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European Journal of Obstetrics & Gynecology and Reproductive Biology 122 (2005) 187–190
EUROPEAN JOURNAL OF OBSTETRICS & GYNECOLOGY AND REPRODUCTIVE BIOLOGY
www.elsevier.com/locate/ejogrb

Predictive power of maternal serum and amniotic fluid CRP and PAPP-A concentrations at the time of genetic amniocentesis for the preterm delivery

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Received 9 January 2004; received in revised form 26 November 2004; accepted 26 February 2005

Abstract

Objective: To investigate whether maternal serum and amniotic fluid CRP and PAPP-A concentrations at the time of genetic amniocentesis are markers of preterm delivery.
Study design: One hundred and forty-one pregnant women were included in this prospective study. Amniotic fluid and maternal serum CRP and PAPP-A concentrations were determined by using commercially available kits. Receiver-operating characteristic (ROC) analysis was performed to determine the efficacy of maternal serum and amniotic fluid CRP and PAPP-A levels in predicting women with preterm delivery.
Results: The prevalence of spontaneous preterm delivery before 37 weeks of gestation was 9.9%. ROC analysis revealed that amniotic fluid CRP level was the only parameter, which had a significant power in the prediction of preterm delivery. The optimum cut-off level was 0.65 mg/L. The sensitivity and specificity were 92.9% and 78.7%, respectively.
Conclusion: The amniotic fluid CRP level has a high sensitivity and specificity in the prediction of preterm delivery and this may be helpful in predicting preterm delivery during genetic amniocentesis.

Keywords: Preterm delivery; CRP; PAPP-A; Amniotic fluid

1. Introduction

Preterm labor is the leading cause of perinatal mortality and morbidity [1]. The physiologic mechanism that initiates preterm labor has not been substantially identified. Placental ischemia and acute inflammation are the most common two pathologies that have been implicated [2]. Abnormal placentation occuring in the first trimester results in uteroplacental hypoperfusion and placental ischemia [3].

C-reactive protein (CRP) is well described as a marker of systemic inflammation and is documented to raise several-fold in response to inflammatory stimuli [4]. Its concentration remains stable over long periods of time and depends almost entirely on the rate of hepatic production rather than factors influencing protein clearance [4,5]. Nevertheless, in contrast to many other inflammatory markers, assay techniques for high sensitivity CRP (hs-CRP) are reliable, fully automated and now, highly sensitive, providing a simple clinical tool for the careful assessment of systemic inflammation [6]. Compelling clinical and experimental evidence has demonstrated an association between intrauterine inflammation markers including hs-CRP and both preterm delivery and premature rupture of membranes [7,8]. Romero et al. [9] described the fetal systemic inflammatory response syndrome, which consists of an elevation of fetal plasma levels of cytokines, impending preterm delivery and adverse neonatal outcome.

The pregnancy-associated major basic protein-A (PAPP-A), which is synthesized by the syncytiotrophoblast and throphoblast-derived septal X cells [10], increases in the maternal serum until the end of pregnancy [11]. PAPP-A is virtually identical to the eosinophil major basic protein [12]. In the first trimester of pregnancy, low maternal serum

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0301-2115/$ – see front matter
doi:10.1016/j.ejogrb.2005.02.021

PAPP-A level is helpful in predicting fetal chromosomal anomalies, such as Down syndrome, trisomy 13, trisomy 18, triploidy [13]. It has also been suggested that serum PAPP-A level is helpful in predicting extremely premature delivery [14].

The purpose of this study was to analyze the power of maternal serum and amniotic fluid CRP and PAPP-A levels at the time of genetic amniocentesis in the prediction of preterm delivery and also, to compare their predictive powers.

2. Materials and methods

The study population consisted of consecutive patients, who underwent standard genetic amniocentesis between March 2002 and May 2003 at our university. Amniocentesis was performed for advanced maternal age, increased risk for aneuploidy (triple test, previous aneuploid child) and increased risk of neural tube defect (high level of alpha-fetoprotein). To qualify for participation, subjects were required to (1) have a singleton pregnancy between 15 and 20 weeks of gestation, (2) have a known gestational age, (3) have a normal pregnancy course before the procedure, (4) have no conditions known to be associated with a higher risk of preterm delivery (i.e., discolored amniotic fluid, placenta previa, history of a preterm delivery), (5) have no congenital malformation or chromosomal abnormality and (6) be older than 18 years of age. Gestational age was calculated on a reliable recollection of the last menstrual period and confirmed at ultrasound imaging within 13 weeks of gestation. Patients were excluded after amniocentesis in the presence of fetal chromosomal abnormalities. The study was approved by the institutional review board at the Marmara University and written informed consent was obtained from each subject.

Amniocentesis was performed by using a 21-gauge needle under ultrasonographic guidance with a free-hand technique. The first 0.5 mL of amniotic fluid was collected by using a 5-mL syringe and discharged to avoid maternal contamination. Subsequently, 15–18 mL of amniotic fluid was collected by using a 20-mL syringe and used for the karyotype analysis and CRP, PAPP-A concentration measurements. The amniotic fluid specimens were centrifuged for 10 min to obtain the cellular components for the karyotype analysis. The supernatant was processed for CRP and PAPP-A measurements. Immediately after amniocentesis, 5 mL of venous samples were withdrawn for serum CRP and PAPP-A determinations. Amniotic fluid and maternal serum CRP and PAPP-A concentrations were determined within 1 h after amniocentesis.

Amniotic fluid and maternal serum CRP concentrations were assessed by using a commercially available high-sensitivity latex-enhanced turbidimetric assay (high sensitivity CRP; Newark, DE, USA). The sensitivity of the assay was 0.5 mg/L. Interassay and intraassay coefficients of variation were between 1.0% and 3.5% for different levels.

Amniotic fluid and maternal serum PAPP-A concentrations were assessed by using a commercially available ELISA kit (Gamma SA, Angluer, Belgium). The sensitivity of the assay was 1.2 mg/L. Interassay and intraassay coefficients of variation were between 4.1–11.6% and 3.1–6.4% for different levels, respectively.

Receiver operating characteristic (ROC) analysis was performed to determine the efficacy of maternal serum and amniotic fluid CRP and PAPP-A levels in predicting women with preterm delivery. Diagnostic sensitivity and specificity were calculated and the ROC curve was constructed by plotting the sensitivity against the false-positive rate (1-specificity) of various cut-off values for predicting preterm delivery. Area under the ROC curve (AUC) was calculated and significance of the difference from a coin test, which is a diagonal from the lower left to the upper right corner of the ROC graph and also, has an AUC of 0.5, was reported. The value with the optimal combination of sensitivity and specificity was chosen as the cut-off value. The ideal screening test is one that approaches or reaches the upper left corner of the graph (100% sensitivity and 100% specificity). The cut-off point for each of the screening tests that has the best combination of sensitivity and specificity is located at the ''knee'' of the graph. Positive and negative predictive values also were calculated.

Univariate comparison between women with term and those with preterm delivery was performed by using Mann–Whitney *U*- and chi-square tests, where appropriate. Statistical analysis was performed by using the software, SPSS, Release 10.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

3. Results

During the study period, 151 patients underwent genetic amniocentesis. Among these, four patients were delivered for fetal or maternal indications, four patients had chromosomal abnormality and two patients could not be reached. The study was completed with 141 subjects, 14 of whom (9.9%) delivered before 37 completed weeks. Characteristics of women with term and those with preterm delivery are shown in Table 1.

ROC analysis revealed that amniotic fluid CRP level had the highest AUC value and also, was the only significant parameter in the prediction of preterm delivery among the amniotic and maternal serum CRP and PAPP-A levels during genetic amniocentesis (Table 2; Fig. 1). Amniotic fluid CRP level with the cut-off value of 0.65 mg/L provided the best combination of sensitivity (93%) and specificity (79%). Neither amniotic fluid PAPP-A nor maternal serum levels of CRP and PAPP-A were significantly different from a simple coin test in predicting women with subsequent preterm delivery.

Table 1
Characteristics of the term and preterm deliveries

	Term delivery ($n = 127$)	Preterm delivery ($n = 14$)	P
Maternal age (years)	35.0 ± 4.8	34.4 ± 4.7	NS
Rate of nulliparous women (%)	47.2	50.0	NS
Gestational age at amniocentesis (weeks)	18.6 ± 0.8	18.8 ± 1.1	NS
Gestational age at delivery (weeks)	38.8 ± 1.3	31.4 ± 3.7	<0.001
Amniotic fluid CRP level (mg/L)	0.51 ± 0.57	1.49 ± 1.73	<0.001
Amniotic fluid PAPP-A level (mg/L)	78.1 ± 156.8	64.7 ± 101.0	NS
Maternal serum CRP level (mg/L)	6.17 ± 4.55	9.52 ± 11.18	NS
Maternal serum PAPP-A level (mg/L)	48.0 ± 86.1	13.7 ± 6.8	NS

Table 2
Results of the ROC analysis and optimum cut-offs for the significant parameters

	AUC ± S.E.	P[a]	95% CI	Optimum cut-off	Sn (%)	Sp (%)	PPV (%)	NPV (%)
Maternal age (years)	0.48 ± 0.07	NS	0.33–0.63	NA	NA	NA	NA	NA
Amniotic fluid CRP level (mg/L)	0.85 ± 0.06	<0.001	0.74–0.97	0.65	92.9	78.7	32.5	99.0
Amniotic fluid PAPP-A level (mg/L)	0.54 ± 0.07	NS	0.41–0.68	NA	NA	NA	NA	NA
Maternal serum CRP level (mg/L)	0.56 ± 0.09	NS	0.38–0.74	NA	NA	NA	NA	NA
Maternal serum PAPP-A level (mg/L)	0.47 ± 0.06	NS	0.36–0.58	NA	NA	NA	NA	NA

Predictive roles for the significant parameters also are shown. *Note:* AUC, area under the curve; SE, standard error; CI, confidence interval; Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.
[a] Significance of the difference from a coin test, which has an AUC of 0.5.

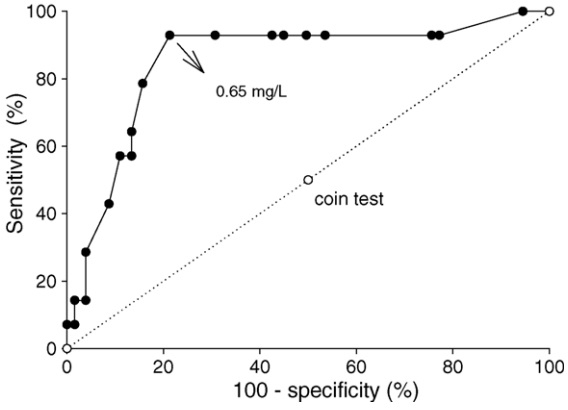

Fig. 1. The ROC curve for the amniotic fluid CRP level, which had a significantly higher AUC value than a coin test, i.e. a test that approximates a coin flip, which is shown by a diagonal from the lower left to the upper right corner of the graph and also signifies an AUC of 0.5. Arrow indicates the optimum cut-off value, which is the closest point to the left upper corner of the graph.

Women with an amniotic fluid CRP levels of >0.65 mg/L had a higher rate of preterm delivery than those with a lower levels (1.0% versus 32.5%, $P < 0.001$) and the odds ratio (OR) for having a preterm delivery was 48.1 in these women. Ninety-nine percent of women with a low amniotic fluid CRP level had a subsequent term delivery.

4. Discussion

Our data demonstrates that amniotic fluid CRP concentration is significantly higher in women with subsequent preterm delivery than in those, who delivered at term. This confirms the recently published study of Ghezzi et al. [7] and also, supports the hypothesis that subclinical fetal inflammatory response might occur very early during gestation in fetuses, who will experience preterm delivery. They have reported the optimum cut-off value of 0.11 mg/L for the amniotic fluid CRP concentration with a sensitivity of 80.8% and a specificity of 69.5% in the prediction of spontaneous preterm delivery at <34 gestational weeks. In our study, CRP concentration of >0.65 mg/L predicted preterm delivery at <37 weeks with a sensitivity of 92.9% and a specificity of 78.7%. Neither Ghezzi et al. [7] nor our group could find any correlation between maternal serum CRP level and preterm delivery. It has been demonstrated that a subclinical intrauterine inflammatory cytokine response might be present very early in gestation [7]. At the time of genetic amniocentesis, increased amniotic fluid concentration of interleukin-6 [15] and angiogenin [16] also have been reported to be higher in women with preterm delivery.

The etiology of a great proportion of spontaneous preterm deliveries is more complex and may involve also chronic villous and uteroplacental vascular disease in addition to, or instead of, lesions of acute inflammation. Placental infarction, chronic villitis and lesions of the uteroplacental vessels (abruption, thrombosis, failure of physiologic change) have been previously shown to be associated with preterm births characterized by decreased fetal growth [17].

The relationship between chromosomal aberrations such as trisomy 21, 13, 18, sex chromosome aneuploidy and triploidy and low maternal serum PAPP-A levels has been well established [13,15]. However, low levels of first trimester maternal serum PAPP-A levels have also been

implicated in ectopic pregnancy and miscarriage [18,19]. Yaron et al. [20] reported that low levels of maternal serum PAPP-A is a predictor of miscarriage, fetal growth restriction, proteinuric pregnancy-induced hypertension, but not associated with preterm delivery. Nevertheless, Smith et al. [14] have reported that women with a PAPP-A level in the lowest 5% in the first trimester had an increased risk of extremely premature delivery with the adjusted odds ratio of 2.9. They have speculated also that, the PAPP-A is not acting as a simple marker of the volume or health of the trophoblast, but that the association reflects a specific property of PAPP-A in the physiological regulation of trophoblast function [14].

In the present study, although women in the term delivery group had a higher mean maternal serum and amniotic fluid PAPP-A concentrations than those in the preterm delivery group, there was no statistically significant difference.

In conclusion, there is a strong correlation between elevated amniotic fluid hs-CRP levels and subsequent preterm delivery. At the time of genetic amniocentesis, we suggest that determination of hs-CRP levels would be an outstanding method for estimating the risk of preterm delivery. This simple clinically useful, cost effective test may assist clinicians in counselling high-risk patients and determine strategies for the prevention of preterm delivery.

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