Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 801–805

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Maternal serum placental growth factor combined with second trimester aneuploidy screening to predict small-for-gestation neonates without preeclampsia

Su Mi Kim [a], Hang Goo Yun [a], Ra Yon Kim [a], Yoo Hyun Chung [a], Ju Young Cheon [a], Jeong Ha Wie [a], Ji Young Kwon [a], Hyun Sun Ko [a], Yeon Hee Kim [a], Eun Hee Han [b], Joon Hong Park [b], Hyun Jung Kim [b], Myung Shin Kim [b], Jong Chul Shin [a], In Yang Park [a, *]

[a] Department of Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[b] Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Accepted 20 August 2017

Keywords:
Placental growth factor
Small for gestational age

ABSTRACT

Objective: To investigate the role of maternal serum placenta growth factor (PlGF) and quadruple test parameters in predicting the risk of small for gestational age (SGA) infants of mothers without preeclampsia.
Materials and methods: We prospectively enrolled 300 pregnant patients who underwent blood sampling at 15–18 weeks gestation and followed them until delivery. Cases with SGA neonate delivery (n = 100) were compared with matched AGA neonate controls (n = 200). The plasma PlGF and quadruple markers were measured by enzyme-linked immunosorbent assay. The results were analyzed with Mann–Whitney U tests, and regression analysis was used to develop a model for the prediction of SGA.
Results: Women who delivered SGA neonates had decreased levels of PlGF (median 0.71 MoM versus 0.7 MoM; $p < 0.01$), hCG (median 0.97 MoM versus 1.06 MoM; $p = 0.046$) and uE3 (median 0.92 MoM versus 1.04 MoM) compared to the AGA group. AFP, hCG and inhibin-A levels did not differ significantly. A PlGF concentration <0.37 MoM had a sensitivity of 28.0% (95% CI: 19.5–37.9) and a specificity of 89.5% (95% CI: 84.4–93.4) for the prediction of SGA neonates without PE.
Conclusion: SGA neonates in the absence of PE could potentially be identified at 15–18 weeks of pregnancy.

Introduction

Small-for-gestational age (SGA) fetuses have an increased risk of perinatal complications such as perinatal asphyxia, hypothermia, hypocalcemia, bronchopulmonary dysplasia, pulmonary hypertension and necrotizing enterocolitis [1]. These risks are reduced in SGA neonates diagnosed antenatally compared to those detected after birth [2]. In the early-detection group, early intensive care and proper decisions regarding delivery timing decreased adverse fetal outcomes by four-fold in SGA fetuses [2]. Thus, there is a need for markers to predict SGA early in pregnancy. In Korea, quadruple markers including inhibin-A, alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), and unconjugated estriol (uE3) in early mid-trimester have been used for routine antenatal care. As many recent reports have suggested that quadruple markers may be associated with fetal growth disorders, we investigated these markers in our study [3].

Placental growth factor (PlGF) is a potent angiogenic factor that affects early placental vascular development. It is produced by trophoblasts [4] and increases throughout gestation until 26–30 weeks of pregnancy. The period of branching angiogenesis in normal pregnancy induces formation of capillaries and highly vascularized terminal villi, followed by “non-branching” angiogenesis in the third trimester [5]. As preeclampsia (PE) results from failure of angiogenesis of the spiral artery, the role of PlGF has mainly been focused on PE. Another study reported that elevated maternal receptor fms-like tyrosine kinase 1 (Flt-1)/PlGF ratio was

* Corresponding author. Department of Obstetrics and Gynecology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul, 137-701, Republic of Korea. Fax : +82 02 595 1549.
E-mail address: oooooobbbbb@catholic.ac.kr (I.Y. Park).

https://doi.org/10.1016/j.tjog.2017.10.017

seen in fetal growth restriction reaching values as high as those observed in preeclampsia or hemolysis, elevated liver enzymes and low platelets syndrome [6]. This reflects the relationship between PlGF and intrauterine growth disorders. We excluded patients with PE because SGA status may be a complication of PE.

The aim of this study was to evaluate early mid-trimester PlGF and quadruple markers in a low-risk population to predict SGA pregnancies in the absence of PE.

Materials and methods

Patients

This cohort study is drawn from prospective observational data on pregnant women who presented for early second-trimester screening and delivered at Seoul St. Mary's Hospital from September 2014 to August 2016. The patients were analyzed as a nested case–control study based on delivery outcomes. Maternal demographics, medical and obstetric history were recorded at the first visit. The gestational age was confirmed from the crown-rump length (CRL) during the early first trimester to avoid a misdiagnosis of SGA. The exclusion criteria included multiple gestation, maternal medical disease (e.g., maternal hypertensive disorder, gestational diabetes, etc.), preterm delivery and fetal anomaly. All of the patients underwent routine antenatal care. Once the estimated body weight of the fetus was below 10 percentile by ultra-sound, we evaluated maternal status and comorbidities, umbilical artery doppler velocimetry and Non Stress Test (NST) and Biophysical profile (BPP). If there was no reversed end-diastolic flow and non-reassuring fetal tracing, we repeated weekly follow up of umbilical artery doppler velocimetry and fetal testing (NST, BPP) and checked fetal growth by sonography every three to four weeks. If this was not the case, we considered optimal time of delivery. The study population was comprised of 100 cases who delivered SGA neonates after 37 weeks. The 200 controls were matched 2:1 for each case based on the date of quadruple marker sampling in order to control for the duration of storage. This study was approved by the Institutional Review Board-Human Research Committee of Seoul St. Mary's Hospital (no. XC15TIMI0004D). Written informed consent was obtained from each patient.

Outcome measures

Maternal demographic characteristics, biochemical results, quadruple markers, and ultrasonographic measurements were recorded in a computer database. Data on pregnancy outcomes were collected from hospital obstetric records. The admission records including nursing data were examined to determine whether the patient developed PE or another medical illness. SGA was defined as a neonatal birth weight below the 10th percentile of a reference range, the newborn centile data of the Royal Prince Alfred hospital newborn care. We selected cases with SGA newborns without PE who delivered after 37 weeks of gestation.

Sample analysis

At the routine quadruple marker sampling during 15–18 weeks of gestation, maternal blood samples (5 cc) were collected under sterile conditions in serum-separating tubes and serum was separated for markers (AFP, hCG, uE3, and inhibin-A) quantification, and in citrate tubes for PlGF quantification. AFP, hCG, and uE3 were analyzed with chemiluminescence assay and inhibin-A was analyzed with radioimmunoassay. Plasmasamples were analyzed for PlGF using the Triage® PlGF Test and the Triage® Meter (Alere, San Diego, California) within 3 h after sampling This is a fluorescent immunoassay that uses two different mouse antibodies against PlGF. First, 250 μL sample of plasma are dropped into the PlGF test cartridge. The cartridge is then inserted into the Triage Meter and the results are displayed in 10–15 min. It is reported that the test has measurable range of 12–3000 pg/mL and the limit of detection of 8 pg/mL. Results were expressed in pg/mL, and the researchers were blinded to clinical diagnosis during analysis.

Statistical analysis

For each patient in the SGA and non-SGA groups, the measured AFP, hCG, u-E3, inhibin-A and plasma PlGF were converted to multiples of the expected normal median (MoM). Comparisons between the SGA and non-SGA groups were conducted with the $\chi 2$ or Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. A receiver operating characteristic curve (ROC) was analyzed to predict SGA neonates in the absence of PE using hCG, u-E3, and PlGF alone as well as the combination of those three markers. Statistical software SPSS 16.0 (SPSS Inc., Chicago, Ill., USA) was used for data analysis.

Results

Clinical characterization

The maternal characteristics of each group are summarized in Table 1. We analyzed 200 patients in the AGA group and 100 patients in the SGA group. There were no significant differences between the two groups in maternal age and gestational age at sampling and delivery. The mean birth weight was 3249 ± 365.5 g in the AGA group and 2624 ± 211.6 g in the SGA group ($p < 0.01$). The percentile of birth weight in each group was significantly different, weighing 40.48 ± 23.67 in the AGA group and 4.64 ± 2.44 in the SGA group ($p < 0.01$).

Table 2 and Fig. 1 show the MoM values of PlGF and the quadruple markers in the AGA and SGA groups without PE. Women who delivered SGA neonates had decreased levels of PlGF (median 0.71 MoM versus 0.7 MoM; $p < 0.01$), hCG (median 0.97 MoM versus 1.06 MoM; $p = 0.046$) and uE3 (median 0.92 MoM versus 1.04 MoM) compared to the AGA group. On the other hand, AFP and Inhibin-A did not show any differences between the SGA and AGA groups.

The area under the ROC curve for the prediction of SGA neonates was analyzed for uE3, hCG, PlGF and the combination of all three markers. The AUC of the combined markers was higher than that of the other three markers, at 0.670 (95% CI: 0.614–0.723, $p = 0.032$). A PlGF concentration <0.37MoM had a sensitivity of 28.0% (95% CI: 19.5–37.9) and a specificity of 89.5% (95% CI: 84.4–93.4) for the prediction of SGA neonates without PE.

Table 1
Baseline characteristics and pregnancy outcome data of patients.

Variable	AGA (n = 200)	SGA (n = 100)	*p* value
Maternal age (years)	33.39 ± 3.27	33.62 ± 3.65	0.573
Gestational age at delivery (weeks)	38.7 ± 1.1	38.72 ± 1.1	0.853
Birth weight (gram)	3249 ± 365.5	2624 ± 211.6	<0.01
Birth weight (percentile)	40.48 ± 23.67	4.64 ± 2.44	<0.01
Gestational age at sampling (weeks)	16.0 ± 0.97	15.9 ± 0.74	0.226
Cesarean deliveries	57 (28.5%)	32 (32%)	0.532
Fetal distress	4 (2%)	4 (4%)	0.325
Prolonged delivery	13 (6.5%)	4 (4%)	0.377
NICU admission	26 (13%)	16 (16%)	0.480

Values are expressed as mean (±standard deviation) for continuous variables and n(%) for categorical values.

Table 2
Maternal serum quadruple markers and PlGF levels in the AGA and SGA groups.

Markers (MoM)	AGA (n = 200)	SGA (n = 100)	*p* value
AFP	0.99 (0.84–1.22)	0.94 (0.77–1.29)	0.554
hCG	1.06 (0.80–1.51)	0.97 (0.72–1.37)	0.046
uE3	1.04 (0.83–1.27)	0.92 (0.76–1.12)	0.003
Inhibin A	0.98 (0.71–1.29)	0.98 (0.74–1.29)	0.797
PlGF	0.79 (0.53–1.19)	0.71 (0.36–0.91)	0.002

All values are expressed as median (Q1–Q3).

Discussion

Fetal growth is a highly complicated process that requires adequate nutrient provision to the fetus. Thus, the functional fetoplacental unit plays an important role in human pregnancy. Placentation is completed by vasculogenesis and angiogenesis during blastocyst implantation, growth of the villous vasculature and remodeling of the uterine arteries [7]. Serious obstetric complications including PE, IUGR and SGA fetuses are related to trophoblast dysfunction and impaired placental vascular

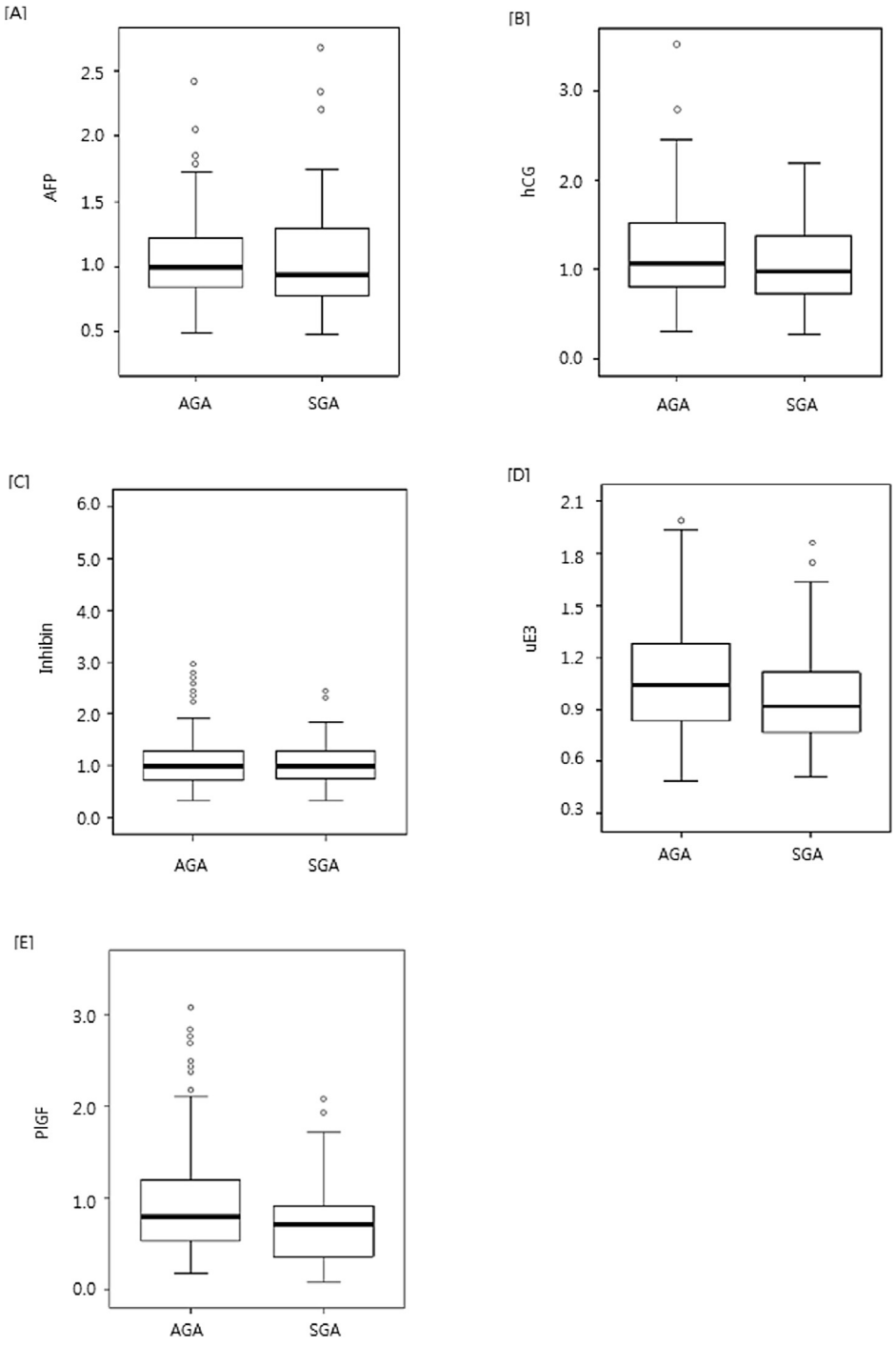


Fig. 1. Median quadruple markers and PlGF MoM levels in the AGA and SGA groups. (A) AFP, (B) hCG, (C) inhibin-A, (D) estriol and (E) PlGF. All values are expressed as MoM levels.

development [8]. As quadruple markers are all generated in normal pregnancy from the fetal organ or placenta, we analyzed these markers to identify SGA fetuses.

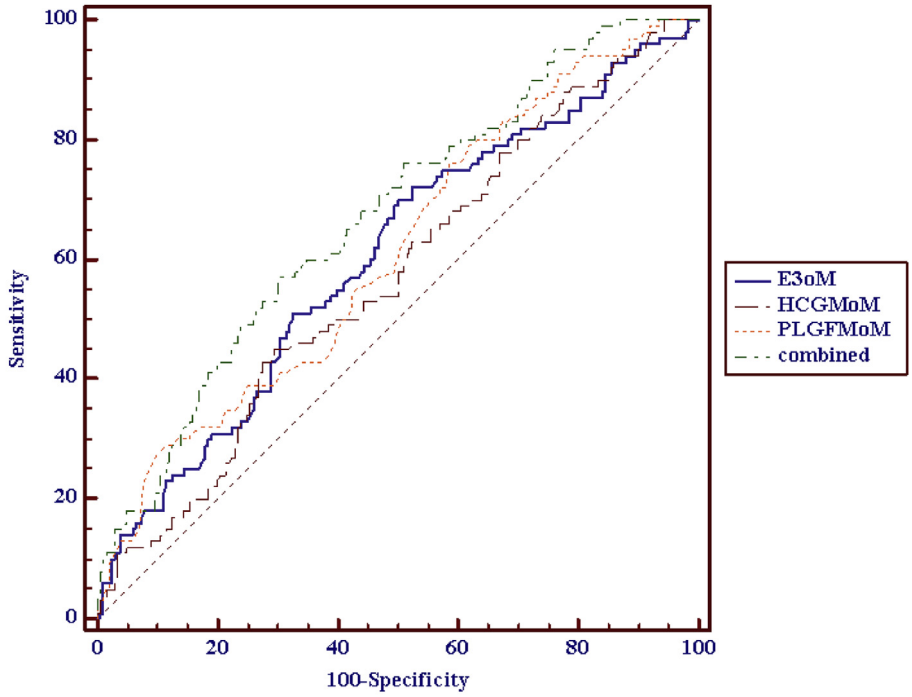
One study reported villous cytotrophoblast depletion and altered syncytiotrophoblast morphology in placentas with elevated 2nd trimester free b-hCG, AFP and inhibin-A [9]. hCG is a peptide hormone which down regulates myometrial gap junctions during pregnancy to maintain uterine quiescence [10]. Fitzgerald et al. suggested that placental immaturity or abnormal placentation were related to increased hCG. Previous studies reported controversial results; either high or low hCG levels in the 2nd trimester were associated with fetal growth restriction [11]. These diverse results may be due to the exclusion of patients with hypertensive disease. In our study excluding PE and gestational hypertension, b-hCG levels were decreased in the SGA group compared to the AGA group (0.97 MoM vs. 1.06 MoM, $p < 0.05$).

Inhibin A is a glycoprotein secreted from the placenta [12], corpus luteum and fetal membrane. Our study shows no relationship between inhibin-A and SGA infant delivery, which is similar to the results of previous studies [3,13]. Itoh et al. reported that inhibin-A was elevated in IUGR fetuses, but they included PE patients [14].

AFP is detectable in maternal serum after six weeks of gestation and is mainly synthesized in the yolk sac and fetal liver [15]. Elevated AFP is related to fetal growth restriction [4]. AFP may act as a proangiogenic factor for endothelial cells within the feto-maternal unit, which may suggest a pathological relationship to SGA. In our study, however, AFP was not significantly correlated with SGA status. Maternal serum AFP (MSAFP) elevations are correlated with abdominal wall and neural tube defects, maternal AFP-producing tumors, and increased feto-maternal transfer or placental damage [16]. These functions of AFP as a predictor of diverse maternal status imply that it would not be specific as a solitary marker for SGA.

Estriol is synthesized in the fetal liver, fetal adrenal cortex and placenta. Decreased unconjugated estriol is related to increased risk of fetal growth restriction and low birth weight [17]. In our study, estriol levels were decreased in the SGA group compared to the AGA group (0.92 ± 0.29 MoM vs. 1.04 ± 0.3 MoM, $p = 0.003$), consistent with to the previous study [17].

Many studies reported that decreased PlGF levels in early pregnancy are correlated with gestational hypertension, including PE, eclampsia and SGA [18–21]. As SGA neonates can result as a complication from PE, we excluded gestational hypertension cases to eliminate bias. PlGF concentrations are regulated by trophoblast levels of PO2 [22], and hypoxic status may downregulate PlGF [23]. Because PlGF stimulates angiogenesis and plasma extravasation [24], if a hypoxic environment occurs in the early stages of pregnancy, low levels of PlGF can lead to branching angiogenesis, which inhibits the development of a low-resistance vascular network [25]. In a normoxic environment, the predominant state is non-branching angiogenesis, and consequently high PlGF levels result



(MoM)	AUC	SE	95% CI
E3	0.604	0.0348	0.546 to 0.659
HCG	0.571	0.0348	0.513 to 0.627
PLGF	0.611	0.0342	0.553 to 0.666
combined	0.670	0.0325	0.614 to 0.723

Fig. 2. Receiver operating characteristic curves for the prediction of SGA neonates without PE using u-E3, free b-hCG, PlGF or a combination of those three factors.

from normal uterine/umbilical flow. This leads to increased impedance and low resistance of the placental vascular network, resulting in optimal fetal growth. As fetal growth is related to uterine/umbilical vascular circulation, PlGF level as a marker for a hypoxic environment may correlate with SGA fetal growth. In our study, decreased PlGF levels was related to the SGA group, which is consistent with previous studies.

According to the results of ROC curve analysis, maternal PlGF, uE3 and hCG were poor predictors of SGA neonates (Fig. 2). The combination of these three markers showed the highest AUC value (0.670, 95% CI: 0.614–0.723). Thus, our study shows decreased levels of PlGF, uE3, hCG and a combination of these three markers were correlated with SGA infants. A more comprehensive investigation of various pathways is required to develop a prediction model for the multi-factorial phenomenon of SGA infants.

Conclusion

The role of PlGF and quadruple markers in normal fetal growth is currently being debated. Low levels of PlGF, uE3, hCG and MoM in maternal circulation during early mid-trimester are associated with impaired fetal growth. Not only its use as PE predictor, PlGF, uE3 and hCG may be useful for initiating prophylactic interventions early in pregnancy.

Conflicts of interest

There is no conflict of interest in this study.

Acknowledgements

We would like to express our deepest gratitude to Dr. Jun Hong Park for his comments on PlGF sampling.

References

[1] Flamant C, Sascoin G. Short-term outcome and small for gestational age newborn management. J Gynecol Obstet Biol Reprod (Paris) 2013;42:985–95.
[2] Visentin S, Londero AP, Grumolato F, Trevisanuto D, Zanardo V, Ambrosini G, et al. Timing of delivery and neonatal outcomes for small-for-gestational-age fetuses. J Ultrasound Med 2014;33:1721–8.
[3] Guducu N, Gonenc G, Isci H, Yigiter AB, Dunder I. Can quadruple test parameters predict SGA infants? J Obstet Gynaecol 2013;33:269–71.
[4] Sung Eui L, Seung Chul K, Ki Hyung K, Man Soo Y, Wan Kyu E, Ari K, et al. Detection of angiogenic factors in midtrimester amniotic fluid and the prediction of preterm birth. Taiwan J Obstet Gynecol 2016;55:539–44.
[5] Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. Reproduction 2009;138:895–902.
[6] Herraiz I, Dröge LA, Gómez-Montes E, Henrich W, Galindo A, Verlohren S. Characterization of the soluble fms-like tyrosine kinase-1 to placental growth factor ratio in pregnancies complicated by fetal growth restriction. Obstet Gynecol 2014;124:265–73.
[7] Huppertz B, Peeters LL. Vascular biology in implantation and placentation. Angiogenesis 2005;8:157–67.
[8] Botsis D, Vrachnis N, Christodoulakos G. Doppler assessment of the intrauterine growth-restricted fetus. Ann N. Y Acad Sci 2006;1092:297–303.
[9] Fitzgerald B, Levytska K, Kingdom J, Walker M, Baczyk D, Keating S. Villous trophoblast abnormalities in extremely preterm deliveries with elevated second trimester maternal serum hCG or inhibin-A. Placenta 2011;32:339–45.
[10] Furcron AE, Romero R, Mial TN, Balancio A, Panaitescu B, Hassan SS, et al. Human chorionic gonadotropin has anti-inflammatory effects at the maternal-fetal interface and prevents endotoxin-induced preterm birth, but causes dystocia and fetal compromise in mice. Biol Reprod 2016;94(136):1–13.
[11] Gagnon A, Wilson RD, Audibert F, Allen VM, Blight C, Brock JA, et al. Obstetrical complications associated with abnormal maternal serum markers analytes. J Obstet Gynaecol Can 2008;30:918–49.
[12] Kondi-Pafiti A1, Grigoriadis C, Samiotaki D, Filippidou-Giannopoulou A, Kleanthis C, Hassiakos D. Immunohistochemical study of inhibin A and B expression in placentas from normal and pathological gestations. Clin Exp Obstet Gynecol 2013;40:109–12.
[13] Boucoiran I, Thissier-Levy S, Wu Y, Wei SQ, Luo ZC, Delvin E, et al. Risks for preeclampsia and small for gestational age: predictive values of placental growth factor, soluble fms-like tyrosine kinase-1, and inhibin A in singleton and multiple-gestation pregnancies. Am J Perinatol 2013;30:607–12.
[14] Itoh Y, Suzuki Y, Yamamoto T, Kojima K, Murakami I, Suzumori N. Increase in serum concentrations of inhibin in early onset pre-eclampsia with intrauterine growth restriction. J Obstet Gynaecol Res 2006;32:80–5.
[15] Newby D, Dalgliesh G, Lyall F, Aitken DA. Alphafetoprotein and alphafetoprotein receptor expression in the normal human placenta at term. Placenta 2005;26:190–200.
[16] Beta J, Bredaki FE, Rodriguez Calvo J, Akolekar R, Nicolaides KH. Maternal serum alpha-fetoprotein at 11-13 weeks' gestation in spontaneous early preterm delivery. Fetal Diagn Ther 2011;30:88–93.
[17] Settiyanan T, Wanapirak C, Sirichotiyakul S, Tongprasert F, Srisupundit K, Luewan S, et al. Association between isolated abnormal levels of maternal serum unconjugated estriol in the second trimester and adverse pregnancy outcomes. J Matern Fetal Neonatal Med 2016;29:2093–7.
[18] Bian Z, Shixia C, Duan T. First-trimester maternal serum levels of sFLT1, PGF and ADMA predict preeclampsia. PLoS One 2015;10:e0124684.
[19] Sung KU, Roh JA, Eoh KJ, Kim EH. Maternal serum placental growth factor and pregnancy-associated plasma protein A measured in the first trimester as parameters of subsequent pre-eclampsia and small-for-gestational-age infants: a prospective observational study. Obstet Gynecol Sci 2017 Mar;60:154–62.
[20] Teixeira PG, Cabral AC, Andrade SP, Reis ZS, da Cruz LP, Pereira JB, et al. Placental growth factor (PlGF) is a surrogate marker in preeclamptic hypertension. Hypertens Pregnancy 2008;27:65–73.
[21] Vaisbuch E, Whitty JE, Hassan SS, Romero R, Kusanovic JP, Cotton DB, et al. Circulating angiogenic and antiangiogenic factors in women with eclampsia. Am J Obstet Gynecol 2011;204:152.
[22] Wu Fan, Tian Fu-ju, Lin Yi. Oxidative stress in placenta: health and diseases. Biomed Res Int 2015;2015:293271.
[23] Bahtiyar MO, Buhimschi C, Ravishankar V, Copel J, Norwitz E, Julien S, et al. Contrasting effects of chronic hypoxia and nitric oxide synthase inhibition on circulating angiogenic factors in a rat model of growth restriction. Am J Obstet Gynecol 2007;196:72. e1-6.
[24] Cai J, Wu L, Qi X, Shaw L, Li Calzi S, Caballero S, et al. Placenta growth factor-1 exerts time-dependent stabilization of adherens junctions following VEGF-induced vascular permeability. PLoS One 2011;6:e18076.
[25] Vrachnis N, Kalampokas E, Sifakis S, Vitoratos N, Kalampokas T, Botsis D, et al. Placental growth factor (PlGF): a key to optimizing fetal growth. J Matern Fetal Neonatal Med 2013;26:995–1002.