Mid-trimester maternal serum markers in predicting adverse pregnancy outcome

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Summary

Objective: In a prospective study, we investigated the association between mid-trimester maternal serum AFP (ms-AFP), maternal serum hCG (ms-hCG) levels and adverse pregnancy outcome in a South-Western Greek population. *Materials and Methods:* 126 healthy Greek women with spontaneous pregnancies were investigated for ms-AFP and ms-hCG levels between the 13th and 24th weeks of gestation and followed for adverse pregnancy outcome. Abnormal outcomes were considered as ms-AFP levels or ms-hCG levels > 2.0 multiples of the median value for gestation (MoM). Statistical analysis was performed by Pearson's chi-square test. *Results:* Elevated ms-AFP levels were detected in a total of 25 out of the 126 women studied (19.84%). Elevated ms-hCG levels were detected in a total of ten of the 126 women studied (7.93%). Elevated ms-AFP and ms-hCG levels were detected in a total of four of the 126 women studied (3.17%). *Conclusion:* Multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology, ms-AFP and ms-hCG screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications.

Key words: Maternal serum markers; AFP levels; hCG levels; Adverse pregnancy outcome.

Introduction

Maternal serum Alpha Fetal Protein (AFP) and human chorionic gonadotropin (hCG) were originally introduced for the detection of neural tube defects and trisomy 21 [1, 2]. However, increased quality of ultrasound equipment and sonographer expertise have greatly reduced the need for maternal serum AFP (ms-AFP) and maternal serum hCG (ms-hCG) screening in mid-trimester [2, 3].

Pregnancies with unexplained mid-trimester elevation in ms-AFP and/or ms-hCG, are at increased risk of pregnancy complications [intrauterine growth restriction (IUGR), intrauterine fetal death (IUFD), preeclampsia (PE)] resulting from placental insufficiency [4-8].

In our prospective study, we investigated the association between mid-trimester ms-AFP levels, ms-hCG levels and adverse pregnancy outcome in a South-Western Greek population.

Material and Methods

Between February 2005 and February 2008, 126 women with spontaneous pregnancies were referred to the Outpatient Clinic of the Obstetrics and Gynaecology Department of the University of Patras Medical School. All women were investigated for ms-AFP and ms-hCG between the 13th-24th weeks of gestation and followed for adverse pregnancy outcome.

Gestational age was estimated from the last menstrual period for women with regular (21-35 days) menstrual cycles or confirmed from ultrasonographic scan in the first trimester for women with irregular menstrual cycles. Women with multiple pregnancies, diabetes mellitus, pregnancy with chromosomal or structural abnormality, hypertension diagnosed before the 20th week of gestation, and history of PE in a previous pregnancy were excluded from the study.

All women had a dating ultrasound (US) examination at their first visit, followed by a detailed examination at the 18th-22nd week of gestation. The study was approved by the Ethical Committee of the hospital. Informed consent was obtained from each woman.

Serum samples were collected from all women between the 13th and 24th weeks of gestation. All serum samples were stored at -20°C. AFP levels were measured with immunoradiometric assay using two highly specific monoclonal antibodies for coating of the solid phase and the tracer. The tracer antibody and the coated antibody react simultaneously with the AFP present in patient samples or standards. Excess tracer is removed by a washing step and the radioactivity bound to the tube wall is measured in a gamma scintillation counter (IRMA-mat AFP, DiaSorin Inc). hCG levels are measured with immunoradiometric assay using two highly specific monoclonal antibodies for coating of the solid phase and the tracer. The tracer antibody and the coated antibody react simultaneously with the b-hCG present in patient samples or standards. Excess tracer is removed by a washing step and the radioactivity bound to the tube wall is measured in a gamma counter (b-hCG IRMA CT, Radim S.p.A.). Abnormal values were considered as ms-AFP levels or ms-hCG levels > 2.0 multiples of the median value for gestation (MoM).

All gestational complications with fetomaternal circulatory disturbances (placental abruption (PA), IUGR, IUFD, PE) were considered as adverse pregnancy outcomes.

Placental abruption (PA) was defined as the separation of the placenta from its site of implantation before delivery of the fetus [10].

Intrauterine growth retardation (IUGR) was defined as a birth weight below the 5th percentile for gestational age [11].

Intrauterine death (IUFD) was defined as fetal loss after 24 weeks' gestation.

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Preeclampsia (PE) was defined by a blood pressure above 140/90 mmHg after 20 weeks' gestation, proteinuria > 300 mg/24 hours or persistent 30 mg/dl (1+ dipstick) in random urine samples. The term severe preeclampsia is used when blood pressure above 160/110 mmHg is recorded at least six hours apart, and proteinuria of more than 5 g during 24 h occurs [12].

Statistical analyses were performed using the SPSS-13 for Windows. The chi-square test was used to assess the association between categoric variables.

Results

Serum samples were collected at a median gestation of 19 weeks (range 13-24). The median weight of the women at the time of serum sampling was 71 kg (range 50–105). The median age at the estimated delivery date was 31 years (range 17-50).

From the 126 women included in the study, 12 (9.52%) developed a gestational complication during the follow-up of their current pregnancy. The demographics of women with gestational complications compared to those without are shown in Table 1.

Table 1. — *Women's demographics (n = 126).*

		Women with complications (n = 12)	Women without complications (n = 114)
Number of pregnancies	1 pregnancy	12 (100%)	96 (84.21%)
	≥ 2 pregnancies	0 (0%)	18 (15.79%)
Age of women	< 25	1 (8.33%)	20 (17.54%)
	25-35	7 (58.33%)	69 (60.53%)
	> 35	4 (33.33%)	25 (21.93%)
Complications in previous pregnancies	No	8 (66.67%)	102 (89.47%)
	Yes	4 (33.33%)	12 (10.53%)
Smoking	No	10 (83.33%)	102 (89.47%)
	Yes	2 (16.67%)	12 (10.53%)

NS: not significant.

Abnormal ms-AFP levels were detected in a total of 25 of the 126 women studied (19.84%). Among them, only four women (16%) developed gestational complications in the current pregnancy (2 PA, 1 IUGR and 1 IUFD). Abnormal ms-hCG levels were detected in a total of ten of the 126 women studied (7.93%). None of them developed gestational complications in the current pregnancy. Abnormal ms-AFP and ms-hCG levels were detected in a total of four of the 126 women studied (3.17%). None of them developed gestational complications in the current pregnancy. These data are shown in Tables 2 and 3.

Discussion

AFP is initially synthesized by the yolk sac, followed shortly thereafter by the fetal liver. Because the human yolk sac involutes at the 9th week, the fetal liver is responsible for most of the AFP production during development [13, 14]. AFP synthesis by the proliferating fetal liver actually increases through the 20th week of gestation, after which it remains fairly constant until the 32nd week [13-15].

Table 2. — *ms-AFP and ms-hCG levels in women with and without gestational complications.*

	Women with complications (n = 12)	Women without complications (n = 114)
ms-AFP > 2 MoM ms-hCG ≤ 2 MoM (n: 25)	4	21
ms-AFP > 2 MoM ms-hCG > 2 MoM (n: 4)	0	4
ms-AFP ≤ 2 MoM ms-hCG > 2 MoM (n: 10)	0	10
ms-AFP ≤ 2 MoM ms-hCG ≤ 2 MoM (n: 87)	8	79

Table 3. — *ms-AFP and ms-hCG levels in women with specific gestational complications in the current pregnancy (n: 12).*

	PA	IUGR	PE	IUFD
ms-AFP > 2 MoM ms-hCG ≤ 2 MoM (n: 25)	2	1	0	1
ms-AFP > 2 MoM ms-hCG > 2 MoM (n: 4)	0	0	0	0
ms-AFP ≤ 2 MoM ms-hCG > 2 MoM (n: 10)	0	0	0	0
ms-AFP ≤ 2 MoM ms-hCG ≤ 2 MoM (n: 87)	2	5	0	1
Total	4	6	0	2

PA = placental abruption; IUGR = intrauterine growth restriction; PE = preeclampsia; IUFD = intrauterine fetal death.

Elevated ms-AFP levels have been strongly associated with congenital abnormalities, placental dysfunction and preterm birth [15, 16]. When the fetus is structurally normal, mid-trimester high ms-AFP levels are thought to reflect a defect in placentation and are associated with an increased risk for complications in later pregnancy, including severe PE, IUGR and IUFD [15-18]. In our study mid-trimester elevated ms-AFP levels were detected in a total of 25 of the 126 women studied (19.84%). Among them, only four women (16%) developed pregnancy complications (2 PA, 1 IUGR and 1 IUFD).

Serum hCG appears early during pregnancy [19]. Its concentration increases gradually by reaching a peak at the end of the first trimester, after which it progressively decreases until delivery [20].

During pregnancy hCG is produced almost exclusively in the placenta, but is also synthesized in the fetal kidney and fetal liver [21]. Most of the hCG in circulation is metabolized by the liver, whereas about 20% is excreted by the kidneys [22].

The etiology of the increased hCG production by the placenta is not clear. Experimental evidence from trophoblastic cells cultured in vitro showed that hypoxia

increases hCG production [23]. Many mechanisms leading to elevations of ms-hCG have been proposed.

Increased ms-hCG concentrations have been related to the presence of placental pathology, such as infarction, ischemic changes, villitis and intervillus thrombosis [9, 24]. Velamentous cord insertion has been described to be associated with elevated mid-trimester ms-hCG concentration [25]. The presence of chromosomally abnormal areas in the placenta known as confined placental mosaicism, has been found to be associated with high mid-trimester ms-hCG levels [26]. All these placental pathologies may be associated with overproduction of hCG [9, 24, 26-28].

Another possible explanation may be inadequate trophoblastic remodelling of the maternal uterine vasculature, with an absence of normal physiologic changes in the spiral arteries leading to placental hypoxia and hCG overproduction [9, 27, 28].

Pregnancies complicated by an unexplained mid-trimester elevation in ms-hCG are at increased risk of perinatal complications resulting from placental insufficiency, including combinations of IUGR, IUFD and PE [7-9, 29, 30]. In our study mid-trimester elevated ms-hCG levels were detected in a total of ten of the 126 women studied (7.93%). None of them developed pregnancy complications.

Combined elevations in ms-AFP and ms-hCG levels suggest a more complex type of placental pathology and they have a stronger association with complications in later pregnancy (PE, IUGR and IUFD) [31, 32, 33]. In our study mid-trimester elevated ms-AFP and ms-hCG levels were detected in a total of four of the 126 women studied (3.17%). None of them developed pregnancy complications.

In our study the main limitation was the small number of cases with gestational complications. According to the results shown in Tables 2 and 3, elevated mid-trimester ms-AFP and/or ms-hCG levels alone cannot detect all pregnant women with increased risk of developing pregnancy complications. However, uterine artery Doppler screening alone is superior to ms-AFP and ms-hCG screening for the identification of significant placental pathology leading to PE and IUGR [17, 34, 35].

In conclusion, multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology, ms-AFP and ms-hCG screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications [34].

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