Mid-Trimester Maternal ADAM12 Levels Differ According to Fetal Gender in Pregnancies Complicated by Preeclampsia

Reproductive Sciences
2015, Vol. 22(2) 235-241
© The Author(s) 2014
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719114537713
rs.sagepub.com

Jenny E. Myers, PhD¹, Grégoire Thomas, PhD²,
Robin Tuytten, PhD², Yven Van Herreweghe, PhD²,
Raoul O. Djiokep, MSc², Claire T. Roberts, PhD³,
Louise C. Kenny, PhD⁴, Nigel A. B. Simpson, MD⁵,
Robyn A. North, PhD⁶, and Philip N. Baker, DM^{1,7}

Abstract

An overrepresentation of adverse pregnancy outcomes has been observed in pregnancies associated with a male fetus. We investigated the association between fetal gender and candidate biomarkers for preeclampsia. Proteins were quantified in samples taken at 20 weeks from women recruited to the SCreening fOr Pregnancy Endpoints (SCOPE) study (preeclampsia $n = 150$; no preeclampsia $n = 450$). In contrast to placental growth factor, soluble endoglin, and insulin-like growth factor acid labile subunit, levels of metalloproteinase domain 12 (ADAM12) at 20 weeks were dependent on fetal gender in pregnancies complicated by preeclampsia, for male ($n = 73$) fetuses the multiples of the median (MoM; interquartile range [IQR] 1.1-1.5) was 1.3, whereas for female fetuses ($n = 75$) MoM was 1.1 (1.0-1.3); $P < .01$. Prediction of preeclampsia using ADAM12 levels was improved for pregnancies associated with a male fetus (area under receiver-operator curve [AUC] 0.73 [95% confidence interval [CI] 0.67-0.80]) than that of a female fetus (AUC 0.62 [0.55-0.70]); $P = .03$. The data presented here fit a contemporary hypothesis that there is a difference between the genders in response to an adverse maternal environment and suggest that an alteration in ADAM12 may reflect an altered placental response in pregnancies subsequently complicated by preeclampsia.

Keywords

preeclampsia, mass spectrometry, biomarkers, fetal sex, ADAM12

Introduction

Many investigators have observed disparities between male and female fetuses in relation to a number of adverse pregnancy outcomes. The ratio of male fetuses to female fetuses has been shown to be increased in fetal loss due to miscarriage¹ and stillbirths.² Disorders of pregnancy associated with poor placentation such as abruption,² preeclampsia,³ and fetal growth restriction (FGR) associated with placental insufficiency^{4,5} have also been shown to occur more frequently when the fetus is male. Furthermore, sex-specific alterations in placental genes (eg, JAK1, IL2RB, Clusterin, LTBP, CXCL1 and IL1RL1, and tumor necrosis factor receptor)⁶ have been observed to be upregulated in female placentas suggesting that aberrations in placental function can occur in a sex-specific manner.⁷

Placental complications such as preeclampsia and FGR represent highly significant but potentially modifiable health and economic burdens throughout the world. Preeclampsia continues to be a major cause of maternal mortality,⁸ and there are strong associations between both conditions and stillbirth.^{9,10} Effects of preeclampsia and FGR are not limited to

very early life; surviving babies are at increased risk of cardiovascular disease, chronic hypertension, type 2 diabetes, and

¹ Maternal & Fetal Health Research Centre, Manchester Academic Health Science Centre, University of Manchester, Central Manchester NHS Trust, Manchester, United Kingdom

² Pronota, Zwijnaarde, Belgium

³ Discipline of Obstetrics and Gynaecology, University of Adelaide, Adelaide, Australia

⁴ The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Ireland

⁵ Department of Obstetrics and Gynaecology, University of Leeds, Leeds, United Kingdom

⁶ Women's Health Academic Centre, King's College London, London, United Kingdom

⁷ Gravida: National Centre for Growth and Development, Liggins Institute, University of Auckland, New Zealand

Corresponding Author:

Jenny E. Myers, Maternal & Fetal Health Research Centre, 5th Floor (Research), St Mary's Hospital, Oxford Rd, Manchester, M13 9WL, United Kingdom.
Email: jenny.myers@manchester.ac.uk

Table 1. Demographic Characteristics.

Characteristics	Preeclampsia (n = 150)	No Preeclampsia (n = 450)	P Value
Maternal age, years	28 (23-32)	29 (24-32)	NS
Fetal gender male	75 (50%)	232 (52%)	NS
Caucasian ethnicity	128 (90%)	402 (90%)	NS
Smoker at 15 wks	12 (8%)	61 (14%)	NS
Body mass index at 15 wks (kg/m ²)	26.6 (23.1-30.5)	24.1 (21.7-27.7)	<.001
Gestation at sampling, wks	20.4 (0.7)	20.4 (0.8)	NS
Systolic blood pressure, mm Hg	116 (11)	109 (10)	<.001
Diastolic blood pressure, mm Hg	69 (9)	65 (7)	<.001
Gestation age at delivery, weeks	38 (37-40)	40 (39-41)	<.001
Preterm birth, <37 wks	42 (28%)	19 (4%)	<.001
Small for gestational age	39 (26%)	49 (11%)	<.001
Gestational hypertension	0 (0%)	39 (9%)	<.001
Gestational diabetes mellitus	8 (7%)	8 (3%)	NS

Abbreviations: IQR, interquartile range; NS, not significant; SD, standard deviation; wks, weeks.

^a Results are expressed as mean (SD), median (IQR), or n (%).

schizophrenia in adulthood.¹¹ To prevent the complications of preeclampsia and FGR, women at high risk of the condition need to be identified early in pregnancy. Consequently, there is much interest in predictive tests using combinations of clinical risk factors, biophysical measurements, and biochemical tests.¹²⁻¹⁶ Although there have been significant advances in the reported performance of these combinations, to date no screening test has achieved the requisite sensitivity and specificity to be useful and cost effective in a clinical setting.

Much of the data on early pregnancy biomarkers is conflicting,¹⁷⁻²⁰ with several studies reporting positive and negative findings for the same markers. We have previously identified the biomarkers placental growth factor (PlGF), soluble endoglin (sEng), metalloproteinase domain 12 (ADAM12), and insulin-like growth factor acid labile subunit (IGFALS) as being relevant to the prediction of preeclampsia and FGR.²¹ In this study, we aimed to investigate whether there was a significant association between fetal gender and the levels of these protein biomarkers. In addition, the influence of fetal gender on the predictive performance of potential biomarkers was assessed.

Methods

Local ethical committee approval was granted, and written informed consent was obtained from all participants. Women recruited into the SCOPE study, a prospective screening study of low-risk nulliparous women recruited in Australia, New Zealand, United Kingdom, and Ireland between November 2004 and February 2011 (ACTRN12607000551493), participated in this study.¹² A research midwife interviewed participants at 14 to 16 weeks and 19 to 21 weeks of gestation, and pregnancy outcomes were prospectively tracked. At the time of interview, blood pressure, height, and weight were measured, and data were entered on an Internet-accessed central database (MedSciNet). Two consecutive manual blood pressure measurements were recorded. Blood samples were collected on EDTA at 14 to 16 weeks and 19 to 21 weeks of gestation, and plasma was stored at -80°C within 4 hours of collection.

Samples

A total of 150 women who developed preeclampsia and 450 controls were randomly selected from the 5605 women recruited in Australia, New Zealand, London, Manchester, Leeds, United Kingdom and Cork, Ireland. Controls were selected from those who did not have preeclampsia at the same center and included women with uncomplicated pregnancies and those with complications such as small for gestational age (SGA), preterm birth, gestational hypertension, and gestational diabetes.²¹ Preeclampsia was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg, or both, on at least 2 occasions 4 hours apart after 20 weeks of gestation but before the onset of labor, or postpartum, with either proteinuria (24-hour urinary protein ≥ 300 mg or spot urine protein:creatinine ratio ≥ 30 mg/mmol creatinine or urine dipstick protein $\geq ++$) or any multisystem complication of preeclampsia.¹² Preterm preeclampsia was defined as women who required delivery prior to 37 weeks of gestation. Small for gestational age was used as a proxy for FGR and was defined as infants weighing <10 th customized centile.¹²

Measurement of Proteins

Proteins were quantified as part of a biomarker validation study using targeted mass spectrometry assays based on an selective reaction monitoring (SRM) peptide quantification method, using custom-built assays as reported previously.²¹ In brief, plasma samples were depleted of albumin and immunoglobulin G, denatured, and spiked with mixture of isotopically labelled peptides serving as internal reference. Following tryptic digestion and peptide separations, quantitative data were obtained using a triple quadrupole MS instrument (Vantage, ThermoFisher, Massachusetts). The readout of an assay in each sample was the ratio of the analyte signal area (endogenous peptide) over the common internal standard signal area. Comparison of the ratios between different

Figure 1. Levels of ADAM12, PIGF, sEng, and IGFALS in samples taken at 20 weeks from women with and without preeclampsia subdivided by fetal sex. The dotted lines represent the median of the control and preeclampsia groups for both sexes combined. ADAM12 indicates metallo-peptidase domain 12; PIGF, placental growth factor; sEng, soluble endoglin; IGFALS, insulin-like growth factor acid labile subunit.

samples represents the relative quantification of the protein. The details of the peptide transitions used are described in the supplementary information of our previous study.²¹

The sample order was randomized prior to every analytical step, and laboratory personnel were blinded to the pregnancy outcome related to each sample. Technical variation was estimated by preparing and measuring 10% of the samples, in duplicate, in a randomized order. Coefficients of variation were 11%, 12%, and 10% for sEng, ADAM12, and IGFALS, respectively, with <5% missing data for each biomarker.²¹

The PIGF was measured in all samples using DELFIA time resolve fluorescence technology (PerkinElmer, Turku, Finland). Interassay coefficients of variation for the PIGF assay were 3% at 16.8 pg/mL and 8% at 852 pg/mL.

Data Analysis

The sample size was determined by the previous study that aimed to identify a panel of predictive markers for preeclampsia.²¹ Bioconductor, using R statistical programming language

(www.bioconductor.org), was used to perform all statistical analyses.²² The characteristics of the preeclampsia group and controls were compared using Student *t* test, Wilcoxon rank-sum test, and chi-square test. Among the controls, there was a significant relationship between ADAM12 concentration and gestational age at sampling and body mass index (BMI), and therefore ADAM12 was converted to multiples of the median (MoM). Concentration of sEng was also expressed as MoM to correct for BMI. Median differences in biomarker levels were assessed using Mann-Whitney *U* test and differences between areas under receiver–operator characteristic curves (AUCs) were assessed using DeLong’s test. For the MoM, the regressed medians were computed using a log-linear regression of the first degree on the control population.

Results

Baseline characteristics and pregnancy outcome for the patient cohort investigated are shown in Table 1. As expected, significant differences between the groups were observed for the parameters known to be associated with preeclampsia, such as early pregnancy BMI and blood pressure. Compared to women who did not develop preeclampsia (controls), there were significantly more preterm births and SGA infants in the preeclampsia group.

Figure 1 shows the levels of ADAM12, s-ENG and IGFALS, and PIGF at 20 weeks in women with preeclampsia compared to controls. There was no effect of gender on sEng, PIGF, or IGFALS levels in the control group or in women with pregnancies complicated by preeclampsia or SGA ($P > .05$). In contrast, levels of ADAM12 at 20 weeks were dependent on fetal gender in women with preeclampsia, with significantly higher ADAM12 levels observed in women carrying male fetuses ($n = 73$) compared to women carrying female fetuses ($n = 75$; 1.3 MoM [IQR 1.1–1.5] vs 1.1 [1.0–1.3]; $P < .01$; Mann-Whitney *U* test). There was no effect of fetal gender in the control group on ADAM12 levels. In the prediction of preeclampsia, ADAM12 levels were associated with significantly higher AUCs for pregnancies associated with a male rather than a female fetus, 0.73 (95% CI 0.67–0.80) versus 0.62 (0.55–0.70), respectively ($P = .03$; DeLong’s test; Figure 2). The MoM levels of ADAM12 were also dependent on fetal gender in pregnancies complicated by SGA (male $n = 39$, female $n = 49$; $P < .01$; Figure 3). The prediction of SGA using ADAM12 was poor, and the AUC was not significantly altered by fetal gender (AUC males 0.60 [0.51–0.69] vs females 0.55 [95% CI 0.45–0.64]; $P > .05$; DeLong’s test). No significant relationship between ADAM12 and the other markers IGFALS (ρ : 0.25), PIGF (ρ : 0.16), or s-ENG (ρ : 0.42) was observed.

Discussion

Previous work has suggested that there are altered responses to pregnancy stressors according to fetal gender. In this study, we aimed to determine the potential impact of fetal gender on levels of a number of pregnancy biomarkers. Of the proteins

Figure 2. Univariate area under the receiver–operator curve (AUC) with 95% confidence interval (CI) for the candidate biomarkers, subdivided by fetal sex.

quantified in this cohort, there was a strong association of ADAM12 with fetal gender in pregnancies subsequently complicated by preeclampsia and/or SGA. The ADAM12 was upregulated in pregnancies complicated by preeclampsia, but discrimination between cases and controls was more distinct for pregnancies with a male rather than a female fetus. This effect was not observed for the other proteins measured.

Both naturally occurring splice variants, ADAM12-L and ADAM12-S, are highly expressed in the human placenta,²³ and ADAM12-S is found in maternal serum from the early first trimester.²⁴ At the beginning of pregnancy (up to week 8), ADAM12 is closely correlated with the size of the placenta.²⁵ In normal pregnancy, the ADAM12-S levels associate with gestational age with a 60-fold increase between 8 weeks and full-term pregnancy (>38 weeks).²⁴

It has been suggested that ADAM12 is involved in the regulation of fetal growth through proteolysis of insulin-like growth factor binding proteins (IGFBPs). Proteolysis of IGFBPs results in the release of insulin-like growth factors (IGFs) that play an important role in the development of the placenta and fetus.²⁶ The cleavage of IGFBP3 (the most abundant IGFBP in serum) and IGFBP5 by ADAM12-S is thought to be one of the factors which regulates the bioavailability of IGF-I and IGF-II, and reduced first-trimester ADAM-12 levels have been reported in SGA pregnancies.²⁷ Several other publications have reported the potential of ADAM12 to be used as an early pregnancy marker for both preeclampsia and FGR, although the results are inconclusive.^{17,28} In these studies, first-trimester maternal serum ADAM12 concentrations were influenced by fetal gender, gestational age (increasing), ethnicity (higher in African American), maternal weight

Figure 3. Levels of ADAM12, PlGF, sEng, and IGFALS in samples taken at 20 weeks from women with (n = 88) and without SGA (n = 512) subdivided by fetal sex. The dotted lines represent the medians of the control and SGA groups for both sexes combined. ADAM12 was significantly different between SGA pregnancies associated with male versus female fetus ($P < .01$), but not different in women without SGA. ADAM12 indicates metallopeptidase domain 12; PlGF, placental growth factor; sEng, soluble endoglin; IGFALS, insulin-like growth factor acid labile subunit; SGA, small for gestational age

(decreasing), smoking (lower in smokers), and maternal age (lower in women ≥ 40 years). The authors, however, commented that smoking and maternal age accounted for the largest intergroup differences in ADAM12, whereas gender-related differences were negligible.²⁸

The data regarding changes in ADAM12 in early pregnancy are conflicting^{17-20,29} with previous studies reporting a

downregulation of ADAM12 in pregnancies subsequently complicated by preeclampsia and more recent reports suggesting an upregulation. Some of these disparities may relate to different sampling methods, particularly different gestational ages. In the current study, significant differences in ADAM12 were observed at 20 weeks, a later gestation than many of the previous reports.

The data presented here fit a contemporary hypothesis stating that when exposed to insults during pregnancy female and male offspring “react” differently.⁷ Previous studies have demonstrated that cord blood levels of IGF I and IGFBP-3 are different between male and female fetuses.³⁰ In addition, a prospective study of pregnancies complicated by maternal asthma investigated the relative influences of asthma, corticosteroid use, smoking, and components of the IGF axis on birthweight.³¹ A gender-specific difference in the relationship between IGF I and IGFBP on birthweight was observed; in pregnancies with a male fetus IGF I levels were not influenced by maternal factors, whereas IGF I was significantly decreased in pregnancies complicated by asthma and smoking when the fetus was female. The authors hypothesized that these data were supportive of different response strategies by male and female fetuses to adverse pregnancy conditions.⁷ Males are believed to adapt for continued growth in an adverse maternal environment, while females reduce growth in an attempt to survive further maternal insults. The data presented here suggest that an alteration in ADAM12 may reflect an altered placental response, perhaps mediated by changes in IGF bioavailability, in pregnancies subsequently complicated by preeclampsia. It is interesting that there was no observed effect of fetal gender on the other biomarkers investigated in this study. This may indicate that regulation of angiogenic factors (sEng and PlGF) is independent of fetal gender, although this would need to be confirmed in independent studies. Although IGFBP3 messenger RNA is expressed by the placenta,³² it is likely that the majority of circulating IGFBP3 is maternal in origin³³ and therefore unlikely to be affected by fetal gender.

Limitations of Study

Although this study has used data from a large prospective cohort, the number of pregnancies studies subsequently affected by preeclampsia is modest. In addition, the analysis described in this study represents a post hoc analysis of data generated as part of a previous study and may therefore be subject to bias.

Significance

Gender differences may account for disparate data relating to ADAM12 as a predictive marker for preeclampsia. Given the heterogeneity of preeclampsia as a diagnosis, the influence of factors such as fetal gender, not traditionally considered in screening algorithms, may be important in the pursuit of more accurate predictive tests for pregnancy complications. Novel technologies that allow us to determine fetal gender from maternal blood are likely to be more affordable in the future; this may allow the inclusion of fetal gender in predictive algorithms. Both prediction studies and ex vivo studies need to consider the effect of fetal gender in the development of pregnancy complications.

Acknowledgments

All women who participated in the SCOPE Study, the SCOPE PIs, Professor L. Poston (King's College London), Professor L. M. E. McCowan (University of Auckland), Professor J. J. Walker (University of Leeds), Professor G. A. Dekker (University of Adelaide), the SCOPE international coordinator Mrs R. Taylor, the SCOPE Country Coordinators Mrs D. Healy, University of Adelaide, Mrs A. Briley, Kings College London, and Mrs N. Murphy and Mrs E. Snapes, University College Cork, and the database and statistical support provided by Ms E. H. Y. Chan, University of Auckland.

Authors' Note

Ethical approval was obtained from local ethics committees (New Zealand AKX/02/00/364, Australia REC 1712/5/2008, London, Leeds and Manchester 06/MRE01/98 and Cork ECM5 (10) 05/02/08).

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: GT, RT, YVH, and ROD are employed by Pronota which has a commercial interest in the development of predictive tests for preeclampsia. JM, PNB, and RAN have received consultancy fees (paid to their institution) from Pronota.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: SCOPE study: New Zealand: New Enterprise Research Fund, Foundation for Research Science and Technology; Health Research Council 04/198; Evelyn Bond Fund, Auckland District Health Board Charitable Trust; Australia: Premier's Science and Research Fund, South Australian Government; London: Guy's and St Thomas' Charity, United Kingdom, Tommy's the Baby Charity; Manchester: UK Biotechnology and Biological Sciences Research Council GT084, UK National Health Services NEAT Grant FSD025, University of Manchester Proof of Concept Funding, Tommy's the Baby Charity, NIHR; Leeds: Cerebra, UK; Cork, Ireland: Health Research Board, Ireland CSA/2007/2. JM is also supported by an Action Medical Research Endowment Fund and the NIHR Manchester Biomedical Research Centre. These study sponsors had no role in study design, data analysis or writing this report. All mass spectrometry data acquisition was funded by Pronota, Zwijnaarde, Belgium.

References

1. Byrne J, Warburton D. Male excess among anatomically normal fetuses in spontaneous abortions. *Am J Med Genet.* 1987;26(3):605-611.
2. James WH. Sex ratios of offspring and the causes of placental pathology. *Human Reproduction.* 1995;10(6):1403-1406.
3. Vatten LJ, Skjaerven R. Offspring sex and pregnancy outcome by length of gestation. *Early Hum Dev.* 2004;76(1):47-54.
4. Edwards A, Megens A, Peek M, Wallace EM. Sexual origins of placental dysfunction. *Lancet.* 2000;355(9199):203-204.
5. Walker MG, Fitzgerald B, Keating S, Ray JG, Windrim R, Kingdom JC. Sex-specific basis of severe placental dysfunction leading to extreme preterm delivery. *Placenta.* 2012;33(7):568-571.

6. Sood R, Zehnder JL, Druzin ML, Brown PO. Gene expression patterns in human placenta. *Proc Natl Acad Sci U S A*. 2006; 103(14):5478-5483.
7. Clifton VL. Review: sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta*. 2010;31 suppl:S33-S39.
8. World Health Organisation. *The World Health Report 2005 - Make Every Mother and Child Count*. Geneva: World Health Organisation; 2005.
9. Centre for Maternal and Child Enquiries (CMACE). *Perinatal Mortality 2009: United Kingdom*. London: CMACE; 2011.
10. Bukowski R. Stillbirth and fetal growth restriction. *Clin Obstet Gynecol*. 2010;53(3):673-680.
11. Barker DJ. Fetal growth and adult disease. *Br J Obstet Gynaecol*. 1992;99(4):275-276.
12. North RA, McCowan LM, Dekker GA, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. *BMJ*. 2011;342(1):d1875.
13. Kenny LC, Broadhurst DI, Dunn W, et al. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. *Hypertension*. 2010;56(4):741-749.
14. Akolekar R, Syngelaki A, Sarquis R, Zvanca M, Nicolaides KH. Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. *Prenat Diagn*. 2011;31(1):66-74.
15. Myatt L, Clifton RG, Roberts JM, et al. First-trimester prediction of preeclampsia in nulliparous women at low risk. *Obstet Gynecol*. 2012;119(6):1234-1242.
16. Myers JE, Kenny L, McCowan L, et al. Angiogenic factors combined with clinical risk factors to predict preterm pre-eclampsia in nulliparous women: a predictive test accuracy study. *BJOG*. 2013; 120(10):1215-1223. doi: 10.1111/1471-0528.12195.
17. Poon LC, Chelemen T, Granvillano O, Pandeva I, Nicolaides KH. First-trimester maternal serum a disintegrin and metalloprotease 12 (ADAM12) and adverse pregnancy outcome. *Obstet Gynecol*. 2008;112(5):1082-1090.
18. Laigaard J, Sorensen T, Placing S, et al. Reduction of the disintegrin and metalloprotease ADAM12 in preeclampsia. *Obstet Gynecol*. 2005;106(1):144-149.
19. Spencer K, Cowans NJ, Stamatopoulou A. ADAM12s in maternal serum as a potential marker of pre-eclampsia. *Prenat Diagn*. 2008;28(3):212-216.
20. Matwejew E, Cowans NJ, Stamatopoulou A, Spencer K, von Kaisenberg CS. Maternal serum ADAM-12 as a potential marker for different adverse pregnancy outcomes. *Fetal Diagn Ther*. 2010; 27(1):32-39.
21. Myers JE, Tuytten R, Thomas G, et al. Integrated proteomics pipeline yields novel biomarkers for predicting preeclampsia. *Hypertension*. 2013;61(6):1281-1288.
22. Gentleman R, Hornik K, Leisch F. R 1.5 and the Bioconductor 1.0 releases. *Comput Stat Data Analysis*. 2002;39(4):557-558.
23. Gilpin BJ, Loechel F, Mattei MG, Engvall E, Albrechtsen R, Wewer UM. A novel, secreted form of human ADAM 12 (meltrin alpha) provokes myogenesis in vivo. *J Biol Chem*. 1998;273(1): 157-166.
24. Laigaard J, Sorensen T, Frohlich C, et al. ADAM12: a novel first-trimester maternal serum marker for Down syndrome. *Prenat Diagn*. 2003;23(13):1086-1091.
25. Sahraravand M, Jarvela IY, Laitinen P, Tekay AH, Ryyanen M. The secretion of PAPP-A, ADAM12, and PP13 correlates with the size of the placenta for the first month of pregnancy. *Placenta*. 2011;32(12):999-1003.
26. Christians JK, Gruslin A. Altered levels of insulin-like growth factor binding protein proteases in preeclampsia and intrauterine growth restriction. *Prenat Diagn*. 2010;30(9):815-820.
27. Cowans NJ, Spencer K. First-trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system. *Prenat Diagn*. 2007; 27(3):264-271.
28. Laigaard J, Larsen SO, Pedersen NG, et al. ADAM 12-S in first trimester: fetal gender, smoking and maternal age influence the maternal serum concentration. *Prenat Diagn*. 2009;29(5): 525-527.
29. Bestwick JP, George LM, Wu T, Morris JK, Wald NJ. The value of early second trimester PAPP-A and ADAM12 in screening for pre-eclampsia. *J Med Screen*. 2012;19(1):51-54.
30. Geary MP, Pringle PJ, Rodeck CH, Kingdom JC, Hindmarsh PC. Sexual dimorphism in the growth hormone and insulin-like growth factor axis at birth. *J Clin Endocrinol Metab*. 2003; 88(8):3708-3714.
31. Clifton VL, Hodyl NA, Murphy VE, Giles WB, Baxter RC, Smith R. Effect of maternal asthma, inhaled glucocorticoids and cigarette use during pregnancy on the newborn insulin-like growth factor axis. *Growth Horm IGF Res*. 2010;20(1):39-48.
32. Iniguez G, Argandona F, Medina P, et al. Acid-labile subunit (ALS) gene expression and protein content in human placentas: differences according to birth weight. *J Clin Endocrinol Metab*. 2011;96(1):187-191.
33. Baxter RC. Circulating levels and molecular distribution of the acid-labile (alpha) subunit of the high molecular weight insulin-like growth factor-binding protein complex. *J Clin Endocrinol Metabol*. 1990;70(5):1347-1353.