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First trimester maternal serum pregnancy-associated plasma protein A and pregnancy-specific β1-glycoprotein in fetal trisomies

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ABSTRACT

Objective To examine the potential value of maternal serum levels of pregnancy-associated plasma protein A (PAPP-A) and pregnancy-specific β1-glycoprotein (SP1) in the detection of fetal trisomy.

Design Cross-sectional study.

Setting The Harris Birthright Research Centre For Fetal Medicine, King's College Hospital Medical School, London, UK and Division of Maternal–Fetal Medicine, Jefferson Medical College, Philadelphia, USA.

Subjects and methods Maternal serum PAPP-A and SP1 concentrations were measured at 10 to 13 weeks gestation in samples from 42 pregnancies with fetal trisomy (trisomy 21, $n = 29$; trisomy 18, $n = 9$; trisomy 13, $n = 4$) and in samples from 210 matched controls.

Results In controls, both maternal serum PAPP-A and SP1 increased significantly with gestation and in trisomic fetuses levels of both hormones were reduced. However, discriminant analysis demonstrated that SP1 did not contribute significantly in the distinction between trisomic and control pregnancies. Although levels of PAPP-A were reduced throughout the gestational range examined (10 to 13 weeks), especially in cases with fetal trisomy 21, the deviation was more pronounced at 10 to 11 weeks than at 12 to 13 weeks gestation. In 45% of pregnancies with fetal trisomy 21 and 70% of pregnancies with trisomies 18 or 13 maternal serum PAPP-A levels at 10 to 11 weeks gestation were below the 5th centile of the normal range.

Conclusion Maternal serum PAPP-A concentration in the first trimester of pregnancy may prove to be useful in the prediction of risk for fetal trisomies.

There is a well-documented association between abnormal maternal serum biochemistry and fetal trisomy 21 in the second trimester of pregnancy (Wald & Kennard 1992). However, the increased demand for early prenatal diagnosis has stimulated the search for first trimester markers (Table 1); there is some evidence that maternal serum levels of pregnancy-associated plasma protein A (PAPP-A) and pregnancy-specific β1-glycoprotein (SP1) are reduced in pregnancies with trisomic fetuses (Brock *et al.* 1990; Wald *et al.* 1992; Brambati *et al.* 1993; Hurley *et al.* 1993; Mackintosh *et al.* 1993; Muller *et al.* 1993).

The aim of the present study was to examine further the potential value of maternal serum levels of PAPP-A and SP1 in the detection of fetal trisomy.

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Subjects and methods

Maternal serum samples were available from 42 pregnancies in which fetal trisomy (trisomy 21, $n = 29$; trisomy 18, $n = 9$; trisomy 13, $n = 4$) was diagnosed at 10 to 13 weeks gestation. Each of these sera was matched for gestation, maternal age and storage time with five samples from mothers with chromosomally normal singleton pregnancies. Gestational age was determined from last menstrual period; in this series only samples from women with regular cycles and certain dates were included.

Maternal blood samples had been taken immediately before chorion villus sampling or early amniocentesis which were performed for parental anxiety or advanced

Table 1. Maternal serum PAPP-A and SP1 in pregnancies with fetal trisomy 21 (Tr21) or trisomies 18 or 13 (Tr18/13) during the first trimester of pregnancy.

		Karyotype							
		Normal		Abnormal					
Study	Gestation (weeks)	*n*	Median (i.u./l)	*n*	Type	Median (MoM)	< median %	< 10th centile %	< 5th centile %
PAPP-A									
Wald & Kennard 1992	9–12	101	—	19	Tr21	0·23	—	63	—
Brambati *et al.* 1993	6–11	445	0·8–1·5	14	Tr21	0·30	86	—	50
Hurley *et al.* 1993	8–12	220	0·6–2·7	7	Tr21	0·33	86	—	—
Muller *et al.* 1993	9–14	66	—	17	Tr21	0·42	—	65	—
Present series	10–11	150	1·7–2·7	20	Tr21	0·47	90	45	40
	12–13	60	2·7–6·0	9	Tr21	0·85	78	22	11
	10–11	150	1·7–2·7	10	Tr18/13*	0·14	100	70	70
	12–13	60	2·7–6·0	3	Tr18/13**	0·14	100	100	100
SP1									
Brock *et al.* 1990	7–14	40	12	21	Tr21	0·79	—	—	—
Mackintosh *et al.* 1993	6–12	662	—	14	Tr21	0·40	71	57	46
				8	Tr18	1·14	38	25	—
Present series	10–11	150	11·7–12·0	20	Tr21	0·94	60	15	15
	12–13	60	12·3–13·7	9	Tr21	0·68	89	44	33
	10–11	150	11·7–12·0	10	Tr18/13*	0·63	80	20	20
	12–13	60	12·3–13·7	3	Tr18/13**	0·75	100	33	33

* Trisomy 18 ($n = 8$), Trisomy 13 ($n = 2$).
** Trisomy 18 ($n = 1$), Trisomy 13 ($n = 2$).

maternal age (median 39 years, 29 to 48 years). Serum was stored at −20 °C, with a maximum of three freeze–thaw cycles. Biochemical analysis was performed in batched runs blind to fetal karyotype.

Immunoassays for PAPP-A and SP1

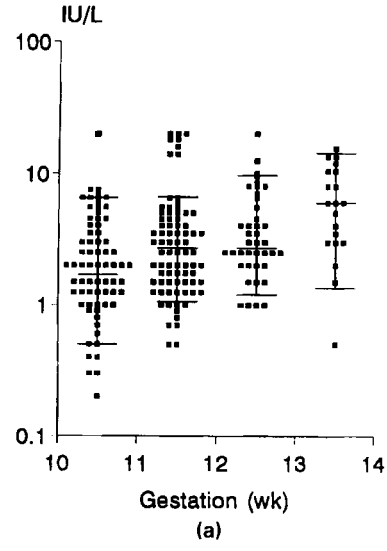
PAPP-A was measured by a double sandwich time-resolved immunofluorometric assay (TR-IFMA) with chelated europium as a label. The antibody used (the only one commercially available, PAPP-A binding immunoglobulin by Dakopatts, Denmark), was a polyclonal rabbit immunoglobulin G in a stabilised solution at 14·3 mg/ml (Batch 11). The purity of the coating antibody was enhanced by negative affinity chromatography over an immobilised normal pooled pregnancy serum fraction obtained by pressure filtration through a 300 kDa cutoff membrane (Sartorius, Germany).

Before immobilisation, the concentrated filtrate presented activities of SP1 and placental lactogen. Very low PAPP-A activity was also detected but no band was seen at 700 to 800 kDa on negative gradient polyacrylamide gel electrophoresis. This absorption purification step removes antibodies against SP1 and haptoglobulin which are present in trace amounts in the anti-PAPP-A preparation and was particularly important in this study because one of the objectives was to examine if SP1 and PAPP-A contribute independently in the detection of fetal trisomies.

Maxisorp microstrips (Nunc, Denmark) were coated overnight with excess absorbed anti-PAPP-A (4 μg/ml) at pH 9·6 in 50 mmol/l sodium carbonate buffer and then blocked with bovine serum albumin (BSA Fraction V, Sigma, USA), 0·5% (w/v) in phosphate-buffered saline (PBS) pH 7·4. Patient sera were diluted 1:5 to 1:20 with PBS containing BSA, 20% (w/v), and nonionic detergent (Emulsit, Japan), 0·05% (v/v). The microstrip wells were washed once after blocking with PBS containing Tween-20, 0·1% (v/v). Then the diluted sera and assay standards (pooled late pregnancy serum calibrated against the international reference preparation 78–610, WHO, France, designated 100 mi.u./ml for PAPP-A), were added in duplicate and the plate was incubated for 2 h at 37 °C.

The tracer was prepared as follows: nonabsorbed anti-PAPP-A immunoglobulin G (2 mg) was incubated with 0·5 mg europium-labelling reagent (Wallac, Finland) in a total volume of 1·25 ml according to the manufacturer's instructions. After overnight reaction at room temperature, the bound label was separated from the free on a column of Sepharose CL-6B (Pharmacia, Sweden). The final tracer, pooled and stored in presence of metal-free BSA (1 mg/ml) in aliquots at −30 °C, had a specific activity of 5×10^9 c.p.s./mg and was used in assay at a 1:100 dilution with DELFIA assay buffer (Wallac, Finland) after filtration through a 0·2-μm membrane. The diluted tracer was added to the washed microstrips (three times with PBS–Tween 20) and incubation was for 1·5 h at 37 °C. The sensitivity of the assay was 0·02 mi.u./ml and the intra- and inter-assay variations were 8·2% and 11·6%, respectively.

SP1 was assayed with a double-antibody microplate enzyme-linked immunosorbent assay developed in our laboratory (NAB) using commercially available reagents.



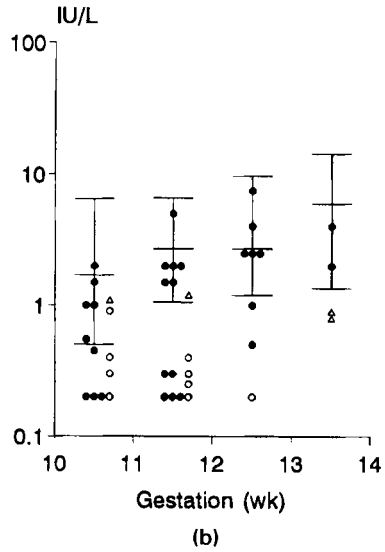
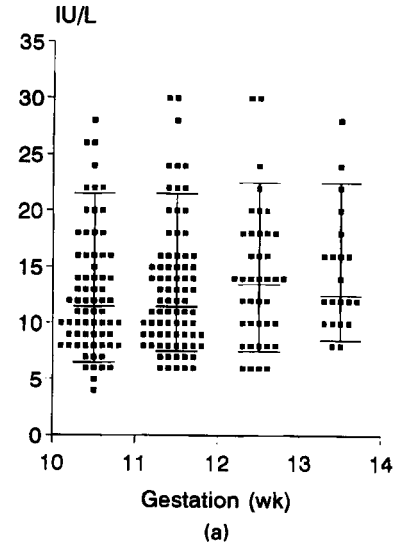


Fig. 1. Maternal serum PAPP-A in control pregnancies (a) increases significantly with gestation ($r = 0{\cdot}354$, $P < 0{\cdot}0001$); (b) the values from pregnancies with trisomy 21 (●), 18 (○) and 13 (△) are plotted on the normal range (median, 10th and 90th centiles).



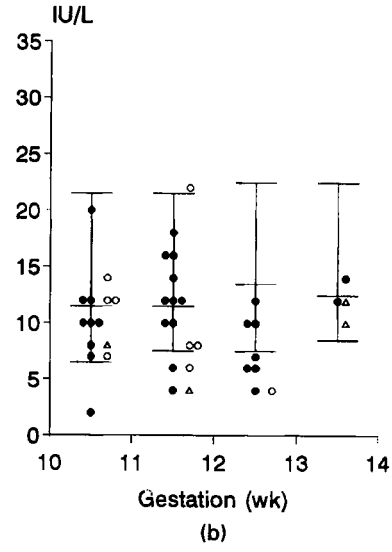


Fig. 2. Maternal serum SP1 in control pregnancies (a) increases significantly with gestation ($r = 0{\cdot}152$, $P < 0{\cdot}05$); (b) the values from pregnancies with trisomy 21 (●), 18 (○) and 13 (△) are plotted on the normal range (median, 10th and 90th centiles).

Maxisorp microtitre plates (Nunc, Denmark) were coated overnight with anti-SP1 immunoglobulin G (Dakopatts, Denmark) at 1·0 μg/ml in 50 mmol/l sodium carbonate buffer (pH 9·6). Postcoating (blocking) buffer, sample incubation buffer, and all incubation times were the same as for PAPP-A. Patient sera were diluted 1:4000, and the tracer used was as a horseradish peroxidase-conjugated rabbit anti-SP1 (Behringwerke, Germany), diluted 1:100 in sample incubation buffer. Plates were then washed four times with PBS–Tween 20 (peroxidase-free, Pierce, USA), the colour reaction was developed with orthophenylene diamine and the signal was read in a Labsystems (Finland) enzyme-liked immunosorbent assay reader. The assay was designed as a replacement of the Behring Enzygnost kit, which has now been withdrawn from the market. The second antibody (conjugate) and the assay standards were the same and, as a consequence, the sensitivity and specificity were identical.

Statistical analysis

Values were expressed as multiples of the median (MoM) of the controls for each week of gestation. Significance of differences between controls and trisomic fetuses were examined using Mann–Whitney test *U*-test (Statistics Package for Personal Computers, P. Royston, Timberlake Clarke Ltd, London). Multiple regression was applied to examine whether SP1 and PAPP-A provided an independent contribution in distinguishing trisomic from control fetuses and to determine the discriminant function. Since measurements of SP1 and PAPP-A in MoM were not normally distributed, multiple regression analysis was performed on log-transformed data with control fetuses assigned the value 1 and trisomic fetuses assigned 0.

Results

In the control group, PAPP-A and SP1 increased significantly with gestational age and in the trisomic pregnancies the values were significantly lower than in the controls (Figs 1 and 2; $z = 6{\cdot}08$, $P < 0{\cdot}0001$ and $z = 3{\cdot}20$, $P < 0{\cdot}01$ respectively). Median values of PAPP-A for trisomy 21, 18 and 13 were 0·53, and 0·07 and 0·28 MoM, respectively and the corresponding values for SP1 were 0·91 and 0·62 and 0·70 MoM, respectively.

In the total population there was a significant association between $\log_{10}$(SP1) and $\log_{10}$(PAPP-A) levels expressed as MoM (Fig. 3; $r = 0{\cdot}334$). Multiple regression analysis demonstrated that $\log_{10}$(SP1) did not contribute independently in the distinction between trisomic and control groups (F to remove SP1 = 3·05, df = 1,250, $t = -1{\cdot}75$, $P = 0{\cdot}081$). Nevertheless, using the classification function [Group = $0{\cdot}132 - 0{\cdot}347 \times \log_{10}$(PAPP-A in MoM) $- 0{\cdot}201 \times \log_{10}$(SP1 in MoM)], 50% of trisomic fetuses would have been identified for a false-positive rate of 8%.

The median maternal serum PAPP-A for trisomy 21 pregnancies at 10 and 11 weeks (0·55 MoM) was significantly lower than at 12 and 13 weeks (0·91 MoM, $z = 2{\cdot}22$, $P < 0{\cdot}05$). Table 2 shows the sensitivity and false positive rate of PAPP-A in the prediction of trisomy 21 and trisomies 21, 18 and 13 using data from the total group and those from pregnancies at 10 and 11 weeks gestation respectively.

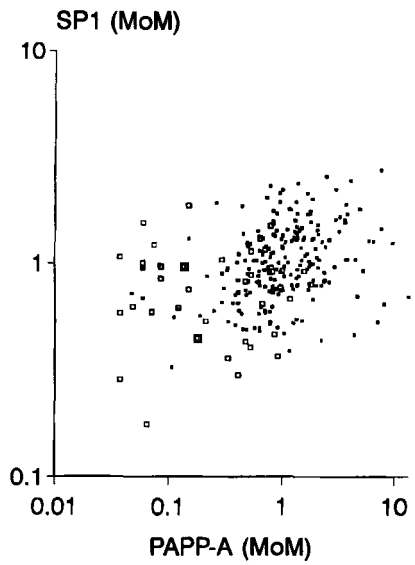


Fig. 3. Association between maternal serum levels of SP1 and PAPP-A in control (■) and trisomic (□) pregnancies.

Discussion

The findings of this study that both maternal serum PAPP-A and SP1 increase with gestation are compatible with those of previous reports and indicate the need to take gestation into account when interpreting results from potentially pathological pregnancies (Brambati *et al.* 1993; Muller *et al.* 1993; Hurley *et al.* 1993).

The finding that maternal serum PAPP-A in the first trimester of pregnancy is significantly lower in trisomic fetuses than in chromosomally normal controls confirms the potential value of this placental protein as a marker for fetal trisomies. However, the deviation from normality is less than in previous studies (Table 1) and, especially for trisomy 21, the deviation seems to decrease with advancing gestation. The latter finding is compatible with previous reports that in the second trimester there is no significant difference in maternal serum PAPP-A between pregnancies with fetal trisomy 21 and controls (Bersinger & Klopper 1984; Knight *et al.* 1993).

Similar to findings for PAPP-A, the mean maternal serum SP1 concentration was found to be significantly lower in trisomic fetuses than in controls. However, multiple regression analysis did not show a significant independent contribution of SP1 in distinguishing between trisomic and control pregnancies after taking into account maternal serum PAPP-A levels. Thus, in cases where measurements of maternal serum PAPP-A are available, little or no extra information is gained by measuring SP1. Nevertheless, an argument in favour of measuring SP1 rather than PAPP-A has been that the assay for SP1 was more robust than the one for PAPP-A. The first trimester serum levels of SP1 found even in trisomic pregnancies are several orders of magnitude above the sensitivity of the assay; this is not the case for PAPP-A which until recently could only be measured by competitive radioimmunoassays, with the drawbacks of short shelf-lives and lability of the iodinated PAPP-A antigen. However, the introduction of a time-resolved fluoroimmunoassay method for the determination of PAPP-A now provides the basis for routine measurement of this marker.

The findings of this study indicate that at 10 to 11 weeks gestation 40% of pregnancies with trisomy 21 and 70% of

Table 2. Percentage of control and trisomic pregnancies with maternal serum PAPP-A (in MoM) below different cut-off levels using data from all pregnancies and pregnancies at 10 and 11 weeks respectively.

	All cases			10 and 11 weeks gestation		
PAPP-A (MoM)	Normal ($n = 210$)	Trisomy 21 ($n = 29$)	Trisomy 21, 18 or 13 ($n = 42$)	Normal ($n = 150$)	Trisomy 21 ($n = 20$)	Trisomy 21, 18 or 13 ($n = 30$)
⩽1·0	51	90	93	50	90	93
⩽0·8	38	72	81	40	85	90
⩽0·6	23	52	64	25	65	73
⩽0·4	10	38	50	11	45	53
⩽0·2	4	28	43	5	40	50

pregnancies with trisomies 18 or 13 have maternal serum PAPP-A levels below the 5th centile of the normal range. Although the population we have studied was of advanced maternal age, it is unlikely that maternal serum PAPP-A in either trisomic or chromosomally normal pregnancies is related to maternal age. For the same false-positive rate (5%), the sensitivity of screening for trisomy 21 by maternal serum PAPP-A at 10 to 11 weeks is potentially higher than that of screening by maternal age (40% compared to 20 to 30%). However, the sensitivity of the first trimester screening by maternal serum PAPP-A is lower than that of second trimester screening by triple biochemistry (50 to 60%, Wald & Kennard 1992). Nevertheless, early diagnosis is preferable to that in the second trimester and therefore screening by maternal serum PAPP-A may gain clinical application, especially if it proves to be complementary to screening by ultrasound for first trimester nuchal translucency (Nicolaides *et al.* 1992).

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