system (Philips Medical Systems, Andover, MA, USA). UtA Doppler ultrasonography with color-flow mapping was performed at the gestational ages 20 weeks and 24 weeks, 6 days. Both left and right UtA blood flows were examined using color Doppler imaging. The Doppler gate was placed at the proximal UtA, according to the Fetal Medicine Foundation guidelines. The PI value was measured and the average of the measurements from the left and right UtA was used for the analysis. The Doppler measurements were performed by 3 well-trained examiners.

Adverse neonatal outcomes regarded as requiring NICU admission were attributed to sub-causes including jaundice, meconium aspiration syndrome, transient tachypnea of newborn, respiratory distress syndrome, necrotizing enterocolitis, sepsis, and use of ventilation. Diagnosis up to 2 months of age was included in the study. The diagnosis of jaundice was made by measuring the serum bilirubin level in the blood; Total bilirubin more than 19.5 mg/dL and increases in the level of total bilirubin by more than 0.5mg/dL per hour or 5mg/dL per 24 hour. Meconium aspiration syndrome was diagnosed when darkly colored amniotic fluid were checked with tachypnea and hypercapnia. Transient tachypnea of newborn was diagnosis of exclusion of respiratory distress syndrome. The diagnosis of respiratory distress syndrome was made by the clinical picture and the chest X-ray, which shows decreased lung volume, absence of the thymus and ground glass appearance

pattern. Necrotizing enterocolitis was diagnosed by both radiographic finding and clinical finding such as poor feeding, bloating, decreased activity, blood in stool and vomiting of biles. The criteria of neonatal sepsis included total leucocyte $< 5000/\text{mm}^3$, bandemia 20%, CRP $> 10\text{ng per ml}$, ESR $>10\text{mm}$ fall in 1hr.

Information on the presence of obstetric complications, pregnancy outcome and fetal outcome, including fetal sex, gestational age at delivery and birth weight, were obtained after delivery.

All of the statistical analyses were performed using SPSS for Windows version 21.0 (SPSS Inc., Chicago, IL, USA) software. All of the values were recorded in means (\pm SD), and the analysis method entailed use of the Mann-Whitney U test. Due to the fact that the sFlt-1/PIGF repeated-measure analysis of the second and third trimesters in each group did not satisfy the requirements for sphericity of data analysis, a mixed version of the general liner model, an analysis technique similar to repeated-measure ANOVA, was employed. Values of $p < 0.05$ were considered statistically significant. Receiver operating characteristic (ROC) curves were constructed using logistic regression analysis. The resulting areas under the ROC curves (AUC) were compared by pairwise analysis.

Results

Among the total 530 study recruits, 22 (4.2%) pregnant women were diagnosed as having PE. After excluding these 22 PE singletons, 508 sets of pregnancy data were analyzed. Among these, 47 SGA singletons and 461 control-group subjects were enrolled and compared to identify the relationship between each of the markers and the customized birth weight.

The neonatal outcome data is presented in Table 1. Fifty-six (56) neonates were admitted to the NICU, and the admission rate was significantly higher in the SGA group (9.5 vs. 25.5%, $p = 0.001$). Concomitant complications were jaundice, meconium aspiration syndrome, Transient tachypnea of newborn, Respiratory distress syndrome, Necrotizing enterocolitis, sepsis, and hypoxia, all of which were more common in the SGA group.

The basic characteristics of the 4 subjects are summarized in Table 2. The mean age and age distribution of the 4 groups were similar. The gestational weeks at delivery, sex dispersion of baby, hemoglobin in early pregnancy, the gestational weeks at 1st, 2nd serum tests and blood pressure in early pregnancy showed no differences between the 4 groups. Pre-pregnancy maternal weight and body-mass index (BMI) were significantly lower in the SGA group (54.8 vs. 49.9kg, $p = 0.00$; 20.2vs. 18.8kg/m², $p = 0.00$). The percentage of primigravidarum women was as high as 80.9% in the SGA group than 64.6% in the control group.

Table 3 compares the angiogenic factors and Doppler results between the 4 groups. The MoM values of the PAPPA of the 1st trimester screening test and the hCG and uE3 levels of the 2nd trimester screening test were significantly lower in the SGA groups. Nuchal translucency of 1st trimester and the AFP and inhibin-A levels of the 2nd trimester showed no difference. Among the serum levels measured at 24–28+6 weeks (early-phase gestation), the sFlt-1 level and sFlt-1/PIGF ratio (ratio I) were higher and the PIGF level was lower in the SGA group, but the differences were not statistically significant. The sFlt-1/PIGF ratio as determined at 29–36+6

weeks (late-phase gestation; ratio II), however, was significantly higher in the SGA groups. And UtA Doppler PI value of ultrasonography was significantly higher in SGA groups.

To determine the clinically applicable cut-off value for prediction of SGA neonates, especially those who showed adverse neonatal outcome, ROC curves were analyzed. Figure 1 (A) plots the ROC curves of sFlt-1/PIGF ratio II for prediction of SGA at birth. The cut-off value was 11.25 with 60.0% sensitivity and 61.9% specificity (AUC area: 0.663 (95% CI, 0.564–0.762)). The Figure 1(B) ROC curve shows a much higher correlation between sFlt-1/PIGF ratio II and prediction of SGA at birth with adverse neonatal outcome among those subsequently admitted for NICU care. The cut-off value of sFlt-1/PIGF ratio II was 28.15 with 76.9% sensitivity and 88% specificity (AUC area: 0.907 (95% CI, 0.829–0.985)).

Discussion

In our study, we demonstrated that the early-sFlt-1/PIGF ratio was not remarkable in predicting obstetric outcome but that increased sFlt-1/PIGF ratio checked at 29 to 36+6 weeks (late-phase gestation) was strongly predictive of adverse neonatal outcome with a cut-off value of 28.15 (76.9% sensitivity and 88% specificity, AUC area: 0.907). Moreover, increased sFlt-1/PIGF ratio checked at the same time with a cut-off value of 11.25 was shown to be a clinically predictive marker of real SGA neonates in cases of ultrasonic SGA (60.0% sensitivity and 61.9% specificity; AUC area: 0.663; 95% CI, 0.564–0.762) beyond the clinical and ultrasonic values.

This study confirmed that in most cases of SGA, especially in those showing adverse neonatal outcome, circulating levels of sFlt-1 are elevated and those of PIGF are diminished, resulting in an increased sFlt-1/PIGF ratio, similarly to what occurs in PE. We demonstrated the possibility of the clinical application of a maternal angiogenic marker (sFlt-1/PIGF) to expect the poor neonatal outcome in cases of ultrasonic suspected SGA in the absence of PE.

Although the exact pathophysiologies of poor fetal growth remain unknown, hypoperfusion from incomplete trophoblastic invasion is known to be the most crucial causative factor for SGA. Impaired trophoblast invasion results in decreased feto-placental perfusion and placental dysfunction, which, finally, leads to poor pregnancy outcomes. The placenta of SGA infants shows reduced intervillous space volume and parenchyme, with few chorionic villies.

What should be the next step for clinicians in cases of incidentally detected ultrasonic SGA? Differential diagnosis between fetuses that are small due to 'placental intrauterine growth restriction' and those that are 'constitutionally small fetuses' has been considered a major area of interest in clinical obstetrics(11–15). The important preliminary step would be to determine PE coexistence. Not only maternal blood pressure and proteinuria, but also ultrasonic Doppler flow indices are used to determine the risk of adverse neonatal outcome. The UtA plays a significant role in the pathophysiology of poor maternal cardiovascular function and PI value is well known to be increased in PE (16). Most studies agree that abnormal Umbilical artery (UA) Doppler flow can suggest fetuses at higher risk of SGA including PE. But Figueras F et al. pointed out that there is a possibility of UA Doppler false positivity. Normal antenatal UA Doppler cannot be taken as a single indicator of low adverse-neonatal-outcome risk and it is insufficient or suboptimal to differentiate the disease

severity or risk of progression of all cases particularly at later gestational ages(17). Another limitation of UA Doppler is that its findings might be affected by individual examiners and thus might be somewhat subjective.

Consequently, angiogenic biomarkers have become an important focus in obstetrics, especially in the diagnosis of placenta-origin diseases such as PE. Many results have shown that imbalances in angiogenic and anti-angiogenic factors as measured in maternal blood are detectable prior to clinical diagnosis, and that such measurements have high prognostic value (18–22). Uteroplacental insufficiency is associated with unbalanced angiogenesis and resultant up-regulation of sFlt-1 and down-regulation of PlGF. Abnormal levels of angiogenic factors, as reflected in the maternal circulation, play a key pathogenic role in the development of the endothelial dysfunction underlying PE. The value of angiogenic biomarkers in the prediction and characterization of early-onset PE and FGR has been demonstrated in a large number of studies; already, in fact, a diagnostic serum kit based on the sFlt/PlGF ratio is being used in outpatient clinics. Gestation-age-specific sFlt/PlGF ratio cut-offs of >85 (20+0 to 33+6 weeks) and >110 (34+0 weeks to delivery) have been shown to be highly suggestive of PE. In the PROGNOSIS study, a single sFlt-1/PlGF ratio cut-off (<38) was validated as accurately and reliably ruling out PE within 1 week (negative predictive value > 96%) and confirming PE (≥ 38) within 4 weeks (positive predictive value >25%). As such, it can be considered to be of great value to both clinicians and patients. The rate of incidence of PE in Asian women, however, is relatively lower than in other ethnic groups (e.g., African-Americans), which fact has resulted in a paucity of PE studies on Asian populations. The present study's population, uniquely, was Asian (i.e., Korean) women. Especially for such a country as Korea, in which the incidence of PE is low(23), our present results suggest an optimal and novel approach that utilizes the sFlt-1/PlGF ratio as a highly effective prognostic indicator of adverse outcome in cases of isolated SGA status.

It must be noted, though, that at the third trimester, unlike the first and second, SGA and placental insufficiency might not be noticeable, because SGA determination is made only by ultrasound biometry routinely. Thus, besides ultrasound biometry measurement for prediction of SGA, objective measurements are needed.

To our knowledge this study is the first to examine, among a cohort of SGA fetuses, the utility of angiogenic factors for prediction of adverse outcome in cases where PE is absent. Over the last few years, several reports have provided evidence that angiogenic markers also differ with respect to late-pregnancy complications. Accordingly, PlGF levels are lower in pregnancies with SGA. However, no studies have evaluated angiogenic factors to determine whether they predict poor outcome among SGA fetuses or how they compare with Doppler ultrasound surveillance. In several preceding studies, maternal serum angiogenic factors in SGA-pregnancy women with PE have been scrutinized. One study reported that among patients suspected of having SGA fetuses, abnormal maternal plasma concentrations of angiogenic/anti-angiogenic factors were 5–9 and 8–9 times more likely to lead to PE or preterm delivery(24). Ignacio et al. reported the following median values of sFlt-1/PlGF: control group 11.0, fetal growth restriction group 116.8, PE 66.5, and PE combined with fetal growth restriction group 165.4(25).

The PAPP-A, which is produced by developing trophoblast cells, is lower in cases of PE, IUGR and impaired placentation(26). In our study, HCG and E3 levels in SGA group were lower than control group as some

studies reported(27, 28) and it is thought to be a good indicator for predicting poor fetal growth if more results are built up in the future.

This study has important clinical implications. Effective screening for detection of adverse perinatal outcome in cases of isolated SGA is an area of unmet clinical need. In the study, we serially evaluated not only UtA Doppler but also sFlt-1/PIGF ratio data for cases of suspected SGA, determining that sFlt-1/PIGF can be an independent marker of poor neonatal outcome. Such biomarkers can be identified from a simple blood test; thus, this approach can be especially useful in areas where access to Doppler examination expertise is limited. If these results are corroborated by others, the strong potential of angiogenic biomarkers for risk stratification in cases of ultrasonic isolated SGA and subsequent reduction of both morbidity and healthcare costs in the management of SGA fetuses will be confirmed.

Conclusion

At the third trimester, unlike the first and second, SGA and placental insufficiency might not be noticeable, because SGA determination is made only by ultrasound biometry. In this study, sFlt-1/PIGF ratio was significantly higher in SGA with adverse neonatal outcome group and also provided sFlt-1/PIGF cutoff value. This study explored the feasibility of the early-third-trimester sFlt/PIGF ratio as an objective measurement and a possibly additional useful predictor of SGA and adverse neonatal outcome besides maternal Doppler indices.

List Of Abbreviations

sFlt-1: soluble fms-like tyrosine kinase-1

PIGF: placental growth factor

PE: preeclampsia

IUGR: Intrauterine Growth Restriction

SGA: small-for-gestational-age

AGA: appropriate for gestational age

PAPP-A:pregnancy-associated plasma protein-A

AFP: alpha-fetoprotein

uE3: unconjugated estriol

hCG: human chorionic gonadotropin

UtA: uterine artery

UA: Umbilical artery

MCA: middle cerebral artery

Declarations

Consents for publication: Not applicable

Competing interests: The authors declare that we have no competing interests

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Authors' contribution: SHS was a major contributor in writing the main texts regarding the maternal sFlt-1/PIGF value for prediction of adverse neonatal outcome. HJJ, HJR and SHK analyzed and interpreted the patient data. SGM and MKK contributed materials and analysis tools. HJP and DHC performed biochemical examination of biomarkers regarding the pathologic fetal growth restriction. All authors read and approved the final manuscript.

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Tables

Table 1. Comparison of pregnancy and neonatal outcome between control and SGA group

		Control (N=461)	SGA (N=47)	p value
Adverse neonatal outcome		46(9.9%)	17(36.2%)	0.001*
NICU admission N,(%)	56	44(9.5%)	12(25.5%)	
Jaundice	41	31(9.0%)	10(21%)	
Meconium aspiration syndrome	43	31(9.0%)	12(22%)	
Transient tachypnea of newborn	15	10(3.0%)	5(10%)	
Respiratory distress syndrome	14	10(3.0%)	4(9.5%)	
Necrotizing enterocolitis	2	1(0.2 %)	1(2.1%)	
Sepsis	2	1(0.2 %)	1(2.1%)	
Hypoxia	15	10(3.0%)	5(10%)	

Table 2. Basic characteristics between control, total SGA, SGA with normal neonatal outcome and SGA with adverse neonatal outcome group

		Control (N=461)	Total SGA (N=47)	SGA with normal neonatal outcome (N=30)	SGA with adverse neonatal outcome (N=17)	p value
Age (year)		32.26±3.2	31.4±3	31.3±3.2	32.12±3.6	0.17
Prepregnancy body weight (kg)		54.81±5.2	49.9±5.1	50.3±5.2	49.9±5.0	<0.001*
Prepregnancy BMI (kg/m ²)		20.21±1.8	18.79±1.8	19.2±1.8	19.0±1.9	<0.001*
Primigravida (%)		64.6	80.9	75.8	82.1	<0.001*
Gestational weeks at delivery		38.43±1.9	38.36±2.0	38.13±1.88	37.76±2.05	0.78
Sex dispersion of baby (%)	Male	48.7	51.3	50.3	52.1	0.42
	Female	42.6	57.4	57.3	57.5	
Hemoglobin in early pregnancy (g/dL)		12±2.3	12.21±2.1	12.1±2.0	12.2±1.9	0.16
Gestational weeks at 1st serum test		11.45±1.5	11.57±1.6	11.5±1.5	11.6±1.5	0.27
Gestational weeks at 2nd serum test		15.8±1.9	15.79±2.0	15.8±2.1	15.5±2.0	0.89
BP in early pregnancy (mmHg)	Systolic	112.39±15.3	110.22±17.2	111.22±18.1	110.1±15.2	0.25
	Diastolic	66.15±10.2	64.76±10.3	65.1±10.2	64.7±10.1	

* : p<0.05. BMI, body mass index; BP, blood pressure.

Table 3. Angiogenic factors and doppler results between control, total SGA, SGA with normal neonatal outcome and SGA with adverse neonatal outcome group

	Control (N=461)	Total SGA (N=47)	SGA with normal neonatal outcome (N=30)	SGA with adverse neonatal outcome (N=17)	p value
1st trimester screening test ^a					
PAPP-A (MoM)	1.25±0.7	1.00±0.7	1.1±0.72	0.96±0.62	0.036*
Nuchal Translucency (MoM)	0.99±0.35	1.02±0.33	1±0.34	1.03±0.39	0.528
2nd trimester screening test ^b					
AFP (MoM)	1.056±0.49	1.085±0.48	1.14±0.54	1.07±0.39	0.618
hCG (MoM)	1.169±0.38	0.93±0.4	0.94±0.36	1.1±0.48	0.001*
Unconjugated estriol (MoM)	1.094±0.29	0.99±0.3	0.99±0.29	0.92±0.32	0.041*
Inhibin-A (MoM)	1.29±0.82	1.34±0.9	1.33±0.65	1.71±1.02	0.614
Maternal angiogenic factors at late- second trimester ^c					
sFlt-1 (pg/mL)	1573.1±1231	1671±1392.2	1735.4±1571.2	1929.4±1027.2	0.543
PlGF (pg/mL)	617±494.2	563±372.2	598.8±383	430.2±277.7	0.321
sFlt-1/PlGF ratio	3.74±2.52	7.62±15.2	8.78±31.14	6.73±8.22	0.63
Maternal angiogenic factors at early- third trimester ^d					
sFlt-1 (pg/mL)	2831.9±2539.2	3237.6±3023.3	3887.5±3449.7	8999.5±7485.4	0.17
PlGF (pg/mL)	399.7±352.2	320.76±292.3	341.2±333.2	116.7±94.2	0.164

sFlt-1/PIGF ratio	14.41±12.5	28.62±37.2	35.7±56.4	109.12±83.96	0.002*
Doppler ultrasonography pulsatile index (PI) of uterine artery ^e	0.93±0.23	1.13±0.5	1.1±0.42	1.36±0.51	0.02*

^a: evaluated between gestational age at 10+0 to 13+6 weeks, ^b: evaluated between gestational age at 15+0 to 20+6 weeks, ^c: evaluated between gestational age at 20+0 to 28+6 weeks, ^d: evaluated between gestational age at 29+0 to 37+6 weeks, ^e: evaluated between gestational age at 20+0 to 24+6 weeks, data given as median,* : P<0.05. MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A; AFP, alpha-fetoprotein; hCG, human chorionic gonadotropin; PIGF, placental growth

Figures

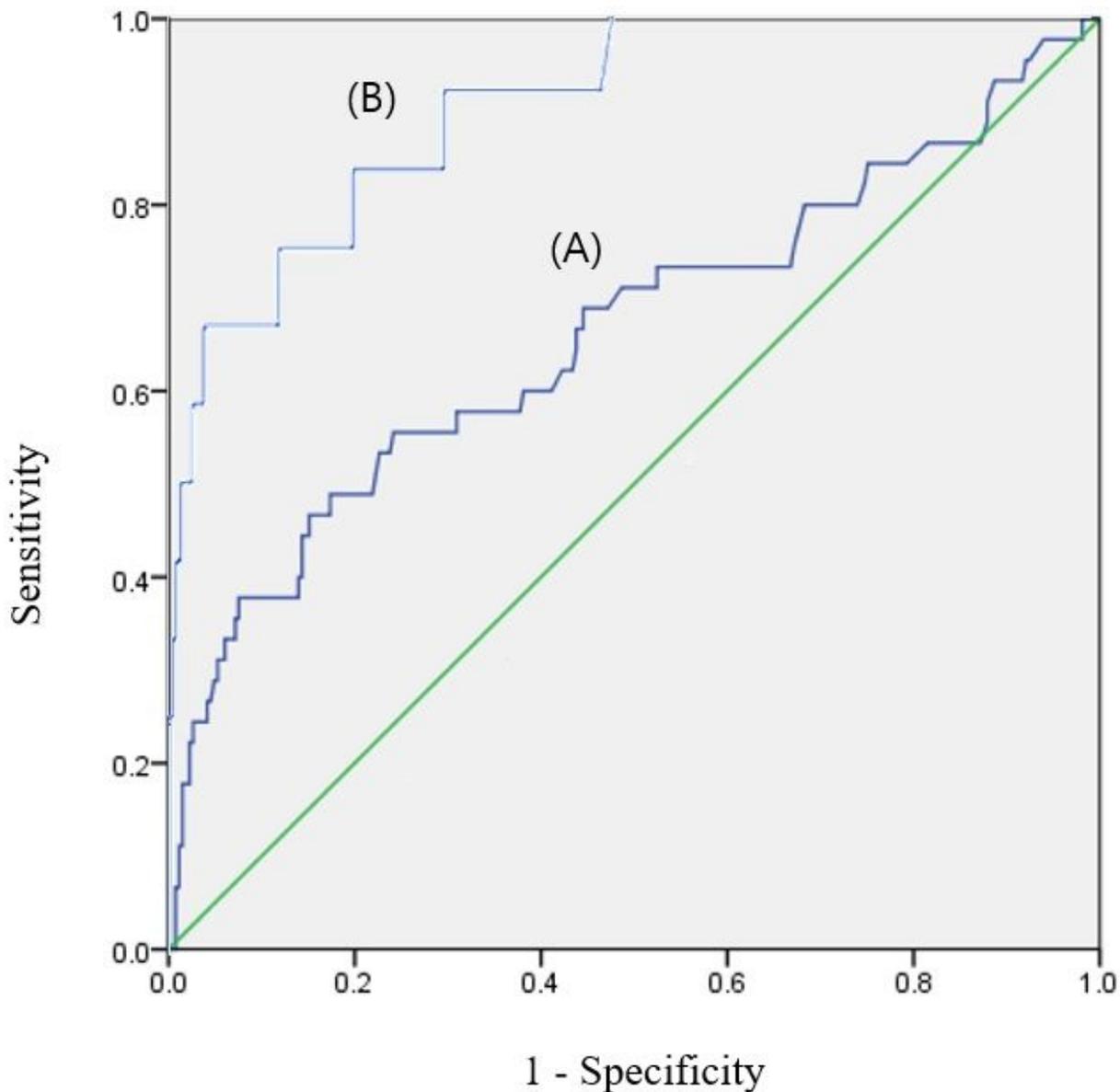


Figure 1

Receiver-operating characteristics curves (A) for prediction of small-for-gestational-age neonates in 508 pregnant women (461 sets of control – group and 47 small-for gestational-age group) by sFlt-1/PIGF ratio at 29 to 36+6weeks of gestation, (B) for prediction of small-for-gestational-age neonates with adverse neonatal outcome in 478 pregnancy women (461 sets of control – group and 17 adverse neonatal group) by sFlt-1/PIGF ratio at 29 to 36+6weeks of gestation.

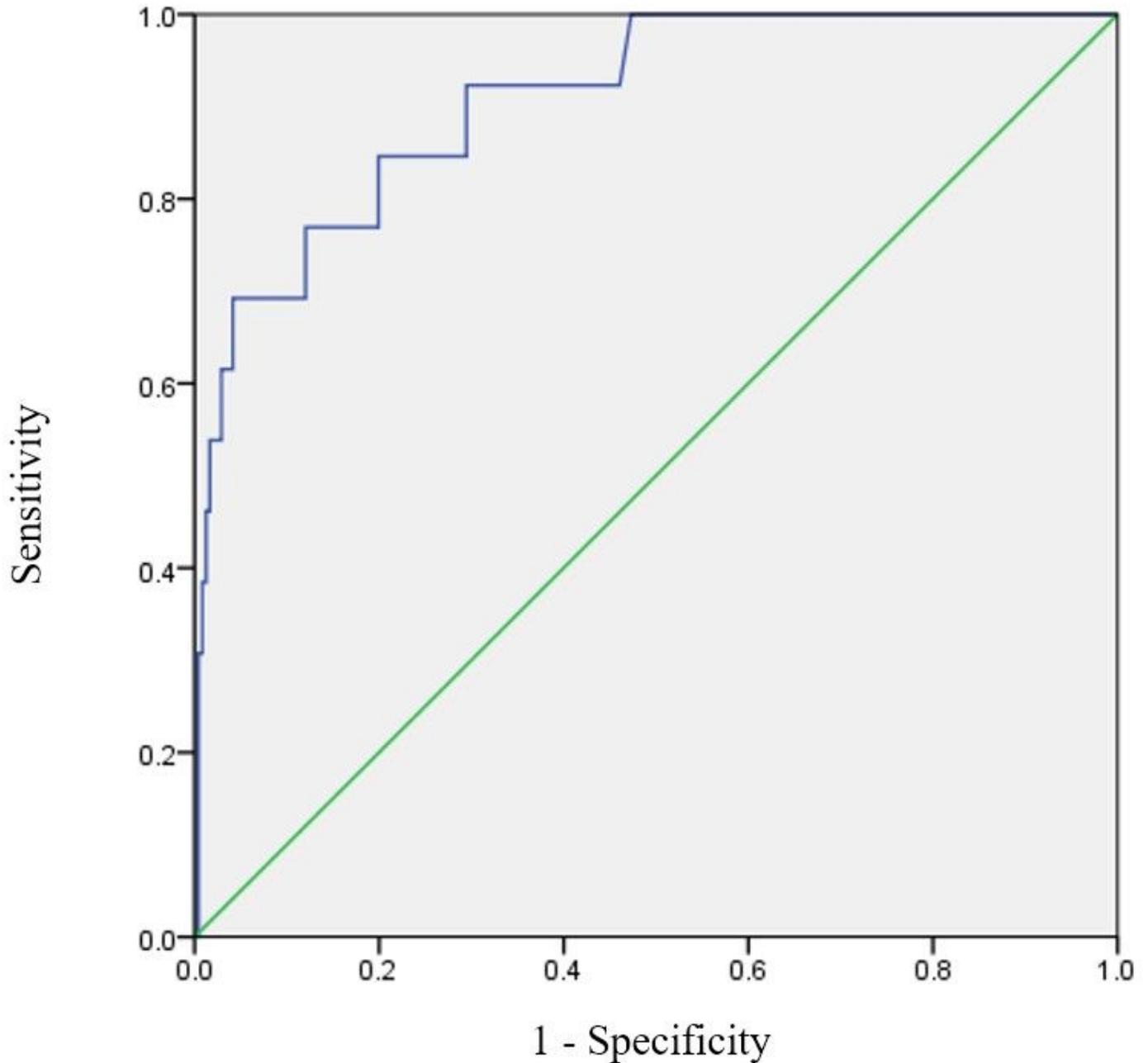


Figure 2

Receiver-operating characteristics curves for prediction of small-for-gestational-age neonates with adverse neonatal outcome in 478 pregnancy women (461 sets of control – group and 17 adverse neonatal group) by sFlt-1/PIGF ratio at 29 to 36+6weeks of gestation.