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FIRST-TRIMESTER BIOCHEMICAL SCREENING FOR DOWN SYNDROME WITH THE USE OF PAPP-A, AFP, AND β-hCG

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SUMMARY

Biochemical screening for Down syndrome (DS) is well established in the second trimester of pregnancy, but there is little information available on its value in the first trimester. This study describes our preliminary results with biochemical screening for DS in the first trimester of pregnancy in order to evaluate its efficacy at this time. Our study population, including 19 DS pregnancies, was evaluated using maternal serum levels of α-fetoprotein (AFP), β-human chorionic gonadotropin (β-hCG), and pregnancy-associated plasma protein A (PAPP-A). At a false positive rate (FPR) of 5 per cent, the detection rate (DR) for DS is 9 per cent for β-hCG, 18 per cent for AFP, and 66 per cent for PAPP-A when considering these parameters individually. With different combinations of the analytes, the best detection rates are obtained with the association of PAPP-A and AFP (85 and 82 per cent DR for a 10 and 5 per cent FPR, respectively). Our data support the value of first-trimester biochemical screening for DS and that of PAPP-A as a single marker.

KEY WORDS: Down syndrome; first trimester; biochemical screening

INTRODUCTION

Several analytes have been shown to be present in abnormally high or low concentrations in the serum of pregnant women whose fetuses are affected by Down syndrome (DS). For some of these analytes, the association is strong enough to form the basis for prenatal screening for DS, which has been shown to be effective in the second trimester of pregnancy (Milunsky, 1992; Haddow and Palomaki, 1993). However, less information is available on its value in the first trimester.

Changes in maternal serum alpha-fetoprotein (MS-AFP) and human chorionic gonadotrophin levels (MS-hCG) in the second trimester of pregnancy have been observed in DS-affected pregnancies (Merkatz *et al.*, 1984; Bogart *et al.*, 1987). The possible association between changes in the levels of these analytes and fetal aneuploidy in the first trimester has also been reported (Milunsky *et al.*, 1984, 1987, 1988; Bogart *et al.*, 1989; Brambati *et al.*, 1986; Johnson *et al.*, 1991; Van Lith, 1992, 1994; Fuhrmann *et al.*, 1993; Crandall *et al.*, 1991, 1993). More recently, depressed maternal serum levels of pregnancy-associated plasma protein A (MS-PAPP-A) and elevated maternal serum levels of free β-hCG (MS-Fβ-hCG) have been described in DS-affected pregnancies in the first trimester (Brambati *et al.*, 1994; Spencer and Macri, 1992; Wald *et al.*, 1992). PAPP-A is detectable in maternal serum by about the 30th day of pregnancy and MS-PAPP-A levels

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increase exponentially until term, then gradually decrease and are undetectable by the sixth postpartum week (Sinosich, 1985). MS-PAPP-A concentrations are significantly reduced in the presence of a fetus with DS, suggesting this as a sensitive marker during the first trimester (Brambati *et al.*, 1991, 1994; Cuckle and Lilford, 1992; Wald *et al.*, 1992; Hurley *et al.*, 1993; Bersinger *et al.*, 1994; Macintosh *et al.*, 1994; El Farra and Grudzinskas, 1995).

MS-Fβ-hCG has also been reported as a useful marker for DS screening in the first trimester, being more sensitive than maternal serum total β-hCG (MS-β-hCG) during this period of pregnancy (Cuckle and Lilford, 1992; Spencer, 1992; Macri *et al.*, 1993; Macintosh *et al.*, 1994; Brambati *et al.*, 1994).

The aim of this study was to examine the value of MS-β-hCG, MS-AFP, and MS-PAPP-A levels in biochemical screening for DS in 19 women carrying a fetus affected by DS during the first trimester of pregnancy in relation to the normal range.

MATERIALS AND METHODS

From 1990 to 1993, maternal serum samples were collected from 1138 women referred to our Unit largely for advanced maternal age (94·4 per cent pregnant women aged more than 35 years), before undergoing first-trimester chorionic villus sampling (CVS). Gestational dating was performed by ultrasound scan in all women prior to sampling. Maternal age has not been considered in this study since in 94.4 per cent of cases it was more than 35 years. First-trimester serum samples of 19 pregnancies carrying a fetus with DS were available for assay.

All chromosomal analyses were carried out at the Cytogenetics Laboratory of our Prenatal Diagnosis Unit. For each week of gestation between 10 and 13 weeks,the medians of the serum analyte levels in continuing chromosomally normal pregnancies ($n \geq 20$ per gestational week) were determined (Table I). The results in DS pregnancies were then compared with this normal range for each gestational week.

MS-β-hCG levels were determined daily by a commercial assay (IMx®) based on microparticle enzyme immunoassay technology. All maternal serum samples were assayed in 1:200 dilutions. The intra-assay coefficient of variation was 6·54 per cent and the inter-assay coefficient of variation 7·03 per cent.

Table I—Median values of the analytes for each gestational week in unaffected pregnancies

Week	AFP (ng/ml)	β-hCG (IU/l)	PAPP-A (IU/l)
10	6·9	117 757	1466
11	9·3	107 075	2457
12	11·5	98 701	3235
13	17·5	73 006	6367

MS-AFP levels were also determined daily by a commercial fluorometric enzyme immunoassay (Stratus®). The intra-assay coefficient of variation was 2·5 per cent and the inter-assay coefficient of variation 4·23 per cent.

MS-PAPP-A levels were determined retrospectively and blindly in 16 cases of DS pregnancies and 20 control cases for each gestational week by radioimmunoassay based on a technique described in detail elsewhere (Sinosich *et al.*, 1982) at the Royal London Hospital, U.K.

The Mann–Whitney *U*-test, with the SPSS statistical analysis package, was used to evaluate the statistical significance of the differences between normal pregnancies and DS pregnancies. All *P* values are two-tailed.

Combinations of arbitrarily chosen cut-off values for two different analytes were used to determine the optimal ratio of detection and false-positive rates.

RESULTS

MS-AFP, MS-β-hCG, and MS-PAPP-A levels in pregnancies with DS fetuses expressed as multiples of the median (MOM) for pregnancies with unaffected fetuses are shown in Table II, whilst Table III shows the means of the MOMs for each of the markers in both DS and normal pregnancies. The mean of the MOM in the DS group was significantly lower for MS-AFP ($\bar{x}$=0·90; P<0·05) and MS-PAPP-A ($\bar{x}$=0·42; P<0·001) and significantly higher for MS-β-hCG ($\bar{x}$=1·38; P<0·001). The means of MOM for gestational weeks and for each analyte are shown in Table IV, excluding the tenth week since only one DS case was available.

Table II—Analyte values expressed as MOM of the normal values for gestational week in 19 Down syndrome pregnancies

Case No.	MA (years)	GA (weeks)	AFP (MOM)	β-hCG (MOM)	PAPP-A (MOM)
1	38	10	1·88	1·34	0·88
2	37	13	0·53	2·09	0·48
3	43	13	1·52	1·33	0·23
4	35	12	0·98	1·03	0·37
5	44	12	0·45	1·41	0·23
6	41	11	0·65	1·33	0·24
7	40	12	0·54	1·55	0·50
8	38	11	1·30	1·25	0·16
9	44	12	0·98	2·20	0·14
10	39	11	0·65	1·04	0·35
11	39	12	0·45	1·48	0·70
12	29	12	0·45	1·45	0·17
13	38	12	1·34	0·65	NA
14	27	13	0·68	1·35	0·68
15	41	13	0·82	1·52	0·11
16	37	13	0·78	1·71	1·48
17	42	11	1·63	1·61	NA
18	39	12	0·54	0·63	NA
19	43	11	1·10	1·21	0·05

MOM=multiple of the median value for unaffected pregnancies of the same gestational age; MA=maternal age; GA=gestational age; NA=not available.

AFP, β-hCG, PAPP-A: in maternal serum, expressed in MOM.

Table III—Mean values of MOMs for maternal serum levels of biochemical markers in Down syndrome pregnancies in the first trimester

Biochemical marker	Mean MOM in normal	Mean MOM in DS	Mann–Whitney (two-tailed probability)
AFP	1·03	0·90	<0·05
β-hCG	1·07	1·38	<0·001
PAPP-A	0·93	0·42	<0·001

A significant correlation was not found between the different analytes: AFP vs. β-hCG ($r=-0·2$, $P=0·66$), AFP vs. PAPP-A ($r=0·22$, $P=0·19$), β-hCG vs. PAPP-A ($r=-0·17$, $P=0·67$).

The potential value of the individual and combined biochemical markers as a screening test for DS in the first trimester was studied. Results obtained with the individual analytes are shown in Fig. 1, using receiver operating characteristic curves (detection rate vs. false positive rate) for tests with varying cut-off values of risk. For a 5 per cent false-positive rate (FPR), the detection rates (DRs) for AFP, β-hCG, and PAPP-A are 18, 9, and 66 per cent, respectively. Assuming a 10 per cent FPR, the DRs for AFP, β-hCG, and PAPP-A are 27, 16, and 73 per cent, respectively.

Table IV—Distribution of mean values for gestational week

Week	AFP (*n*)	β-hCG (*n*)	PAPP-A (*n*)
11	1·07 (5)	1·29 (5)	0·20 (4)
12	0·72 (8)	1·30 (8)	0·35 (6)
13	0·87 (5)	1·60 (5)	0·60 (5)

n=number of DS cases.

Results obtained combining biochemical analytes are shown in Fig. 2. Assuming a 5 per cent FPR, the combined analysis gives a DR of 15 per cent for AFP+β-hCG, 82 per cent for AFP+PAPP-A, and 42 per cent for β-hCG+PAPP-A. For a 10 per cent FPR, the DRs are 24, 85, and 54 per cent, respectively.

DISCUSSION

Earlier screening for DS with the use of maternal serum markers would be desirable for several reasons because of the benefits to be derived from earlier diagnosis. First-trimester screening with MS-AFP levels has been evaluated by some authors (Brambati *et al.*, 1986; Milunsky *et al.*, 1988; Cuckle and Wald, 1988; Brock *et al.*, 1990; Crandall *et al.*, 1991, 1993; Berry *et al.*, 1995; Powell and Grudzinskas, 1996), suggesting that measurement of this analyte is less discriminatory for DS than in the second trimester (Van Lith, 1994). Our results are in agreement with these reports, with a mean of MOM in DS cases of 0·90. The distribution of the means for AFP values shown in Table IV suggests that the effectiveness of AFP increases with gestational age, but the small number of cases precluded any conclusions being possible here.

Previous reports have shown that an increase of hCG in DS pregnancies is observed from 14 weeks' gestation but not earlier (Cuckle and Wald, 1988; Brock *et al.*, 1990; Van Lith, 1992; Crandall *et al.*,

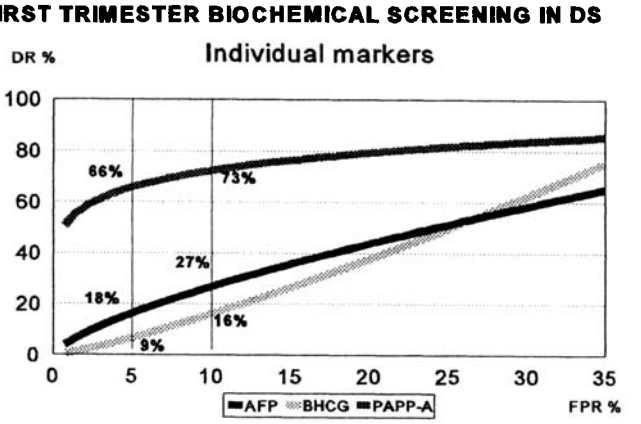

Fig. 1—Receiver operator characteristic curves depicting the variation of the detection rate with the false-positive rate for markers analysed independently (AFP, β-hCG, PAPP-A)

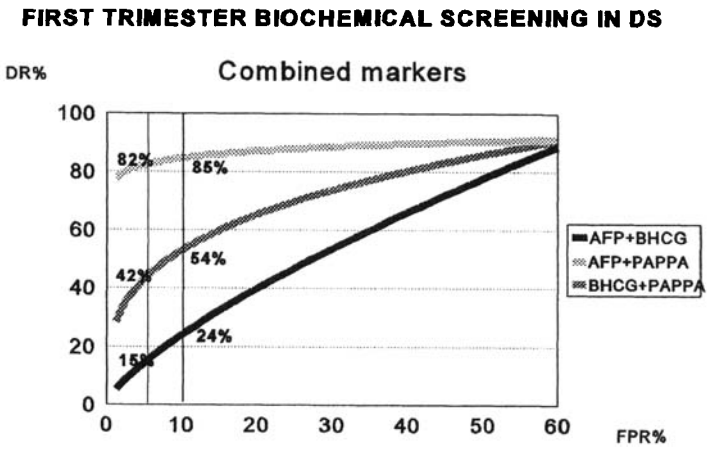

Fig. 2—Receiver operator characteristic curves depicting the variation of the detection rate with the false-positive rate for combined markers: AFP+PAPP-A: AFP≤0·7 MOM and variable cut-off for PAPP-A; β-hCG+PAPP-A: β-hCG≥2·5 MOM and variable cut-off for PAPP-A; AFP+β-hCG: AFP≤0·5 MOM and variable cut-off for β-hCG

1993; Macintosh *et al.*, 1994). One reason may be that since our DS cases were diagnosed by first-trimester CVS, some of the pregnacies may have been predetermined to miscarry and for this reason lower values of β-hCG do not necessarily reflect the chromosomal anomalies (Macintosh *et al.*, 1994). An alternative explanation could be a different contribution in the synthesis of the α and β subunits at this time of pregnancy (Hay, 1988). According to our results, when considered separately, β-hCG levels have little value for screening purposes in the first trimester. However free β-hCG measurements would be of value at this time, according to previous reports (Spencer, 1992; Macri *et al.*, 1993; Aitken *et al.*, 1993; Brambati *et al.*, 1994; Berry *et al.*, 1995). As observed for AFP, the β-hCG value for this purpose seems to increase with gestational age.

PAPP-A has been suggested as the single most effective biochemical marker in first-trimester screening for DS to date. Our findings are in agreement with recent reports showing that PAPP-A concentrations are significantly reduced in cases of DS in the first trimester, but not later in

pregnancy (Cuckle and Lilford, 1992; Wald *et al.*, 1992; Knight *et al.*, 1993; Hurley *et al.*, 1993; Muller *et al.*, 1993; Aitken *et al.*, 1993; Van Lith *et al.*, 1996; Brambati *et al.*, 1994; El Farra and Grudzinskas, 1995). In our experience, PAPP-A is the most sensitive and specific marker for DS in the first trimester, but becomes less discriminatory as pregnancy progresses.

In order to try to improve the detection rates, we combined different analytes since these appeared to be independent of each other. Combining β-hCG with either AFP or PAPP-A did not seem to produce a significant increase in the detection rate. The best detection rates were obtained with the combination of PAPP-A and AFP values. This combination of PAPP-A and AFP increases by 16% the detection rate obtained using only PAPP-A at a 5 per cent FPR (66 per cent for PAPP-A vs. 82 per cent for AFP+PAPP-A).

In conclusion, our data support earlier reports suggesting that PAPP-A is the best single biochemical marker for DS screening in the first trimester of pregnancy. The value of ultrasound markers combined with the use of these analytes is currently being explored in our Unit.

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