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Maternal Serum α-Fetoprotein at 11–13 Weeks' Gestation in Spontaneous Early Preterm Delivery

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Key Words

α-Fetoprotein · Preterm delivery · Biochemical markers · First-trimester screening · Pyramid of antenatal care

Abstract

Objective: To examine the potential value of maternal serum level of α-fetoprotein (AFP) in the first trimester of pregnancy in the prediction of spontaneous early preterm delivery. ***Methods:*** Maternal serum concentration of AFP at 11–13 weeks' gestation was measured in a case-control study of singleton pregnancies delivering phenotypically normal neonates, including 33 cases with spontaneous delivery before 34 weeks and 99 matched controls delivering after 37 weeks. The median multiple of the median (MoM) serum AFP in the two outcome groups was compared and the bivariate gaussian distributions were simulated in a previously described screened population of 33,370 pregnancies to estimate the performance of screening for early delivery by a combination of maternal characteristics and obstetric history with serum AFP. ***Results:*** In the preterm delivery group compared to the term delivery group, the median serum AFP MoM was higher (1.33 vs. 0.97, p = 0.006). The estimated detection rate of preterm delivery, at a false-positive rate of 10%, from maternal characteristics and obstetric history was 27.5% and this increased to 36.0% with the addition of serum AFP. ***Conclusions:*** Measurement of serum AFP at 11–13 weeks improves the prediction of early preterm delivery provided by maternal characteristics and obstetric history.

Introduction

Preterm delivery is the leading cause of perinatal death and handicap in children and the vast majority of mortality and morbidity relates to early delivery before 34 weeks, which occurs in about 2% of singleton pregnancies [1, 2]. In two-thirds of the cases this is due to spontaneous onset of labour or preterm prelabour rupture of membranes and in the other one-third it is iatrogenic, mainly due to preeclampsia [3]. The patient-specific risk for spontaneous delivery before 34 weeks can be determined at 11–13 weeks by an algorithm combining maternal characteristics and obstetric history with an estimated detection rate of 18% in nulliparous women and 38% in parous women, at a false-positive rate (FPR) of 10% [4]. This performance of screening has not been improved by the addition of a series of biophysical and biochemical markers of uteroplacental perfusion or placental function, including uter-

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Table 1. Studies reporting on the association between maternal serum concentration of AFP and preterm delivery

Reference (first author)	GA weeks	Definition of preterm delivery, weeks	Serum AFP cut-off, MoM	Controls	Cases	RR or OR 95% CI	p
Roop, 1991 [5]	15–20	all <37	2.3	1,533	–	2.2 (1.5–3.2)	–
Bernstein, 1992 [6]	17–21	all <37	2.0	197	27	4.1 (1.7–9.8)	<0.01
Davis, 1992 [7]	14–22	all <37	2.0	6,398	–	2.4 (1.9–3.1)	<0.001
Phillips, 1992 [8]	15–20	all <37	2.5	144	20	1.0 (0.3–3.8)	NS
Williams, 1992 [9]	14–24	all <37	2.0	357	55	4.5 (2.3–8.9)	<0.05
Brazerol, 1994 [10]	15–20	all <37	2.0	735	41	1.4 (0.5–4.1)	NS
Tanaka, 1994 [11]	15–19	all <37	2.0	955	69	4.1 (1.4–12.2)	<0.05
Morssink, 1995 [12]	15–20	all <37	2.5	7,992	477	2.4 (1.4–5.1)	<0.01
Simpson, 1995 [13]	15–20	all <37	2.0	650	9	1.1 (0.3–4.3)	NS
Akinbiyi, 1996 [14]	16–18	all <37	2.0	289	11	4.9 (1.5–16.2)	<0.005
Waller, 1996 [15]	15–19	all <37	2.0	44,663	3,711	3.2 (2.9–3.5)	<0.01
Wenstrom, 1996 [16]	14–24	all <37	2.5	4,574	671	2.8 (2.3–3.4)	<0.001
Hsieh, 1997 [17]	14–22	all <37	2.0	5,533	352	2.4 (1.5–3.8)	<0.001
Yaron, 1999 [18]	14–22	all <37	2.5	58,077	1,963	1.8 (1.5–2.4)	<0.001
Chitayat, 2002 [19]	14–24	all <37	2.2	1,044	90	5.9 (3.8–9.3)	0.001
Duric, 2003 [20]	15–22	all <37	2.0	613	34	0.5 (0.1–3.4)	NS
Smith, 2006 [21]	15–21	all <37	1.7	8,058	425	2.0 (1.5–2.8)	<0.001
Van Rijn, 1999 [22]	15–20	all <34	2.5	5,721	149	5.0 (2.5–9.8)	<0.05
Dugoff, 2005 [23]	14–24	all <32	2.0	32,888	257	4.5 (3.1–6.4)	<0.001
Moawad, 2002 [24]	24	spontaneous <35	2.0	119	119	3.5 (1.8–6.7)	<0.001
Jelliffe-Pawlowski, 2010 [25]	15–20	spontaneous <32	2.0	101,745	1,116	5.4 (4.6–6.4)	<0.001

ine artery pulsatility index and maternal serum or plasma concentration of pregnancy-associated plasma protein-A (PAPP-A), free β-human chorionic gonadotrophin (β-hCG), placental growth factor, placental protein 13, a disintegrin and metalloprotease 12 (ADAM12), inhibin-A or activin-A [4].

α-Fetoprotein (AFP), the fetal equivalent of albumin, is synthesized in the yolk sac and fetal liver, and enters the maternal circulation through the placenta and across the fetal membranes. Increased maternal serum levels of AFP are the consequence of (a) increased amniotic fluid concentration in association with fetal defects including exomphalos and open spina bifida; (b) increased transfer from the fetal to the maternal circulation as a consequence of placental damage, and (c) increased production in the mother from germ cell tumours, hepatocellular carcinoma and metastatic cancer in the liver. Several studies reported that elevated maternal serum levels of AFP during the second trimester are associated with increased risk for subsequent preterm delivery. A systematic review of PubMed for studies written in English on the association between maternal serum AFP and preterm delivery identified 21 publications on a combined total of 291,854 pregnancies (table 1) [5–25]. Serum AFP above a cut-off of 2.0 multiples of the normal median (MoM) was associated with a tripling in risk for preterm delivery. However, most studies did not distinguish between spontaneous and iatrogenic preterm delivery and only two reported on spontaneous delivery. The gestational age cut-off for the diagnosis of preterm delivery was 37 weeks in 17 studies, 35 weeks in 1, 34 weeks in 1, and 32 weeks in 2 studies.

The aim of this study was firstly to investigate whether the maternal serum concentration of AFP at 11–13 weeks is increased in pregnancies that subsequently deliver spontaneously before 34 weeks and secondly to examine the potential value of this biomarker in combination with maternal characteristics and obstetric history in screening for preterm delivery.

Methods

The data for this study were derived from a prospective screening study for adverse obstetric outcomes in women attending their routine first hospital visit in pregnancy. In this visit, which is held at 11^{+0}–13^{+6} weeks of gestation, we record maternal characteristics and medical history and perform an ultrasound scan to (a) determine gestational age from the mea-

Table 2. Maternal characteristics and obstetric history in the case-control study

Characteristics	Delivery ≥37 weeks (n = 99)	Delivery <34 weeks (n = 33)
Median maternal age, years (IQR)	32.2 (29.1–36.0)	30.4 (24.9–34.5)
Median maternal weight, kg (IQR)	65.0 (58.6–74.0)	63.6 (56.0–73.0)
Median maternal height, cm (IQR)	165.1 (160.0–170.2)	164.0 (159.5–170.6)
Racial origin		
Caucasian	57 (57.6)	17 (51.5)
African	42 (42.4)	16 (48.5)
Cigarette smoker	7 (7.1)	3 (9.1)
Conception		
Spontaneous	97 (98.0)	33 (100)
Ovulation drugs	2 (2.0)	0
Parity		
Nulliparous	46 (46.5)	17 (51.5)
Parous with previous preterm delivery	3 (3.0)	11 (33.3)*
Parous without previous preterm delivery	50 (50.5)	5 (15.2)*

Data are presented as numbers with percentages in parentheses, unless otherwise indicated. Comparisons between the groups were by χ^2 test or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables. * $p < 0.05$.

surement of the fetal crown-rump length (CRL); (b) diagnose any major fetal abnormalities, and (c) measure fetal nuchal translucency (NT) thickness as part of screening for chromosomal abnormalities [26, 27]. In addition, the maternal serum PAPP-A and free β-hCG are determined and the results are combined with the fetal NT to calculate the patient-specific risk for trisomy 21 [28]. Samples of serum were stored at –80°C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women and were also recorded in our database. The obstetric records of all patients delivering before 34 weeks were examined to determine if the preterm delivery was iatrogenic or spontaneous.

In this case-control study of singleton pregnancies delivering phenotypically normal neonates, we measured maternal serum concentration of AFP at 11–13 weeks' gestation in 33 cases with spontaneous delivery before 34 weeks and 99 matched controls delivering after 37 weeks. The cases were drawn at random from the screening study population with available stored serum. The controls were from pregnancies with no complications and normal outcome matched to the cases for storage time. None of the samples were previously thawed and refrozen.

Maternal serum concentration of AFP was measured by the chemiluminescent method using AFP Immulite 2500® (Siemens Healthcare Diagnostics Ltd, Surrey, UK). The inter-assay coefficient of variation varied between 2.0 and 4.4%. All samples were analyzed in duplicate and those with a coefficient of variation exceeding 10% were reanalyzed.

Statistical Analysis

Comparisons between the early preterm and term delivery groups were by χ^2 test or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables.

In the case-control study, the following steps were taken. First, the distribution of AFP was made gaussian after square root (sqrt) transformation and the normality of distribution was assessed using probability plots and Shapiro-Wilk test ($p = 0.751$). Second, in the control group, multiple regression analysis was used to determine which of the factors amongst maternal characteristics and gestation provided a significant contribution in predicting sqrt AFP and then the measured concentration of AFP in each case and control was converted into a MoM in the control group. Third, Mann-Whitney U test was used to compare the median MoM value of serum AFP in the two outcome groups. Fourth, the means and SDs of the gaussian distributions of sqrt AFP MoM in the preterm delivery group and the control group were estimated and then simulated in a previously described screened population of 33,370 pregnancies [4]. Fifth, likelihood ratios for spontaneous early preterm delivery were calculated from the fitted bivariate gaussian distributions of AFP. Sixth, in each patient in the screened population the a priori odds for preterm delivery based on maternal history and characteristics were multiplied by the likelihood ratio for AFP to derive the a posteriori odds. The a posteriori risks were used to calculate detection rates of spontaneous early preterm delivery and FPRs and the performance of screening was determined by receiver operating characteristic (ROC) curves analysis. The performance of different methods of screening was compared by the areas under the ROC curves (AUROC) [29].

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, Ill., USA) and MedCalc software package version 9.6.2.0 (MedCalc Software, Mariakerke, Belgium) were used for data analyses.

Table 3. Performance of screening for spontaneous delivery before 34 weeks in all pregnancies and in the subgroups of nulliparous and parous women expressed as detection rate (DR) at 10% FPR and AUROC curves

Method of screening	DR (95% CI) at 10% FPR	AUROC (95% CI)
Maternal history in all pregnancies	27.5 (19.7–36.9)	0.668 (0.639–0.698)
Maternal history + AFP	36.0 (27.3–45.8)	0.709 (0.680–0.738)
Maternal history in nulliparous women	19.5 (12.9–28.3)	0.607 (0.566–0.649)
Maternal history + AFP	29.9 (21.8–39.5)	0.685 (0.643–0.726)
Maternal history in parous women	35.8 (27.1–35.6)	0.706 (0.663–0.749)
Maternal history + AFP	41.3 (32.2–51.1)	0.729 (0.689–0.779)

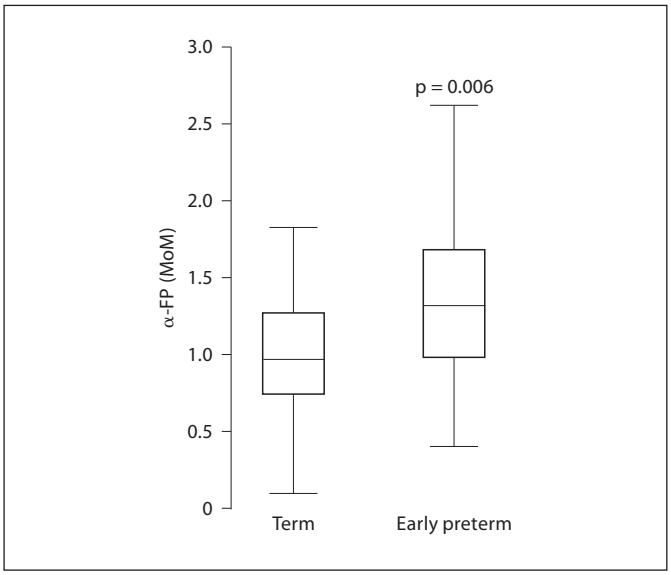

Fig. 1. Box (median, interquartile range) and whisker (range) plot of maternal serum AFP in pregnancies delivering at term and those with spontaneous delivery before 34 weeks.

Results

The maternal characteristics of the early preterm and term delivery groups in the case-control study are compared in table 2.

Multiple regression analysis in the term delivery group demonstrated that for sqrt AFP a significant independent contribution was provided by fetal CRL but not by maternal age (p = 0.289), weight (p = 0.099), height (p = 0.052), racial origin (p = 0.219), smoking status (p = 0.667), method of conception (p = 0.437) or parity (p = 0.689):

Sqrt AFP expected = 1.463 + 0.048 × fetal CRL in mm;
$R^2 = 0.116$, $p < 0.0001$.

In the spontaneous early preterm delivery group compared to the term delivery group, the median serum AFP MoM was higher (1.33 vs. 0.97, p = 0.006; fig. 1). In the early preterm delivery group, there was spontaneous onset of contractions with intact membranes in 22 (66.7%) cases and preterm prelabour rupture of membranes in 11 (33.3%) cases; there was no significant difference in the median AFP MoM between the two subgroups (1.34 and 1.33 MoM, p = 0.778).

In the simulated screened population the a posteriori odds for preterm delivery were derived by multiplying the a priori odds by the likelihood ratio for AFP. The a posteriori risk was calculated using the formula: $\beta/(1+\beta)$, where β = a posteriori odds. The estimated detection rate of preterm delivery at a fixed FPR of 10% from the a priori risk alone was 27.5% (AUROC 0.668, 95% CI 0.639–0.698) and this increased to 36.0% (AUROC 0.709, 95% CI 0.680–0.738) when the a priori risk was combined with serum AFP MoM (table 3).

Discussion

The findings of this study demonstrate that (a) in pregnancies resulting in spontaneous early preterm delivery the maternal serum AFP at 11–13 weeks' gestation is increased and (b) measurement of serum AFP improves the prediction of preterm delivery provided by maternal characteristics and obstetric history alone.

Our results are in general agreement with those of previous second-trimester studies; however, in all but two of such studies the outcome measure was total rather than spontaneous preterm delivery. Iatrogenic early delivery is usually undertaken in pregnancies complicated by preeclampsia and/or fetal growth restriction and in these conditions there is a well-described association with impaired placentation [30]. Inadequate trophoblastic inva-

sion of the maternal spiral arteries and failure in their conversion from narrow muscular vessels to large non-muscular channels leads to placental hypoxia and necrosis which inevitably results in interruption of the physiological barrier between the fetal and maternal circulations and escape of AFP from the fetus to the mother. Placental ischemia and damage is also thought to be the underlying mechanism for the observation that in pregnancies that develop preeclampsia there is an increase in the maternal serum cell-free fetal DNA which is apparent from the first trimester and this increase is strongly associated with high impedance to flow in the uterine arteries [31, 32].

We could identify only two previous studies reporting on the association of spontaneous preterm delivery with elevated serum AFP in the second trimester [24, 25]. In pregnancies complicated by spontaneous early delivery there is no evidence of impaired placental perfusion and function [4] and there is, therefore, no obvious explanation for the finding of increased serum AFP in such pregnancies. It is possible that a mechanism other than impaired placental perfusion may be implicated in placental damage resulting in increased serum AFP and also leading to preterm delivery. One study reported that in pregnancies delivering spontaneously before 36 weeks the serum concentration of cell-free fetal DNA at 30–34 weeks is increased and this was attributed to increased cell trafficking across the placenta and/or trophoblast damage [33].

In screening for spontaneous early preterm delivery, inclusion of maternal serum AFP at 11–13 weeks' gestation in an algorithm combining maternal characteristics and obstetric history improved the estimated detection rate, at a FPR of 10%, from about 20 to 30% for nulliparous women and from 36 to 41% for parous women. Future studies will define firstly, whether the overall performance of early screening can be improved further by the addition of the sonographic measurement of the endocervical length at 11–13 weeks, which is reduced in pregnancies that subsequently deliver preterm [34], and secondly, whether early identification of the high-risk group could potentially improve outcome by earlier intervention with such measures as prophylactic use of progesterone or cervical cerclage [35].

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