Original Paper

Fetal Diagnosis and Therapy

Fetal Diagn Ther 2011;30:215–218
DOI: 10.1159/000330198

Received: May 17, 2011
Accepted after revision: June 19, 2011
Published online: September 23, 2011

First-Trimester Screening for Trisomy 21 Using Alpha-Fetoprotein

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Key Words
First-trimester screening · Trisomy 21 · Alpha-fetoprotein · Free β-human chorionic gonadotropin · PAPP-A · Nuchal translucency

Abstract
Objective: To investigate the potential value of adding maternal serum alpha-fetoprotein (AFP) to free β-human chorionic gonadotropin (β-hCG) and PAPP-A and fetal nuchal translucency (NT) thickness in first-trimester screening for trisomy 21. ***Methods:*** In this case control study, serum AFP was measured in 100 trisomy 21 and 1,500 euploid pregnancies in which screening for trisomy 21 had been performed by a combination of serum free β-hCG and PAPP-A and fetal NT at 11–13 weeks' gestation. We examined the effect of adding AFP on the performance of screening by the combined test. ***Results:*** In the trisomy 21 pregnancies, the median multiple of the normal median AFP, adjusted for gestational age, maternal weight, racial origin, smoking status and method of conception, was significantly reduced (0.7037, 95% CI: 0.6398–0.7739). Adding AFP to the combined test improved the performance of screening and for a risk cut-off of 1 in 100, the false-positive rate was reduced from 2.8 by 0.4% (95% CI: 0.13–0.77%) without a significant change in detection rate. ***Conclusions:*** Inclusion of serum AFP improves the performance of the first-trimester combined test in screening for trisomy 21.

Introduction

In trisomy 21 pregnancies, the maternal serum level of alpha-fetoprotein (AFP) is reduced and measurement of this protein is an integral part of second-trimester biochemical screening for aneuploidies [1, 2]. A meta-analysis of studies on screening for trisomy 21 at 15–18 weeks' gestation, involving a total of about 120,000 pregnancies, reported that the median multiple of the normal median (MoM) AFP in 1,140 trisomy 21 pregnancies was 0.73 [2]. In the first trimester, effective screening for fetal trisomy 21 is provided by a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotropin (β-hCG) and PAPP-A with a detection rate of about 90% at a false-positive rate of 5% [3, 4].

The aim of this study is to assess the potential value of adding maternal serum AFP to free β-hCG and PAPP-A and fetal NT thickness in first-trimester screening for trisomy 21.

Methods

This was a case control study drawn from a large prospective screening study for aneuploidies at King's College Hospital, London, UK by a combination of maternal age, fetal NT thickness and maternal serum PAPP-A and free β-hCG at 11^{+0}–13^{+6} weeks' gestation [4]. Gestational age was determined from the measurement

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1015–3837/11/0303–0215$38.00/0

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Table 1. Characteristics of the cases and controls

	Euploid pregnancies (n = 1,500)	Trisomy 21 pregnancies (n = 100)
Median maternal age (IQR), years	31.7 (27.3–35.5)	38.8 (34.9–40.5)
Median maternal weight (IQR), kg	62.5 (56.0–71.0)	65.0 (60.0–71.3)
Ethnicity, n (%)		
African	300 (20.0)	19 (19.0)
East Asian	300 (20.0)	0 (0.0)
South Asian	300 (20.0)	1 (1.0)
Caucasian	600 (40.0)	80 (80.0)
Cigarette smoker, n (%)	100 (6.7)	8 (8.0)
Mode of conception, n (%)		
Spontaneous	1,300 (86.6)	88 (88.0)
In-vitro fertilization	100 (6.7)	4 (4.0)
Ovulation drugs	100 (6.7)	8 (8.0)
Median gestational age (IQR), days	89 (86–91)	91 (88–95)
Parity, n (%)		
Nulliparous	744 (49.6)	32 (32.0)
Multiparous	756 (50.4)	68 (68.0)

Table 2. Distributional characteristics of log MoM AFP values in euploid and trisomy 21 pregnancies

Parameter	Euploid		Trisomy 21	
	estimate	95% CI	estimate	95% CI
Mean	0		–0.1526	–0.1939 to –0.1113
Standard deviation	0.1642	0.1442 to 0.1908	0.1967	0.1899 to 0.2040
Correlation with PAPP-A log MoM	0.0592	–0.1388 to 0.2527	0.0084	–0.1883 to 0.2045
Correlation with free β-hCG log MoM	0.0894	–0.1089 to 0.2809	–0.0020	–0.1984 to 0.1945
Correlation with NT log MoM	–0.1363	–0.3240 to 0.0618	0.0137	–0.1832 to 0.2096

of the fetal crown-rump length at the time of screening [5]. Serum and plasma from women agreeing to participate in research were stored at –80°C for subsequent biochemical analysis. Written informed consent was obtained for participation in the study, which was approved by the King's College Hospital ethics committee.

In this study we measured maternal serum AFP in 100 trisomy 21 and 1,500 euploid pregnancies. Cases were selected at random from our database of stored samples and matched for storage time. None of the samples were previously thawed and refrozen. Maternal serum AFP was measured using the DELFIA Xpress analyzer (PerkinElmer Life and Analytical Sciences, Waltham, Mass., USA).

Statistical Analyses

MoM values for AFP were computed from our previously reported multiple regression model in euploid pregnancies [6]. The mean log MoM in trisomy 21 pregnancies was estimated from the case control sample. Taking previously published parameter estimates for PAPP-A and free β-hCG [7], trivariate Gaussian distributions were obtained for the joint distribution of log MoM values for PAPP-A, free β-hCG and AFP. The adequacy of the fitted distributions was assessed by inspecting histograms of the squared Mahalanobis distances with the χ^2 distribution superimposed. Gross outliers beyond the 99.99th centile were removed.

Standardised detection rates were computed by obtaining the likelihood ratios for biochemistry alone or biochemistry and fetal NT [8] in trisomy 21 pregnancies in the sample and then applying these to each year of maternal age from 12 to 50 to estimate the age-specific detection rates. These were then weighted according to the maternal age distribution of trisomy 21 pregnancies in England and Wales in 2000–2002 [9]. Similarly, standardised false-positive rates were computed by obtaining the likelihood ratios for biochemistry and NT, as appropriate, in unaffected pregnancies in the sample and then applying these to each year of maternal age from 12 to 50 to estimate the age-specific false-positive rates. These were then weighted according to the maternal age distribution of unaffected pregnancies in England and Wales in 2000–2002 [9]. Confidence intervals (CI) were obtaining by bootstrapping.

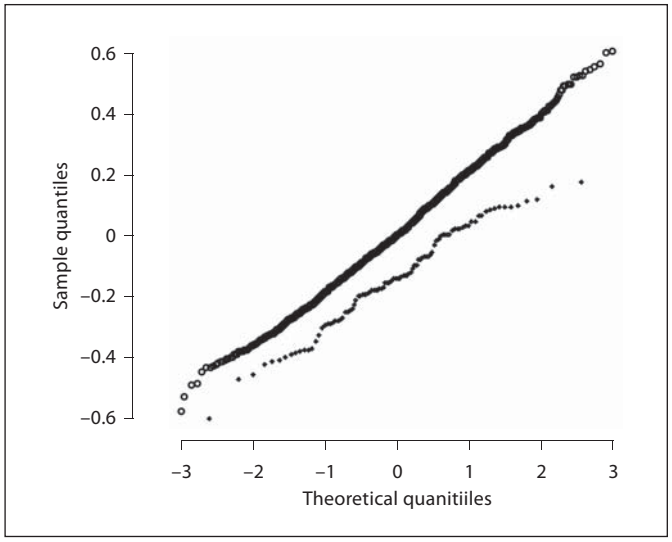

Fig. 1. Gaussian probability plot for AFP (log scale) for euploid (circles) and trisomy 21 (diamonds) pregnancies.

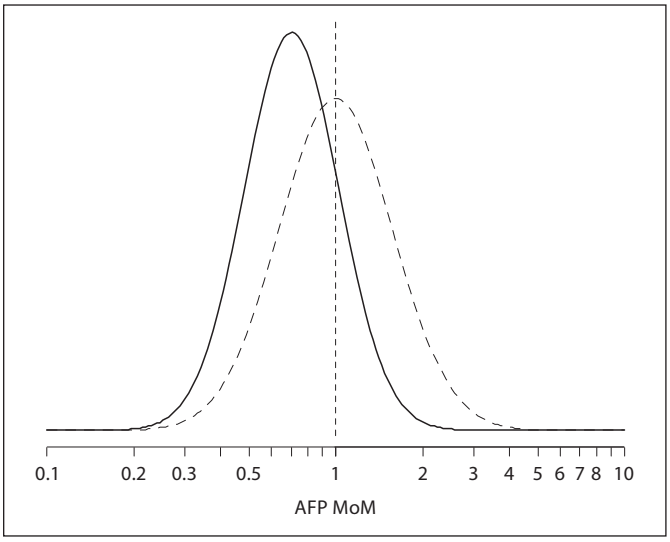

Fig. 2. Fitted Gaussian distribution of AFP (log scale) for euploid (interrupted) and trisomy 21 (solid) pregnancies.

Table 3. Estimated detection and false-positive rates, with 95% confidence limits, for the first-trimester biochemical and combined testing with and without AFP

Risk cut-off	Performance	Screening test, %			
		biochemistry	biochemistry and AFP	biochemistry and NT	biochemistry, NT and AFP
1 in 50	FPR	3.2 (2.5–3.9)	2.9 (2.3–3.6)	1.6 (1.2–2.1)	1.4 (1.0–1.9)
	DR	55.4 (47.7–62.6)	61.3 (54.0–68.3)	91.6 (86.4–94.8)	92.2 (86.5–95.9)
1 in 100	FPR	5.6 (4.7–6.6)	5.1 (4.3–6.1)	2.8 (2.2–3.4)	2.4 (1.9–2.9)
	DR	65.5 (58.0–72.2)	71.7 (64.5–78.0)	94.7 (90.4–97.2)	95.1 (89.3–98.0)
1 in 150	FPR	7.4 (6.5–8.5)	7.0 (6.0–8.1)	3.7 (3.1–4.5)	3.4 (2.7–4.0)
	DR	71.5 (64.4–77.6)	77.0 (70.2–82.8)	96.0 (91.7–98.2)	96.1 (89.9–98.7)
1 in 200	FPR	9.3 (8.2–10.5)	8.7 (7.6–9.9)	4.7 (4.0–5.5)	4.2 (3.5–4.9)
	DR	75.8 (68.9–81.5)	80.2 (73.6–85.6)	96.6 (92.2–98.7	96.5 (89.4–99.0)
1 in 250	FPR	11.0 (9.9–12.3)	10.1 (8.9–11.4)	5.5 (4.7–6.4)	5.0 (4.2–5.7)
	DR	78.6 (72.0–83.9)	82.3 (76.0– 87.5)	97.0 (92.3–99.0)	96.7 (89.7–99.1)
1 in 300	FPR	12.7 (11.4–14.0)	11.3 (10.1–12.7)	6.3 (5.5–7.3)	5.5 (4.7–6.3)
	DR	80.7 (74.3–85.9)	84.2 (78.1–89.0)	97.1 (92.5–99.0)	96.9 (90.1–99.2)

Rates are standardised so that they relate to the population of England and Wales 2000–2002.
FPR = False-positive rate; DR = detection rate.

Results

The characteristics of the cases and controls are presented in table 1. Distributional parameters for AFP in trisomy 21 and euploid pregnancies are given in table 2. The estimated mean log MoM in trisomy 21 pregnancies of –0.1526 (95% CI: –0.1939 to –0.1113) corresponds to a median MoM of 0.7037 (95% CI: 0.6398–0.7739). The probability plots shown in figure 1 demonstrate that the distributions of $\log_{10}$MoM AFP in euploid and trisomy 21 pregnancies can be represented by Gaussian distributions, and the fitted Gaussian distributions are shown in figure 2.

Estimated standardised detection and false-positive rates in screening by biochemical testing with serum free

β-hCG and PAPP-A and combined testing with biochemistry and fetal NT, with and without inclusion of serum AFP, are given in table 3. Adding AFP to biochemical testing with a risk cut-off of 1 in 100 reduced the false-positive rate from 5.6 by 0.4% (95% CI: 0.06–0.83%) and increased the detection rate from 65.5 by 6.2% (95% CI: 3.4–8.5%). In combined biochemical and fetal NT testing, addition of AFP reduced the false-positive rate from 2.8 by 0.4% (95% CI: 0.13–0.77%) and the detection rate increased from 94.7 by 0.4% (95% CI: –0.7 to 1.4%).

Discussion

The finding of this study that in first-trimester trisomy 21 pregnancies maternal serum AFP is reduced is compatible with the results of previous studies. A meta-analysis of studies on a total of about 34,000 pregnancies at less than 14 weeks' gestation reported that the median AFP in 542 trisomy 21 pregnancies was 0.79 [10]. Our results demonstrate that this decrease in serum AFP in trisomy 21 pregnancies can be exploited to improve the already high performance of screening for this aneuploidy by the combination of maternal age, fetal NT and serum free β-hCG and PAPP-A at 11–13 weeks' gestation.

In the case of biochemical testing alone, addition of AFP resulted in significant reduction of the false-positive rate and increase in the detection rate. Our results are compatible with a modeled prediction based on findings from a meta-analysis [10] that in first-trimester biochemical testing addition of AFP and unconjugated estriol to free β-hCG and PAPP-A could increase the detection rate, at a false-positive rate of 5%, from about 65 to 70% [11]. In combined screening by biochemistry and fetal NT, addition of AFP reduced the false-positive rate but there was no significant improvement in detection rate, presumably because in this particular data set screening performance was exceptionally good even without AFP. In the modeled prediction based on findings from the meta-analysis [10] it was estimated that addition of AFP and unconjugated estriol in first-trimester combined testing could increase the detection rate, at a false-positive rate of 5%, from 86.6 to 88.4% [11].

Traditionally, measurement of serum AFP was restricted to the second trimester of pregnancy where it has been shown to be useful in screening both for aneuploidies and neural tube defects. Recent evidence suggests that in pregnancies resulting in spontaneous early preterm delivery, the maternal serum AFP at 11–13 weeks' gestation is increased and this measurement improves the prediction of preterm delivery provided by maternal characteristics and obstetric history alone [12]. This finding as well as the results of our study on screening for trisomy 21 suggest that inclusion of AFP in routine first-trimester testing could be beneficial and the measurement can be performed by the same automated machines used for free β-hCG and PAPP-A at little extra cost.

Acknowledgment

The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No. 1037116).

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