OBSTETRICS

Combined screening for open spina bifida at 11-13 weeks using fetal biparietal diameter and maternal serum markers

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OBJECTIVE: Screening at 11-13 weeks with ultrasound biparietal diameter (BPD) can detect half of open spina bifida cases. Maternal serum α-fetoprotein (AFP) levels at 15-19 weeks are increased 3- to 4-fold, in open spina bifida. We assessed whether combined screening using BPD, AFP, and other serum markers at 11-13 weeks would increase detection.

STUDY DESIGN: Maternal AFP levels were measured on serum stored at 11-13 weeks in 44 open spina bifida and 182 unaffected pregnancies, and results were expressed in multiples of the median (MoM) for gestational age. All samples had been measured for free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein (PAPP)-A. A multivariate Gaussian model was used to predict screening performance from the serum data and BPD measurements on 80 cases, including 36 previously published.

RESULTS: The median AFP level in cases was 1.201 MoM, significantly higher than in unaffected pregnancies ($P < .01$, 1 tail). The median free β-hCG was significantly reduced to 0.820 MoM ($P < .02$), but the median PAPP-A was similar in cases and controls. Modeling predicted the following: BPD alone would detect 50% of cases for a 5% false-positive rate or 63% for 10%; adding AFP increases detection by 2%; and a combined test with BPD, AFP, and free β-hCG detects 58% for 5% or 70% for 10%.

CONCLUSION: Combining AFP and BPD with free β-hCG as part of first-trimester aneuploidy screening would also allow early detection about two-thirds of cases with open spina bifida.

Key words: fetus, first trimester, neural tube defects, ultrasound

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Prenatal detection of neural tube defects (NTD) began 40 years ago with the discovery of high levels of α-fetoprotein (AFP) in amniotic fluid and maternal serum from pregnancies with open fetal spina bifida and anencephaly.[1] Maternal serum AFP soon became an established screening test when performed at 15-19 weeks' gestation.[2-5] In recent years screening resources have largely been shifted toward the first trimester of pregnancy, primarily to improve the detection rate for Down syndrome.[6-10]

Ultrasound diagnosis of spina bifida is feasible in the first trimester.[11-15] However, the skills required for direct identification as well as that of related cerebral changes have precluded effective population screening at 11-13 weeks.[16,17] In contrast, we have previously shown that a simple biparietal diameter (BPD) measurement at 11-13 weeks could identify half of spina bifida cases for a 5% false-positive rate.[18]

Although the increase in maternal serum AFP levels in open neural tube defects is higher, 3- to 4-fold, at 15-19 weeks, a smaller increase, 75%, has also been shown in the first trimester.[19] We therefore aimed to investigate whether the combination of BPD with serum levels of AFP would increase spina bifida detection. We also investigated the combined test markers, free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein (PAPP)-A. Although there was no specific reason to expect levels to differ in open neural tube defects, the data were readily available in all cases.

Materials and Methods

Samples and clinical data

A collaborative study was carried out in 9 French fetal medicine units to identify cases of open spina bifida following combined screening for aneuploidy performed at 11-13 weeks. Maternal serum samples are legally required to be

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kept frozen at −80°C up until after delivery, and cases in which samples were still available were eligible for inclusion in the study. Those known to have a chromosomal abnormality were not included.

Forty-four cases of open spina bifida fulfilled the entry criteria and included 8 cases from the series previously published.[18] In addition, 182 serum samples from unaffected pregnancies that resulted in the birth of a normally grown child at term were used as controls. Up to 5 samples were chosen for each case to have a similar gestational age at sampling and duration of freezing; the median gestation was 88 days for both cases and controls.

Serum samples were thawed and AFP was measured on a simple PLC Kryptor (BRAHMS Thermo Fisher Scientific, Berlin, Germany) using the TRACE technology (time-resolved amplified cryptate emission).[20,21] The linearity extended from 0.23 to 700 ng/mL with a sensitivity of functional 1.2 ng/mL (coefficient of variation [CV] 20%). Testing was carried out without knowing the outcome of pregnancy. Quality assurance comprised both reproducibility on internal quality control (3 levels of control: 11.4, 38.9, and 110 ng/mL) with CVs of 2.3%, 3.4%, and 2.2%, respectively. External monthly quality control (ProBioQual, Lyon, France) aimed at evaluating the interlaboratory variability (CV 2% target at 39.6 ng/mL). Variability assessment was performed during the dosing period on 6 control sera from patients serially measured 5 times. AFP concentrations, ranging from 14.1 ng/mL to 36.1 ng/mL, showed a CV of between 1.4% and 3.7%.

Following established practice, AFP levels were expressed as multiples of the normal median (MoM) value for gestation, using a regression equation derived from the unaffected pregnancies. Results were adjusted for maternal weight, ethnicity, and smoking status.

As part of the combined test, maternal serum free β-hCG and PAPP-A had been measured in 9 of the 55 laboratories accredited by the French health authorities on the basis of internal and external quality control results. Results had been expressed in MoMs using locally derived algorithms.

BPD measurements were available for the 44 open spina bifida cases with serum analyzed here. In addition, 36 cases from our previous study of BPD[18] were added to increase the precision of the model. As previously described, BPD was expressed in MoMs based on the normal median for crown-rump length (CRL), rather than gestation, using a regression equation from 34,951 control pregnancies[22] that underwent ultrasound examination by sonologists certified both nationally and internationally by the Fetal Medicine Foundation. The institutional review board (Comité Consultatifs de Protection des Personnes se Prêtant à des Recherches Biomédicales Ile-de-France no. 12067) approved the study.

Statistical analysis

The MoM median values and distributions of each of the 3 serum markers were compared between spina bifida cases and unaffected pregnancies. Statistical significance was evaluated using the nonparametric Wilcoxon rank sum test with $P < .05$. A 2-tail test was used except for AFP in which a 1-tail test was used because there is prior reason to expect an increase in levels.

Potential performance of the significant serum markers and BPD was estimated by multivariate Gaussian modeling.[23] Model parameters were derived from the distributions of each marker in spina bifida cases and unaffected controls for the serum markers after log transformation. The mean was estimated by the median and the standard deviation by the 10th to the 90th range divided by 2.563. The correlation coefficients were estimated directly after removing outliers exceeding 3 standard deviation from the mean; among serum marker controls, only 42 had BPD measurements. Numerical integration was used whereby the theoretical range is divided into a number of equal sections, thus forming a grid in multidimensional space. The Gaussian distributions are then used to calculate for each section the proportion of spina bifida and unaffected pregnancies that are summed over the range to compute a detection rate for a fixed 5% or 10% false-positive rate. The area under the receiver-operating characteristic (ROC) curve was derived and positive and negative likelihood ratios were computed.

Results

The median maternal weight was significantly smaller in cases than controls (59.0 vs 62.6 kg, $P < .02$). Only 1 case (2.2%) was in a woman of African ethnic origin, whereas there were 37 (20%) in controls. There were 6 smokers among cases (14%) and 14 in controls (7.7%); all smokers were non-African.

The best-fitting normal median AFP concentrations regression equation was $-50.1912 + 0.76195^{*}d$, where d is the gestation in days and the best-fitting MoM equation was $10^{0.32613 - 0.00509^{*}w}$ where w is the maternal weight in kilograms. After weight correction, among controls the median AFP level was 1.24 MoM for women of African ethnic origin and in non-Africans 1.06 MoM for smokers and 0.94 MoM in nonsmokers ($P < .02$ for the 3 groups). These values were used to adjust all AFP MoMs.

The median AFP level in spina bifida cases was 1.201 MoM compared with 1.005 MoM in unaffected pregnancies, a statistically significant difference ($P < .01$, 1 tail). The median free β-hCG was significantly reduced to 0.820 MoM compared with the median in the controls of 0.994 MoM ($P < .02$). PAPP-A levels were not significantly different between cases and controls with medians 1.105 MoM and 1.111 MoM, respectively ($P = .72$). The median BPD in both the 44 cases and the total series of 80 cases was 0.918 MoM ($P < .0001$). For open spina bifida cases, the 95% confidence intervals on the medians for statistically significant markers were as follows: AFP, 1.00-1.44 MoM; free β-hCG, 0.65-1.04; and BPD, 0.88-0.96.

Table 1 shows the model parameters. None of the correlations were statistically significant, although the negative association between free β-hCG and BPD in the unaffected pregnancies approached significance ($P = .06$). All the correlations, regardless of significance, were used in the modeling.