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Very low alpha-fetoprotein in Down syndrome maternal serum screening

Françoise Muller[1]*, Sophie Dreux[1], Corinne Sault[2], Armelle Galland[2], Hugues Puissant[3], Gisèle Couplet[4], Catherine Lemay[5], Marie-Estelle Larcher[6] and Gilles Renom[7], for the ABA Group[†]

[1]*Service de Biochimie, Hôpital Ambroise Paré, Boulogne, France*
[2]*Laboratoire Marcel Mérieux, Lyon, France*
[3]*Génétique, CHU Angers, France*
[4]*Laboratoire Biolille, Lille, France*
[5]*Biologie Endocrinienne et Osseuse, CHU Amiens, France*
[6]*Laboratoire Stahl-Kuntzel, Metz, France*
[7]*Biochimie, Hôpital Calmette, CHRU Lille, France*

Objective To establish the frequency of very low maternal serum AFP and to differentiate congenital AFP deficiency from those diseases known to be associated with low AFP.

Methods AFP values below 2 μg/L and borderline values up to 3 μg/L were retrospectively analysed in 839 773 singleton pregnancies included in a programme for routine screening of trisomy 21 maternal serum markers.

Results Serum AFP was undetectable (≤2 μg/L) in 8 cases, giving a frequency of 1/105 000. The calculated risk of Down syndrome was ≥1/250 in 5 cases. Fetal karyotype was normal. Seven of these pregnancies went to term (39–41 weeks) uneventfully, and birth weight was normal (3050–4110 g). In the 8th case, fetal death occurred at 35 weeks due to severe maternal diabetes. AFP levels between 2.1 and 3.0 μg/L were noted in 7 other cases. The calculated risk of Down syndrome was ≥1/250 in 5 cases, and fetal karyotype was normal. Pregnancies went to term in 4 cases (33–41 weeks), and birth weight was normal (3000–3380 g). In 3 cases, low hCG (<0.6 MoM) was associated with low AFP, and fetal death occurred at 15 to 16 weeks.

Conclusion Once technical errors have been excluded (repeat assay in a second run, calcium assayed to exclude the interference of EDTA for fluorimetric methods, dilution to exclude interfering antibodies, running on an alternative analyser, checking a second sample), very low second-trimester maternal serum AFP should prompt ultrasound examination in order to check fetal viability. Congenital AFP deficiency, an extremely rare disorder (1/100 000), should be suspected. It has no consequences for fetal and infant development, and parents should be reassured. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: prenatal diagnosis; AFP; maternal serum markers; Down syndrome screening; congenital deficiency

INTRODUCTION

Alpha-fetoprotein (AFP) is a serum glycoprotein produced at high levels during fetal life by the liver and the visceral endoderm of the yolk sac and at lower levels by the developing gastrointestinal tract (Mizejewski, 2001). AFP may play a role in fetal immune function and in maintenance of osmotic pressure, but its exact function is unknown. Owing to fetal renal immaturity, AFP filtered through the glomeruli is present in fetal urine and therefore in the amniotic fluid. From here, AFP reaches the maternal serum by two routes. About two-thirds reach maternal serum by transplacental diffusion and the remainder by transamniotic membrane diffusion. The AFP concentration ranges from gram per litre in fetal serum, milligram per litre in amniotic fluid to microgram per litre in maternal serum. Maternal serum AFP levels are lower in trisomy 21–affected pregnancies compared to controls, and this forms the basis for prenatal screening for Down syndrome (Haddow *et al*., 1992; Aitken *et al*., 1993; Wald *et al*., 1997; Palomaki *et al*., 1997; Walton *et al*., 1999; Cuckle, 2000; Spencer, 2000; Muller *et al*., 2002a). Low levels of maternal serum AFP are also observed in trisomy 18, sex chromosome aneuploidies, other less common chromosome anomalies and adverse obstetrical outcome (*in utero* fetal death, stillbirth) or in maternal disease (preeclampsia, diabetes, thyroid disease) (Bennett *et al*., 1979; Davenport and Macri, 1983; Simpson *et al*., 1987; Drugan *et al*., 1989; Greenberg *et al*., 1992; Baschat *et al*., 2002; Muller *et al*., 2002b). In addition, undetectable maternal serum AFP has been described in pregnancies in which the infants presented no anomalies at birth, and congenital AFP deficiency was suspected (Greenberg *et al*., 1992; Sher and Shohat, 1997).

The aim of the present study was to establish the frequency of undetectable maternal serum AFP and to differentiate congenital AFP deficiency from those diseases known to be associated with low AFP.

*Correspondence to: Françoise Muller, Biochimie, Hôpital Ambroise Paré, 92104, Boulogne, France.
E-mail: francoise.muller@apr.ap-hop-paris.fr
† See appendix.

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MATERIAL AND METHODS

The cohort consisted of 839 773 women with singleton pregnancies included in a programme for routine screening of trisomy 21 maternal serum markers, over a five-year period (1997–2001). Data were collected by 35 of the 72 French accredited laboratories. Information collected for each pregnancy included maternal age, gestational age at sampling (14–18 weeks) and pregnancy outcome. Gestational age was estimated by first-trimester ultrasonography in 95% of cases, and underestimated gestational age can therefore be excluded.

All laboratories used AFP as a marker plus total hCG or free ß-hCG, and in some cases estriol was also used (AxSym Abbott, ACS180 Bayer, Centaur Bayer, Kryptor Brahms, RIAgnost CisBio, Amerlex Ortho Clinical Diagnostic, Immulite DPC, Elecsys Roche, Delfia Perkin-Elmer). Median values determined by each manufacturer were used to express each marker as multiples of the median (MoM). Down syndrome risk was calculated on the basis of the combination of the risk due to maternal age and to markers, using the manufacturers' software. For very low AFP values, MoMs were insignificant, and the results were expressed in μg/L. Assays had an AFP detection limit of 2 μg/L, which was therefore used as the cutoff. We also collected borderline values up to 3 μg/L. Two groups were subsequently defined, group A with AFP value $\leq$2 μg/L and group B with AFP value from 2.1 μg/L to 3.0 μg/L. In all cases, assays were repeated in a second run with and without dilution. Calcium was also assayed in order to exclude the interference of EDTA for fluorimetric methods. In all cases, AFP was checked using a second method.

RESULTS

Among the 839 773 pregnancies included in routine trisomy 21 maternal serum screening, 8 presented undetectable levels ($\leq$2 μg/L) of serum AFP, and in 7 others AFP levels were between 2.1 and 3.0 μg/L (Table 1). The frequency of undetectable AFP was 1/105 000.

In group A, hCG was $\geq$2.5 MoM in 2 cases. The calculated risk of Down syndrome was $\geq$1/250 in 5 cases. Pregnancies went to term uneventfully in seven of the eight cases (39–41 weeks), and birth weight was normal (3050–4110 g). In the 8th case, fetal death occurred at 35 weeks due to severe maternal diabetes.

In group B, hCG was $\geq$3.5 MoM in 2 cases and was low (<0.6 MoM) in 4 cases. The calculated risk of Down syndrome was $\geq$1/250 in 5 cases. Karyotyping was normal in all the cases. Pregnancies went to term in four cases (33–41 weeks), and birth weight was normal (3010–3380 g). In 4 cases, in which low hCG was associated with low AFP, fetal death occurred at 15 to 16 weeks in 3 of them.

DISCUSSION

Second-trimester Down syndrome maternal serum screening is widely used, AFP being one of the two or three markers. When risk calculation combining maternal age and markers is over a cutoff (1 in 250 in most cases), amniocentesis for fetal karyotyping is offered to parents. In addition to this group of patients, special attention should be paid to abnormally high (generally >2.5 MoM) or abnormally low AFP values (most often <0.5 MoM). Once assay conditions have been optimised, accurate gestational dating is critical because maternal serum AFP levels normally increase steadily throughout the second trimester. High AFP values determine a group of patients at high risk of neural tube defect, and low AFP values determine a group of patients at high risk of maternal or fetal abnormalities (Stein *et al*., 1981; Haddow *et al*., 1987; Simpson *et al*., 1987; Drugan *et al*., 1989). In the low AFP group, it is necessary to distinguish undetectable values from low values. Undetectable AFP values can be defined as being under 2 μg/L (the detection cutoff defined by most instruments for routine use). Low AFP values can be defined as values between 2 μg/L and under 0.5 MoM. Measurement error must be excluded by reassay, as must errors due to the presence of EDTA or citrate for fluorimetric assays (chelating effect with europium) by measuring calcium. In our series, 8 cases met the definition of undetectable AFP, giving a frequency of 1 in 105 000 pregnancies. When fetal death due to severe unbalanced maternal diabetes was excluded, thriving newborns were observed in seven cases. These observations are comparable with the two cases of congenital AFP deficiency described in the literature (Greenberg *et al*., 1992), emphasising the absence of a major role of AFP in fetal development. Although assay of amniotic fluid AFP was not performed in this study, it would be interesting because low values would support the diagnosis of congenital AFP deficiency. AFP is thought to be related evolutionarily to albumin. Genes for the two proteins are located on chromosome 4, and AFP has a similar molecular weight and structure to albumin. Absence of AFP is most probably a result of a gene deletion similar to that seen with analbuminaemia. Maternal serum hCG was normal, but this hormone of placental origin is independent of AFP synthesis. However, when AFP was between 2 and 3 μg/L, 7 additional cases were observed, and 3 ended in fetal death. Depending on the software and on the presence of a low AFP limit in risk calculation, the calculated risk can be greatly modified explaining why only 10 of the 15 cases have a calculated risk $\geq$1/250. Sher and Shohat (1997) described one case of trisomy 21 associated with undetectable AFP, probably a fortuitous association.

In conclusion, once technical errors have been eliminated (repeat assay in a second run, calcium assayed to exclude the interference of EDTA for fluorimetric methods, dilution to exclude interfering antibodies, running on an alternative analyser, checking a second sample), very low second-trimester maternal serum AFP should prompt ultrasound examination in order to check fetal viability. Congenital AFP deficiency, an extremely rare disorder, should be suspected. As it has no consequence for fetal and infant development, parents should be reassured.

Table 1—Characteristics of 15 cases with undetectable maternal serum AFP

	No.	Maternal age	AFP value (μg/L)	hCG value (MoM)	Risk for trisomy 21	Outcome			
						Gestational age at delivery (weeks)		Weight at birth (kg)	Sex
Group A	1	37	0.00	0.89	1/75	41	Healthy baby	3.300	F
	2	38	0.53	1.09	1/48	35	IUFD	—	M
	3	23	1.04	2.93	>1/10	40	Healthy baby	3.250	F
	4	33	1.05	3.15	>1/10	39	Healthy baby	3.200	M
	5	29	1.12	0.51	1/620	41	Healthy baby	4.110	F
	6	28	1.32	1.13	1/127	39	Healthy baby	3.820	F
	7	22	1.82	0.74	1/434	40	Healthy baby	3.050	F
	8	30	2.00	1.18	1/130	40	Healthy baby	3.930	M
Group B	9	29	2.10	0.40	1/556	15	IUFD before AC	—	
	10	23	2.24	0.57	1/570	16	IUFD before AC	—	
	11	29	2.52	0.22	1/383	41	Healthy baby	3.320	F
	12	30	2.65	4.09	>1/10	40	Healthy baby	3.380	M
	13	31	2.76	0.52	1/230	15	IUFD before AC	—	
	14	33	2.78	5.23	>1/10	40	Healthy baby	3.300	M
	15	29	3.00	1.47	>1/10	33	Healthy baby	3.010	F

AC, Amniocentesis; MoM, multiple of median; IUFD, *in utero* fetal death.
Group A = AFP ≤ 2 mg/L, group B = 2 < AFP ≤ 3 mg/L.

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APPENDIX

ABA study group

This is an association of the French laboratories authorised by the Ministry of Health to carry out Down syndrome screening: Albi (C. Gassier, M. B. Bleuven), Amiens (C. Lemay, N. Roussel-Mizon), Angers (H. Puissant, A. Larget-Pied), Arras (A. Gruson, M. Baillet), Bézier (J. Y. Réal, P. Dumas), Brest (M. P. Moineau, J. F. Morin), Calais (P. Andlauer, E. Gaeremynck), Chambéry (B. Dingeon, C. Doche), Dax (I. Peraud, H. Chahine), Dijon (J. Desgres, M. F. Frigère), Dreux (J. C. Cartron), Le Havre (E. Berreville), Lille (J. M. Perini, G. Renom), Lille (G. Couplet, F. Dancoine, A. Mainardi-Leduc), Lille (P. Jaumain, P. Duchateau), Lons-le-Saulnier (B. Veyrat, A. Piedimonte), Lorient (F. Cornu), Lyon Croix-Rousse (C. Boisson),

Lyon Hôtel Dieu (F. Poloce, M. C. Gelineau), Lyon Mérieux (C. Sault, L. Guilloux, F. Forestier), Marseille (C. Giorgetti, D. Caparros), Marseille (F. Roux), Metz (M. E. Larcher, M. Wasel), Mulhouse (O. Michotey), Nantes (S. Mirallié), Nice (P. Soubiran), Paris A. Paré (F. Muller, S. Dreux), Paris Pitié (M. Bernard, C. Brochet), Paris R. Debré (J. Guibourdanche), Poitiers (C. Millet), Saint-Etienne (H. Dupoizat, P. Guiardiola), Saint-Etienne (N. Rabi, A. Chamson), Toulouse (A. Blanchet, F. Fortenfant), Tours (D. Dudragne, B. Cara), Vitry-le-François (K. Tang).