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First-Trimester Screening for Neural Tube Defects Using Alpha-Fetoprotein

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Key Words

First-trimester screening · Alpha-fetoprotein · Neural tube defects · Acrania · Spina bifida

Abstract

Objective: To assess the potential value of maternal serum alpha-fetoprotein (AFP) at 11–13 weeks' gestation in early screening for fetal neural tube defects (NTDs). ***Methods:*** Maternal serum AFP at 11–13 weeks' gestation was measured in 32 cases of fetal NTDs, including 18 cases of acrania and 14 cases of spina bifida, and 1,500 unaffected controls. The measured serum AFP was converted into multiple of the expected median (MoM) after adjustment for gestational age and maternal characteristics and Mann-Whitney test was used to determine the significance of difference in the mean MoM of serum AFP in the NTD group to that in the controls. ***Results:*** The mean AFP MoM in the NTD group (1.76, 95% CI 1.39–2.23) was significantly higher than in the controls ($p < 0.0001$). The mean AFP MoM was not significantly different between the cases of acrania and cases of spina bifida (1.78 vs. 1.75; $p = 0.722$). The detection rates of NTD in screening by serum AFP were 50.0% (95% CI 31.9–68.1) and 37.5% (95% CI 21.1–56.3) at fixed false-positive rates of 10 and 5%, respectively. ***Conclusion:*** Measurement of maternal serum AFP at 11–13 weeks' gestation may be useful in screening for fetal NTDs.

Introduction

Measurement of maternal serum alpha-fetoprotein (AFP) is an integral part of second-trimester biochemical screening for both aneuploidies and neural tube defects (NTDs). In pregnancies with fetal trisomy 21 serum AFP is reduced, whereas in neural tube defects AFP is increased [1, 2]. A UK multicenter study reported that at 16–18 weeks' gestation 88% of cases of anencephaly, 79% of cases of open spina bifida and 3% of unaffected singleton pregnancies had AFP levels equal to or greater than 2.5 multiples of the median (MoM) for unaffected pregnancies [3]. In the last few years the introduction of successful second-trimester sonographic diagnosis of neural tube defects and the improved detection of aneuploidies by a combination of fetal nuchal translucency (NT) thickness and maternal serum free β-hCG and PAPP-A at 11–13 weeks' gestation, compared to second-trimester biochemical testing, have reduced the use of serum AFP [4–7]. However, recent evidence suggests that in trisomy 21 pregnancies the maternal serum level of AFP is reduced not only in the second trimester but also in the first trimester and it is possible that measurement of serum AFP may improve the performance of the combined test at 11–13 weeks [8]. There is also evidence that in pregnancies resulting in spontaneous early preterm delivery the maternal serum AFP at 11–13 weeks' gestation is in-

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creased and this measurement improves the prediction of preterm delivery provided by maternal characteristics and obstetric history alone [9]. Consequently, measurement of serum AFP is likely to become an integral part of first-trimester screening for aneuploidies and pregnancy complications.

The aim of this is study to assess the potential value of maternal serum AFP at 11–13 weeks' gestation in early screening for fetal neural tube defects.

Methods

This study is drawn from a large prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy at King's College Hospital, London, UK. In this visit, which is held at 11^{+0} to 13^{+6} weeks of gestation, we recorded maternal characteristics and performed an ultrasound scan to confirm gestational age from the measurement of the fetal crown-rump length (CRL) to diagnose any major fetal abnormalities, and to measure fetal NT thickness and maternal serum free β-hCG and PAPP-A as part of the screening for chromosomal abnormalities [10, 11]. Additionally, blood was collected for research and the separated plasma and 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.

Maternal characteristics recorded were age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), smoking status during pregnancy (yes or no) and method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or in vitro fertilization). The maternal weight and height were measured at the time of screening.

Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women.

Study Population

During the study period (March 2006 to July 2010), we examined 44,982 singleton pregnancies with a live fetus at 11^{+0} to 13^{+6} weeks. We searched the database and found 32 cases of NTD with 18 cases of acrania and 14 cases of spina bifida. These affected cases are compared to 1,500 pregnancies which were not complicated by hypertensive disorders or diabetes mellitus and resulted in the live birth at or after 37 weeks of phenotypically normal neonates with birth weight above the 5th and below the 95th percentile [12].

Maternal serum AFP was measured using the DELFIA XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, Mass., USA). None of the samples were previously thawed and refrozen.

Statistical Analyses

The median gestational age calculated from fetal CRL was compared to that derived from the last menstrual period (LMP) by Wilcoxon signed ranks test. In each case and control, the measured serum AFP was converted into multiple of the expected median (MoM) after adjustment for gestational age, maternal weight, racial origin, smoking status and method of conception as previously described [11]. The Mann-Whitney test was used to determine the significance of difference in the mean MoM of serum AFP in the NTD group to that in the controls. Likelihood ratios were computed from the fitted distributions of AFP MoM values in the NTD group and controls. The a priori risk for NTDs of 1 in 1,000, 1 in 2,000 and 1 in 5,000 was then multiplied with the appropriate likelihood ratio for different AFP cut-offs to derive the a posteriori risks for NTDs. The detection rate and false-positive rate were calculated as the respective proportions of NTD (detection rate) and unaffected pregnancies (false-positive rate) with MoM values above given cut-offs. The statistical software package SPSS 18.0 (SPSS Inc., Chicago, Ill., USA) was used for all of the data analyses.

Results

The characteristics of the cases with fetal acrania and open spina bifida are summarized in table 1. In the cases of spina bifida there was no significant difference in median gestational age calculated from fetal CRL from that derived from the LMP ($p = 0.875$), whereas in the cases of acrania the gestational age calculated from fetal CRL was significantly shorter ($p = 0.008$).

AFP in Neural Tube Defects

The estimated mean AFP MoM was not significantly different between the cases of acrania and cases of spina bifida (dating by CRL: 1.78 vs. 1.75; $p = 0.722$; dating by LMP: 1.62 vs. 1.75; $p = 0.561$). In the cases of spina bifida, the estimated mean MoM was 1.75 with 95% CI 1.32–2.32, when pregnancy dating was by CRL, and 1.75 with 95% CI 1.35–2.28, when dating was by LMP and they were both significantly higher than in the controls ($p < 0.0001$). In the total NTD group, the estimated mean MoM was 1.76 (95% CI 1.39 to 2.23) when dating by CRL and 1.68 (95% CI 1.32 to 2.14), when dating by LMP and they were both significantly higher than in the controls ($p < 0.0001$; fig. 1, 2).

There was no evidence to suggest that the effect of NTD on AFP MoM levels changes with gestation at 11^{+0} to 13^{+6} weeks (dating by CRL: $p = 0.578$, dating by LMP: $p = 0.276$) or with maternal racial origin (dating by CRL: $p = 0.392$, dating by LMP: $p = 0.464$).

Performance of Screening

The receiver operating characteristic (ROC) curve by AFP in the prediction of NTD is shown in figure 3. The area under the ROC curve for the detection of NTDs was 0.750 (95% CI 0.653–0.847) when dating by CRL and 0.736 (95% CI 0.632–0.840) when dating by LMP. The de-

Table 1. Characteristics of the cases with fetal acrania and open spina bifida

Maternal characteristics	Acrania (n = 18)	Spina bifida (n = 14)	p
Maternal age in years, median (IQR)	29.5 (24.7–34.3)	35.6 (26.3–38.9)	0.054
Maternal weight in kg, median (IQR)	66.7 (55.0–78.0)	64.2 (57.0–86.3)	0.837
Gestational age from CRL in days, median (IQR)	85 (83–87)	89 (84–94)	0.050
Gestational age from LMP in days, median (IQR)	87 (85–90)	88 (85–94)	0.464
Racial origin			
Caucasian, n (%)	14 (77.8)	10 (71.4)	0.704
Afro-Caribbean, n (%)	2 (11.1)	3 (21.4)	0.631
South Asian, n (%)	2 (11.1)	1 (7.1)	0.999
Cigarette smokers, n (%)	2 (11.1)	1 (7.1)	0.999
Method of conception			
Spontaneous, n (%)	16 (88.9)	11 (78.6)	0.631
Ovulation induction	2 (11.1)	2 (14.3)	0.999
In vitro fertilization	0	1 (7.1)	0.438

Comparison between outcome groups by Mann-Whitney U test and χ^2 test or Fisher's exact test for categorical variables; significance level * $p < 0.05$.

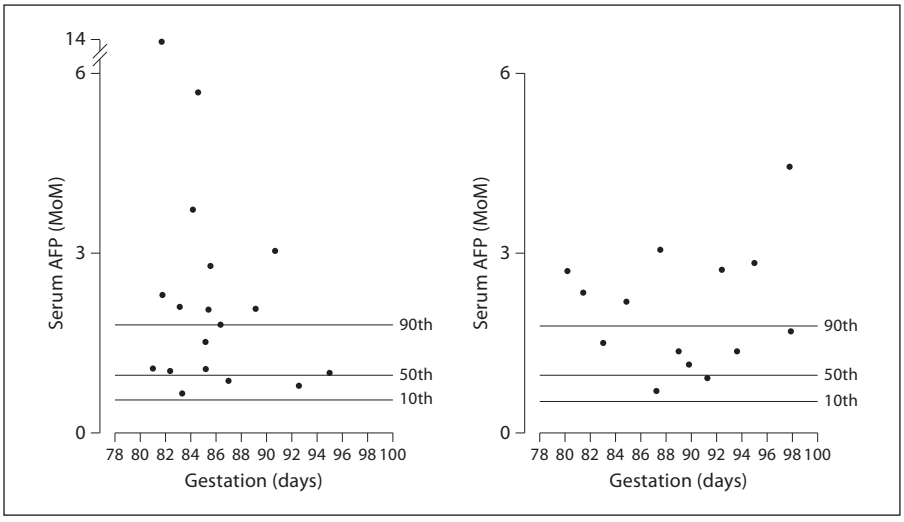

Fig. 1. Distributions of AFP MoM values in pregnancies with fetal acrania (left) and open spina bifida (right) plotted on the normal range (median, 90th and 10th centiles) for unaffected pregnancies. Gestational age was derived from the fetal crown-rump length.

tection rates of NTD in screening by serum AFP were 50.0% (95% CI 31.9–68.1) and 37.5% (95% CI 21.1–56.3) when dating by CRL and 43.8% (95% CI 26.4–62.3) and 31.3% (95% CI 16.1–50.0), when dating by LMP, at fixed false positive rates of 10 and 5%, respectively (table 2).

The likelihood ratio for fetal NTDs increased with maternal serum AFP MoM and at a cut-off of 1.5 MoM the detection rate was 56.3% at a FPR of 20.5% (table 3). The respective values at 2.5 MoM were 31.3 and 2.2%. At a given AFP MoM the patient specific risk for NTDs was related to the population prevalence of NTDs (table 3). For example, at 2.5 MoM the risk for NTDs is 1 in 220 if the population prevalence is 1 in 1,000 and the risk decreases to 1 in 1,120 if the prevalence is 1 in 5,000.

Discussion

The findings of this study demonstrate that at 11–13 weeks' gestation maternal serum AFP is increased in pregnancies with fetal NTDs and the increase is similar in cases of acrania and those with open spina bifida. Se-

Table 2. Performance of screening with fixed percentiles of AFP

Percentile	Cut-off	FPR %	DR %	LR at cut-off	Risk at cut-off for a prior risk of		
					1 in 1,000	1 in 2,000	1 in 5,000
Pregnancy dating from fetal crown-rump length							
90th	1.800	10	50.0	1.51	1 in 660	1 in 1,320	1 in 3,300
95th	2.122	5	37.5	2.47	1 in 400	1 in 810	1 in 2,020
99th	2.891	1	18.8	7.53	1 in 130	1 in 260	1 in 660
Pregnancy dating from maternal last menstrual period							
90th	1.800	10	43.8	1.47	1 in 680	1 in 1,360	1 in 3,400
95th	2.122	5	37.5	2.37	1 in 420	1 in 840	1 in 2,100
99th	2.891	1	15.6	7.06	1 in 140	1 in 280	1 in 700

False-positive rates (FPR), detection rates (DR), likelihood ratios (LR) and patient-specific risk at the cut-off associated with different percentile levels for AFP. The FPR and DR are for population screening performance using the given percentile value to define positivity. The LR is the ratio for pregnancies with fetal neural tube defects relative to unaffected pregnancies.

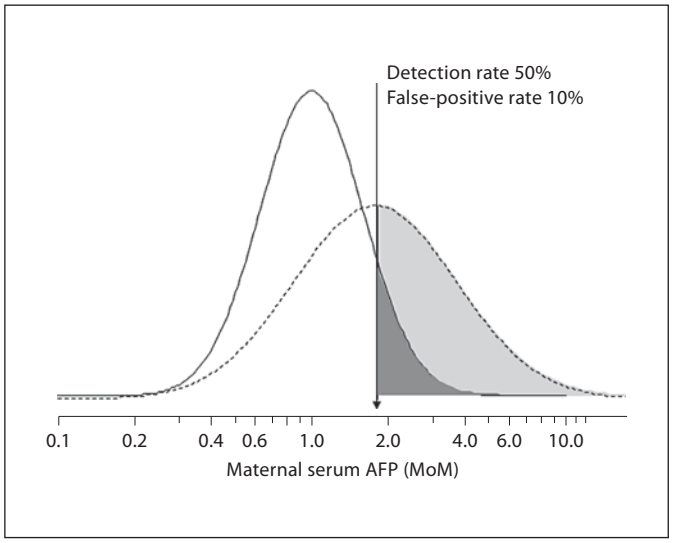

Fig. 2. Fitted distributions of AFP MoM for unaffected (continuous line) and neural tube defect (interrupted line) pregnancies.

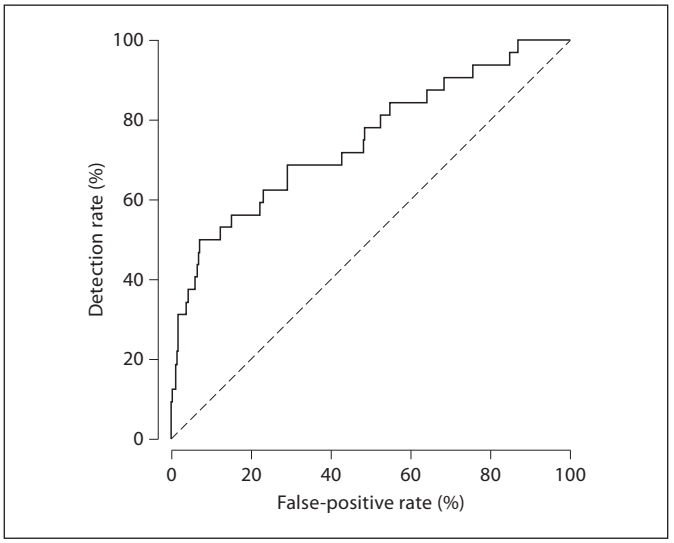

Fig. 3. Receiver-operating characteristic curve in the prediction of fetal neural tube defects by maternal serum AFP.

rum AFP was at or above the 90th and 95th percentiles in about 50 and 40%, respectively, of pregnancies with fetal NTD.

Maternal serum AFP normally increases with gestational age [12]. In both biochemical and sonographic first-trimester screening for aneuploidies gestational age is derived from the measurement of the fetal CRL [10, 11]. Although in spina bifida gestational age derived from fetal CRL and maternal LMP is similar, in the case of acrania the CRL is reduced on average by the equivalent of 2 days. As shown by our results, this underestimation of the true gestational age in acrania contributes to the observed increase in serum AFP and therefore a policy of pregnancy dating by CRL is beneficial in biochemical screening for this defect.

The detection rate of first-trimester biochemical screening for NTDs, at a given false positive rate, is considerably lower than in the second trimester. In a previous

Table 3. Performance of screening and patient-specific risk at different AFP MoM values

AFP MoM	FPR %	DR %	LR at cut-off	Risk according to prior prevalence of NTD		
				1 in 1,000	1 in 2,000	1 in 5,000
Pregnancy dating from fetal crown-rump length						
1.5	20.5	56.3	0.95	1 in 1,050	1 in 2,105	1 in 5,260
2.0	6.9	43.8	2.06	1 in 485	1 in 970	1 in 2,430
2.5	2.2	31.3	4.47	1 in 220	1 in 440	1 in 1,120
3.0	1.1	12.5	9.48	1 in 105	1 in 210	1 in 525
3.5	0.3	12.5	17.84	1 in 56	1 in 110	1 in 280
4.0	0.1	9.4	31.90	1 in 32	1 in 62	1 in 156
Pregnancy dating from maternal last menstrual period						
1.5	20.5	59.4	0.94	1 in 1,060	1 in 2,120	1 in 5,320
2.0	6.9	31.3	1.99	1 in 500	1 in 1005	1 in 2,510
2.5	2.2	25.0	4.22	1 in 230	1 in 470	1 in 1,180
3.0	1.1	12.5	8.86	1 in 110	1 in 225	1 in 560
3.5	0.3	9.4	16.59	1 in 60	1 in 120	1 in 300
4.0	0.1	6.3	29.55	1 in 34	1 in 68	1 in 170

False-positive rates (FPR), detection rates (DR), likelihood ratios (LR) and patient-specific risk at the MoM cut-off. The FPR and DR are for population screening performance using the given MoM value to define positivity. The LR is the ratio for pregnancies with fetal neural tube defects relative to unaffected pregnancies.

multicentre collaborative study in the 1970s, when AFP was measured by radioimmunoassay, the values were at or above the 95th percentile in 19% of cases at 10–12 weeks' gestation and this increased to 61% at 13–15 weeks and 88% at 16–18 weeks [3]. In a study of 13 cases of NTDs, the median AFP at 8–13 weeks was 0.95 MoM and in the same cases the median AFP at 16–18 weeks increased to 4.38 MoM [13]. Another study of 19 cases of NTDs at 6–14 weeks reported that the median serum AFP was only mildly increased to 1.21 MoM [14].

In the 1970s and 1980s, the main method of screening for fetal NTDs was by serum AFP at around 16 weeks and the method of diagnosis was amniocentesis and measurement of amniotic fluid AFP and acetyl cholinesterase [15, 16]. Amniocentesis, being an invasive test, was reserved for women with serum AFP at or above 2.5 MoM, which constituted about 3% of pregnancies. In the late 1980s, biochemical assessment was replaced by second-trimester ultrasonography because improved resolution of machines and training of sonographers allowed easy identification of anencephaly. In the case of open spina bifida effective screening was provided by the realization that the condition is associated with scalloping of the frontal bones (the lemon sign) and caudal displacement of the cerebellum (the banana sign) [17, 18].

In the last 10 years screening for aneuploidies has shifted from second-trimester biochemistry to the first-trimester combined test [11]. In addition to aneuploidies, the 11–13 weeks' scan can identify the majority of all major fetal abnormalities [19]. However, spina bifida is usually not detectable at this scan. A screening study at 11–13 weeks in 61,972 pregnancies undergoing measurement of fetal NT reported that none of the 29 fetuses with spina bifida were detected [20]. However, recent evidence suggests that effective first-trimester sonographic screening for open spina bifida can be provided by examination of the same mid-sagittal view of the fetal head as currently used for measurement of the fetal NT and assessment of the nasal bone. In this view fetuses with open spina bifida demonstrate an increase in the diameter of the brain stem, decrease in the diameter of the fourth ventricle and cisterna magna, as well as a decrease in the fronto-maxillary facial angle [21–23]. Consequently, increased serum AFP, unlike in the 1980s, will not lead to amniocentesis but rather to more careful ultrasonographic examination of both the mid-sagittal view of the head and of the spine. If the necessary expertise for ultrasonographic exclusion or diagnosis of open spina bifida at 11–13 weeks is not available then an additional scan can be carried out at 15–16 weeks. In this respect, in first-trimester biochemical screening for NTDs, it would be preferable to maxi-

mize the detection rate by use of a lower serum AFP cut-off, such as the 90th percentile, rather than the 97th percentile which was traditionally used in second-trimester screening.

Measurement of maternal serum AFP is likely to be incorporated in routine first-trimester testing because of its beneficial effects in screening for aneuploidies and preterm delivery [8, 9]. The findings of our study suggest that serum AFP may also be beneficial in the early identification of fetal NTDs. The performance of screening for fetal NTDs by serum AFP alone and in combination with the sonographic features observed in the mid-sagittal view of the fetal head will ultimately be determined by large prospective studies.

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