

Frequency and clinical consequences of extremely high maternal serum PAPP-A levels

H. Cuckle^{1*}, S. Arbuzova², K. Spencer³, J. Crossley⁴, G. Barkai⁵, D. Krantz⁶, F. Muller⁷, M. Nikolenko², D. Aitken⁴, T. Hallahan⁶, J. Macri⁶ and P. D. Buchanan⁸

¹*Reproductive Epidemiology, University of Leeds, UK*

²*Interregional Medico-Genetics Centre, Donetsk, Ukraine*

³*Clinical Biochemistry Department, Harold Wood Hospital, Romford, Essex, UK*

⁴*Department of Medical Genetics, Yorkhill Hospital, Glasgow, UK*

⁵*Danek Gertner Institute of Human Genetics, Sheba Medical Center, Tel Hashomer, Israel*

⁶*Research Division, NTD Laboratories Inc., Huntington Station, New York, USA*

⁷*Biochemie, Hôpital Ambroise Paré, Boulogne Cedex, France*

⁸*GeneCare Medical Genetics Center, Chapel Hill, North Carolina, USA*

A multicentre study was carried out to determine the frequency and clinical consequences of extremely high maternal serum pregnancy-associated plasma protein (PAPP)-A. There was a total of 79 pregnancies with PAPP-A exceeding 5.0 multiples of the gestation-specific median in a series of 46 776 pregnancies tested (0.2%) at the 7 collaborating centres. Five pregnancies were lost to follow-up, one miscarried and one with Noonan's syndrome was terminated. Of the remaining 72 that ended in a live birth, one infant had gastroschisis and five pregnancies had obstetric complications: pre-eclampsia, pregnancy-induced hypertension, gestational diabetes and two with growth retardation. Among women with high PAPP-A and no complications or adverse outcomes, there was no evidence of a substantial change in the levels of other Down syndrome markers or the extent of nuchal translucency. Three analytical methods were used to assay PAPP-A and yielded different frequencies of extremely high levels (0.05%, 0.4% and 0.6%) possibly owing to cross-reaction with another substance. We conclude that women with high PAPP-A can be reassured that there is no reason to suppose that the outcome of pregnancy will differ from those with normal levels, provided other markers are normal. If, as more centres move their Down syndrome screening practice to the first trimester, additional cases emerge with Noonan's syndrome or gastroschisis and raised PAPP-A, this advice will need to be modified. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome; screening; maternal serum; extremely high; PAPP-A

INTRODUCTION

In Down syndrome screening programs, the incidental finding of an extremely high or low maternal serum marker level can generate anxiety and uncertainty. Often, the clinical consequence is increased surveillance, which can itself fuel the problem. Recently, a woman undergoing first-trimester screening in Leeds had a maternal serum pregnancy-associated plasma protein (PAPP)-A level of 9.0 multiples of the normal gestation-specific median (MoM). In the absence of aneuploidy, pregnancies with extremely low PAPP-A levels are at high risk of fetal death (Westergaard *et al.*, 1983; Ruge *et al.*, 1990; Brambati *et al.*, 1991; Hurley *et al.*, 1993; Wheeler and Sinosich, 1998; Cuckle *et al.*, 1999; Ong *et al.*, 2000; Smith *et al.*, 2002), but to our knowledge there have been no comparable reports of an adverse outcome in pregnancies with extremely high levels. This case prompted us to carry out a multicentre study in order to provide

information on the chances of having a normal outcome of pregnancy.

METHODS

All singleton pregnancies with maternal serum PAPP-A levels of 5.0 MoMs or higher were sought from 7 centres carrying out first-trimester Down syndrome screening. Testing was carried out with three different assay methods: time-resolved fluorescent assay using a Delfia analyser (Perkin-Elmer, Turku, Finland), time-resolved amplified cryptate emission with a Kryptor analyser (Brahms Diagnostica, Berlin, Germany) and enzyme-linked immunosorbent assay on blood spot (NTD Laboratories, NY, USA). Samples were tested prospectively, but in two of the centres, for all or most of the samples, this was part of a non-intervention research project. In one centre, the series is restricted to samples from referring clinicians who are able to obtain information on the outcome. Each centre expressed results in MoMs using their own regression equations and weight-correction formulae. Gestational age was generally based on ultrasound biometry, but in a proportion of cases menstrual dates were used. Table 1 shows the methods used

*Correspondence to: Professor H. Cuckle, Reproductive Epidemiology, Leeds University, 3 Gemini Park, Sheepscar Way, Leeds LS7 3JB, UK. E-mail: h.s.cuckle@leeds.ac.uk

Table 1—Method, numbers tested and characteristics of high PAPP-A, according to the centre

Centre	Assay	Intervention study	Median age ^a	Gravid I(%)	Tested	PAPP-A \geq 5 MoM
Leeds, UK	Delfia	Yes	36	27	2395	12
Donetsk, Ukraine	NTD labs	Yes	26	27	1857	9
Romford, UK	Kryptor	Yes	30	33	17 013	6
Sheba, Israel	Delfia	Yes	30	51	907	2
Glasgow, UK	Kryptor	No	30	36	16 953	11
	Delfia	Yes	32	N/k	1305	2
Long Island, USA	NTD labs	Yes	33	28	5102	30
Paris, France	Delfia	No	30	23	1244	7

^a At estimated date of delivery.

N/k, Not known—information was not collected.

in each centre, the number of cases tested and their characteristics.

RESULTS

There were 79 pregnancies with PAPP-A exceeding 5.0 MoM in a total of 46 776 tested (0.2%). Of these, 20 had levels of 5.0 to 5.9 MoM, 30 were 6.0 to 9.9 MoM, 14 were 10.0 to 19.9 MoM, 6 were 20.0 to 39.9 MoM and 9 were exceeding 40 MoM. There was one termination of pregnancy, one miscarriage, 72 ended in a live birth and five were lost to follow-up. The termination was carried out because of suspected Noonan's syndrome based on second-trimester ultrasound findings: hydramnios, increased nuchal skin fold and abnormal facies. The results of a post-mortem examination were in agreement with the diagnosis. The maternal serum PAPP-A level was 9.6 MoM, similar to the whole series. The miscarriage was not associated with any congenital abnormality: the PAPP-A level was 7.2 MoM. Among the live births, the only serious abnormality was a case of gastroschisis with a very high PAPP-A level of 66 MoM. There was also one infant with a minor inter-ventricular communication that regressed spontaneously (PAPP-A 8.9 MoM). In each of the five pregnancies, there was an obstetric complication: pre-eclampsia (PAPP-A 6.8 MoM), pregnancy-induced hypertension (6.8 MoM), gestational diabetes (22 MoM) and two with growth retardation (5.7 and 11 MoM), leading to early delivery

at 35 and 34 weeks, respectively. In three cases, ultrasound revealed an unusual obstetric feature, in two there was a spontaneous resolution of triplets to a singleton prior to screening (5.1 and 7.3 MoM) and in the other, the corpus luteum persisted until 17 weeks (253 MoM). The Leeds patient whose 11-week 9.0 MoM PAPP-A level had initiated this study was tested on 3 subsequent occasions and the level remained high: 6.8, 5.1 and 6.0 MoM at 13, 14 and 18 weeks, respectively. Inhibin was also measured in the repeat samples and the levels fell during this period: 1.8, 1.1 and 0.7 MoM, respectively.

The proportion of cases with extremely high PAPP-A levels was about tenfold lower for samples tested with the Kryptor analyser (0.05%) than for the other assays: Delfia (0.4%) or the NTD laboratories method (0.6%). Each of the comparisons with Kryptor was statistically significant (both $P < 0.0001$), but the difference in rates between the other assays did not reach significance ($P = 0.17$).

All maternal serum samples had also been tested for free β -human chorionic gonadotrophin (hCG) and the median level was 0.97 MoM. In addition, 19 samples had been tested for α -fetoprotein (AFP) with a median of 1.26 MoM ($P = 0.17$, 2-tail test) and in 12, the median unconjugated oestriol (uE₃) level was 0.95 MoM. Ultrasound nuchal translucency (NT) had been measured in 56 pregnancies and the median was 0.96 MoM. The complete marker profile for the eight pregnancies with obstetric complications or severe adverse outcomes is shown in Table 2.

Table 2—Marker levels (MoMs) in eight pregnancies with obstetric complications or serious adverse outcomes

Pregnancy	PAPP-A	Free β -hCG	AFP	uE ₃	NT
Gastroschisis	66	0.56	ND	ND	ND
Gestational diabetes	22	0.18	ND	ND	0.82
Intrauterine growth retardation	11	0.25	ND	ND	2.01
Noonan's syndrome ^a	9.6	2.36	1.12	ND	1.10
Miscarriage	7.2	0.56	ND	ND	ND
Pregnancy-induced hypertension	6.8	0.41	ND	ND	0.81
Pre-eclampsia	6.8	2.84	ND	ND	1.86
Intrauterine growth retardation	5.7	0.90	1.03	0.66	0.95

^a A second-trimester sample yielded free β -hCG 1.59 and AFP 1.15 MoM.

ND, Not determined.

The median maternal age at the expected date of delivery was 34, which is older than the average of 31 for all women having first-trimester screening in our centres. Information on gravidity and parity was available for 75 women: 25 (33%) were primigravid, 19 (25%) were gravid 2, 16 (21%) were gravid 3 and 15 (20%) were of higher gravidity. There is a higher proportion of primigravidas than in all women screened in our centres, particularly in view of the older maternal age. Of the 106 previous pregnancies of these women, 59 (56%) ended in births, which is a lower proportion than the 64% for all women tested in four centres where this information was available.

More detailed information on previous obstetric history was available for all except one of the 27 women from 3 centres. Of the 9 primigravid women, 3, aged 22, 28 and 39, were known to have a history of infertility, in the younger 2 lasting for 4 years and involving disturbed periods. Of the 17 multigravidas, 12 (71%) had previous fetal losses, a higher rate than for all screened women in those centres: 36, 46 and 47%.

DISCUSSION

We found that most pregnancies with extremely high maternal serum PAPP-A levels in the first trimester of pregnancy have a generally benign course. There was one miscarriage, and five pregnancies had an obstetric complication. This is not an excessive number; for example, in one of the participating centres, 10% of women having first-trimester screening had a similar complication (Ong *et al.*, 2000).

Two pregnancies were associated with a severe congenital abnormality—Noonan's syndrome and gastroschisis. Since PAPP-A levels have not previously been reported in Noonan's syndrome, it is not possible to judge whether this was a chance occurrence. However, it is noteworthy that other maternal serum markers are known to be abnormal, on average, in this condition. Maternal serum marker levels have previously been reported in two pregnancies with Noonan's syndrome (Aranguren *et al.*, 1996). One was tested at 14 weeks with an hCG level of 5.39 MoM and an AFP level of 0.50 MoM, the other at 16 weeks with levels of 2.28 MoM and 0.77 MoM, respectively. The syndrome is associated with hydropic changes and in one series, two of the three affected pregnancies scanned in the first trimester of pregnancy were found to have nuchal oedema (Nisbet *et al.*, 1999). In our case of Noonan's syndrome, the maternal serum free β -hCG was also raised (2.4 MoM), whilst the AFP level and the NT were normal (both 1.1 MoM).

There are no published reports on PAPP-A levels in gastroschisis. However, one of our centres has screened 7 cases during the first trimester and the median PAPP-A level was 1.0 MoM, which suggests that the current case with extremely raised PAPP-A is a chance occurrence. The median level for other first-trimester markers in this small series was: free β -hCG 1.15 MoM and NT 0.75 MoM; in the current case, the free β -hCG level was 0.56

MoM. In the second trimester of pregnancy, abdominal wall defects tend to have elevated serum marker levels; in one series of 16 second-trimester cases, the median was 6.8 MoM for AFP, 1.6 MoM for uE₃ and 1.7 MoM for hCG (Schmidt *et al.*, 1993).

The large differences in the proportion of samples with extremely high PAPP-A according to the assay techniques do not reflect the relative precision of the methods. The standard deviation of log₁₀ PAPP-A when determined in a large series of unaffected pregnancies was almost identical: using Delfia 0.237 (Tsukerman *et al.*, 1999), Kryptor 0.243 (Spencer *et al.*, 1999) and NTD laboratories 0.230 (Krantz *et al.*, 2000). One possible explanation is that in these pregnancies, there are interfering substances that cross-react with the PAPP-A antibodies in the different assays to a varying extent. For example, it is known that the polyclonal antibody (Daco A230) in the NTD laboratories assay cross-reacts with haptoglobin and Schwangerschafts protein 1 (Bueller and Bersinger, 1989; Bersinger *et al.*, 1995). To examine this further, we retested, using Kryptor, 20 of the samples originally found to have PAPP-A exceeding 5.0 MoM using Delfia, and 9 (45%) remained elevated. When Delfia was used to retest 5 samples originally tested by Kryptor, 3 (60%) remained elevated. It is not possible to test blood spot using Kryptor or Delfia, so a similar comparison between these assays and the NTD laboratories method could not be performed.

Two studies have reported a tendency for PAPP-A levels to increase slightly with both gravidity and parity (de Graaf *et al.*, 2000; Spencer *et al.*, 2000). One of them also examined the number of previous miscarriages and found the same tendency (de Graaf *et al.*, 2000). In our series of women with extremely high PAPP-A levels, there were more primigravidas than expected from the overall population screened locally. The series also had an apparent excess of women with fetal loss, particularly early intrauterine death, in a previous pregnancy.

In our series, one case was identified as having a corpus luteum persisting until 17 weeks and in two others, there had been a multiple pregnancy that resolved to a singleton, possibly retaining corpus lutea. Since not all women had detailed ultrasound examination early in pregnancy, there may be further unidentified cases with corpus luteum involvement. To examine this possibility, we measured progesterone in stored serum samples from women with extremely raised PAPP-A. In the first weeks of pregnancy, progesterone is secreted by the corpus luteum, but by the late first trimester the placenta is the major source of this hormone. We tested 26 women and used published gestation-specific mean values to express each progesterone concentration as a MoM (Dawood, 1976). The median progesterone level was 1.48 MoM (95% confidence interval, 1.35–1.62 MoM). The Leeds patient who initiated this study has, as a consequence, been screened at 11, 13, 14 and 18 weeks' gestation: the progesterone levels in these 4 samples were 4.33, 2.61, 2.16 and 2.45 MoM, respectively. The finding of relatively high progesterone levels in some of the women we have studied may also reflect a general endocrine disturbance perhaps related to the history of infertility and early intrauterine death seen in some cases. Some

causes of raised progesterone, such as adreno-genital syndrome, polycystic ovaries, adjuvant progesterone or other hormonal therapy, were not reported in our series.

In the first trimester, there is a correlation between maternal serum PAPP-A and free β -hCG levels. However, in our series of women with extremely high PAPP-A levels, the median level of free β -hCG was not elevated. This observation lends weight to the view that extremely high PAPP-A identifies a distinct group with a different endocrine profile compared to other women.

To investigate further the connection between PAPP-A and progesterone, we carried out a case-control study using stored serum samples. The cases comprised 13 women with moderately elevated PAPP-A levels, in the range of 2 to 5 MoM, with a median of 2.82 MoM. The controls were 28 women, with a median PAPP-A of 1.06 MoM, chosen to have similar gestational age, maternal age and duration of sample storage to the cases. The median progesterone levels in the cases was 1.30 MoM, a statistically significant increase compared with the controls ($P < 0.001$, Wilcoxon Rank Sum Test). As with the extremely elevated PAPP-A series, there was no difference in the median free β -hCG level between the cases and the controls. In contrast, among the controls, PAPP-A and free β -hCG were correlated with an r -value of 0.39 ($P < 0.05$).

It is well established that low PAPP-A is associated with an adverse outcome of pregnancy. The mechanism for this is not understood, although a plausible suggestion is that it relates to the role of PAPP-A as a protease for insulin-like growth factor (IGF)-binding protein (Smith *et al.*, 2002). Low PAPP-A is expected to result in low free IGF, which is an important determinant of fetal growth and trophoblast invasion. In contrast, high levels of PAPP-A would lead to IGF-binding protein being broken down more rapidly than usual and increased circulating IGF, without any particularly harmful effect.

In the course of a first-trimester Down syndrome screening program, about one-half percent or less of women will have a PAPP-A level exceeding 5.0 MoM. On the basis of our study, such women should be informed that there is no reason to suppose that the outcome of pregnancy will differ from those with normal levels, particularly if other markers are all normal. The only serious adverse outcomes in our series were the cases of Noonan's syndrome and gastroschisis. If similar cases emerge as more centres move their Down syndrome screening practice to the first trimester, this advice will need to be modified.

REFERENCES

- Aranguren G, Garcia-Minaur S, Loridan L, Urribarren A, Vargas LM, Rodriguez-Soriano J. 1996. Multiple-marker screen positive results in Noonan syndrome. *Prenat Diagn* **16**: 183,184.
- Bersinger NA, Zakher A, Huber U, Pescia G, Schnelder H. 1995. A sensitive enzyme immunoassay for pregnancy associated plasma protein A: a possible first trimester method of screening for Down syndrome and other trisomies. *Arch Gynecol Obstet* **256**: 185–192.
- Brambati B, Lanzani A, Tului L. 1991. Ultrasound and biochemical assessment of the first-trimester pregnancy. In *The Embryo: Normal and Abnormal Development and Growth*, Chapman M, Grudzinskas G, Chard T (eds). Springer-Verlag: Berlin; 181–194.
- Bueler MR, Bersinger NA. 1989. Antiserum to pregnancy associated plasma protein A (PAPP-A) recognizes human haptoglobin. *Br J Obstet Gynaecol* **96**: 867–869.
- Cuckle HS, Sehmi IK, Jones R, Mason G. 1999. Low maternal serum PAPP-A and fetal viability. *Prenat Diagn* **19**(8): 788–790.
- Dawood MY. 1976. Circulating maternal progesterone in high-risk pregnancies. *Am J Obstet Gynecol* **125**: 832–840.
- de Graaf IM, Cuckle HS, Pajkrt E, Leschot NJ, Bleker OP, Van Lith JMM. 2000. Co-variables in first trimester maternal serum screening. *Prenat Diagn* **20**: 186–189.
- Hurley PA, Ward RHT, Teisner B, Iles RK, Lucas M, Grudzinskas JG. 1993. Serum PAPP-A measurements in first-trimester screening for Down's syndrome. *Prenat Diagn* **13**: 903–908.
- Krantz DA, Hallahan TW, Orlando F, Buchanan P, Larsen JW, Macri JN. 2000. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* **96**: 207–213.
- Nisbet DL, Griffin DR, Chitty LS. 1999. Prenatal features of Noonan syndrome. *Prenat Diagn* **19**: 642–647.
- Ong CYT, Liao AW, Spencer K, Munim S, Nicolaidis KH. 2000. First trimester maternal serum free β human chorionic gonadotropin and pregnancy-associated plasma protein A as predictors of pregnancy complications. *Br J Obstet Gynaecol* **107**: 1265–1270.
- Ruge S, Pedersen JF, Sorensen S, Lange AP. 1990. Can pregnancy-associated plasma protein A (PAPP-A) predict the outcome of pregnancy in women with threatened abortion and confirmed fetal viability? *Acta Obstet Scand* **69**: 589–595.
- Schmidt D, Rose E, Greenberg F. 1993. An association between fetal abdominal wall defects and elevated levels of human chorionic gonadotropin in mid-trimester. *Prenat Diagn* **23**: 9–12.
- Smith GCS, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. 2002. Early pregnancy levels of pregnancy associated plasma protein A and the risk of intrauterine growth restriction, premature birth, preeclampsia and stillbirth. *J Clin Endocrinol Metab* **87**: 1762–1767.
- Spencer K, Souter V, Tul N, Snijders R, Nicolaidis KH. 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free β -human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* **13**: 231–237.
- Spencer K, Ong CYT, Liao AWJ, Nicolaidis KH. 2000. The influence of parity and gravidity on first trimester markers of chromosomal abnormality. *Prenat Diagn* **20**: 792–794.
- Tsukerman GL, Gusina NB, Cuckle HS. 1999. Maternal serum screening for Down's syndrome in the first trimester: experience from Belarus. *Prenat Diagn* **19**: 499–504.
- Westergaard JG, Sinosich MJ, Bugge M, Madsen LT, Teisner B, Grudzinskas JG. 1983. Pregnancy-associated plasma protein A in the prediction of early pregnancy failure. *Am J Obstet Gynecol* **145**: 67–69.
- Wheeler DM, Sinosich MJ. 1998. Prenatal screening in the first trimester of pregnancy. *Prenat Diagn* **18**: 537–543.