

Abnormal First Trimester Maternal Serum Biochemical Markers and Prediction of Adverse Pregnancy Outcomes

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Objective: To review the obstetric outcomes associated with abnormal first trimester maternal serum markers, including pregnancy-associated plasma protein A and beta-human chorionic gonadotrophin.

Methods: A retrospective review of all singleton pregnancies with first trimester Down syndrome screening by a combination of fetal nuchal translucency thickness, and maternal serum pregnancy-associated plasma protein A and beta-human chorionic gonadotropin done at 11+0 to 13+6 weeks of gestation in a public hospital from 1 July 2010 to 31 December 2011 was conducted. The biochemical markers were converted to multiples of the expected normal median for a pregnancy of the same gestation. The associations between abnormal biochemical markers and adverse pregnancy outcomes, including small for gestational age, preterm delivery, low Apgar score, neonatal intensive care unit admission rate, miscarriage, and stillbirth were studied.

Results: A total of 4367 women were included in the study. Low pregnancy-associated plasma protein A level (<0.4 multiples of the expected normal median) was significantly associated with an increased rate (adjusted odds ratios) of small-for-gestational-age infants (4.8; 95% confidence interval, 2.8-8.2), preterm deliveries (2.0; 1.3-3.2), neonatal intensive care unit admissions (3.1; 1.8-5.3), and stillbirths (7.7; 2.0-29.1), but not low Apgar scores (2.6; 0.8-8.6) or miscarriages (1.3; 0.7-2.6). A low beta-human chorionic gonadotrophin level (<0.4 multiples of the expected normal median) was not associated with any of these adverse outcomes, except in a subgroup analysis of low Apgar score in gestation at or after 37 weeks.

Conclusion: Low pregnancy-associated plasma protein A level was significantly associated with increased rates of small-for-gestational-age infants, preterm deliveries, neonatal intensive care unit admissions, and stillbirths. These results may help in counselling women and consideration of increased fetal surveillance in such cases.

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Introduction

In the first trimester of pregnancy the placentally derived biochemical markers, pregnancy-associated plasma protein A (PAPP-A) and beta-human chorionic gonadotropin (beta-hCG), are increasingly being used in conjunction with ultrasound measurement of nuchal translucency (NT) thickness as part of screening programmes for trisomy 21 and other aneuploidies.

Independent of the presence of aneuploidy, women undergoing biochemical screening and are found to have markedly reduced PAPP-A levels in the first trimester are increasingly recognised as being at increased risk for other pregnancy complications. Such adverse outcomes include

miscarriage, preterm delivery, small-for-gestational-age (SGA) infant, low Apgar score of <7 at 5 minutes, neonatal intensive care unit (NICU) admission, and stillbirth¹⁻¹⁶. The results for low beta-hCG levels and other pregnancy complications are more controversial¹⁻⁵.

The association between PAPP-A or beta-hCG levels and various adverse obstetric outcomes has been explained by the fact that both hormones are produced in

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the placenta soon after implantation, and low levels could possibly reflect abnormal placentation. This may, in turn, account for or be associated with adverse obstetric and neonatal outcomes³⁻⁷.

Most studies addressing this issue have concentrated on outcomes of pregnancies where PAPP-A and beta-hCG levels are less than the fifth centile of a normal population (approximately 0.41 multiples of the expected normal median [MoM])¹⁻⁸. However, similar studies have not been reported within the Chinese population, which is especially relevant as it is well known that the normal range of serum markers varies among different populations^{1-11,13-16}.

This study aimed to assess whether low levels of PAPP-A or beta-hCG, measured as part of first trimester screening for chromosomal aneuploidies, were related to increased risk of SGA infants, preterm deliveries, low Apgar scores, NICU admissions, and stillbirths in a Chinese population.

Methods

This was a retrospective cohort study of all singleton pregnant women who had undergone first trimester Down syndrome screening by a combination of fetal NT thickness, and maternal serum PAPP-A and beta-hCG levels. Screening was performed between 11+0 and 13+6 weeks of gestation in a public hospital from 1 July 2010 to 31 December 2011.

This study included only women for whom there was complete information on serum markers and primary outcome measures, including fetal loss (miscarriage or stillbirth), birth weight, maturity at delivery, Apgar score, and NICU admission. Women with pregnancies with abnormal karyotype, multiple pregnancies, blood sample taken before 11+0 weeks or after 13+6 weeks of gestation, type 1 or 2 diabetes, and non-Chinese ethnicity were excluded from the final analysis.

Miscarriage was defined as fetal loss before 24 completed weeks of gestation, and stillbirth as delivery of a fetus with no cardiac activity after 24 weeks. Fetal loss included all miscarriages and stillbirths. Small for gestational age was defined as birth weight of less than the 10th centile for gestational age. The study was reviewed and approved by the local research ethics committee.

All pregnant women who accepted first trimester screening test had a blood sample taken between 11+0 and 13+6 weeks. All the serum samples were analysed

at a single laboratory (Tsan Yuk Hospital), and levels of PAPP-A and beta-hCG were measured using the Kryptor analyser (Thermo Fisher Scientific, Waltham [MA], US). The levels of the biochemical markers were converted into MoMs by expressing the absolute concentration relative to the median value of the gestational age on the day of blood sampling. The MoM values were not calculated until the gestational age was determined by crown-rump length (CRL) at the first trimester scan. Furthermore, the MoM values were corrected for maternal weight, as high maternal weight is known to be associated with low PAPP-A and beta-hCG levels. In the analyses, PAPP-A and beta-hCG MoM values were both included as continuous variables and dichotomised by a cut-off at 0.4 MoM (corresponding to the fifth percentile). Data regarding the analyses of PAPP-A and beta-hCG levels were obtained from the Tsan Yuk Hospital Down syndrome screening database. An ultrasound examination was performed during 11+0 to 13+6 weeks of gestation and, at this time, the gestational age of the fetus was estimated by means of the CRL using the formula of Robinson and Fleming¹⁷. The gestational age determined at this scan was used to calculate the expected date of delivery and thereby the gestational age at delivery.

Outcome information was obtained through the Obstetrics Clinical Information System, which is part of the Clinical Data Analysis and Reporting System, where all obstetric patients' data are recorded. This system is used in all hospitals under the Hospital Authority in Hong Kong for recording, data retrieval, statistics, and audit. Women were considered lost to follow-up when they delivered in other private hospitals and details of the pregnancy outcome were not known.

Information for other potential explanatory variables (maternal age, weight, parity, and maternal lifestyle factors such as smoking and alcohol consumption) was obtained from questionnaires completed by the pregnant women.

Dichotomised data were analysed by linear logistic regression. Results for PAPP-A and beta-hCG levels in relation to SGA, preterm delivery, low Apgar scores, NICU admission rate, miscarriage, and stillbirth were presented as adjusted odds ratios (adjusted ORs) with 95% confidence interval (95% CI).

To investigate whether a potential association between the placental hormones and neonatal outcomes could be due to the newborns being born preterm, we performed all the analyses for the entire study population

and then only for newborns with gestational ages of more than 37 weeks at birth. The prevalences of various adverse outcomes, including spontaneous miscarriage, stillbirth, SGA, prematurity, low Apgar scores, and NICU admission were determined from the available data.

Results

A total of 5979 women were recruited into the first trimester Down syndrome screening programme during the study period. Of them, six were confirmed to have abnormal karyotypes, 11 had multiple pregnancies, 12 had pre-existing type 1 or type 2 diabetes, 514 were of non-Chinese ethnic origin, 971 had incomplete obstetric outcome information, and 98 were subsequently noted to have date problem with gestation not eligible for first trimester Down's screening. After all the exclusions, 4367 pregnancies were investigated.

Of these 4367 pregnancies, 204 (4.7%) had low PAPP-A level, and 190 (4.4%) had low beta-hCG level. There were 128 (2.9%) infants requiring NICU admission, 27 (0.6%) with low Apgar scores, 265 (6.1%) preterm deliveries, 100 (2.3%) SGA infants, 148 (3.4%) miscarriages, and 11 (0.3%) stillbirths. Of the 971 women with incomplete data, five had low PAPP-A levels and six had low beta-hCG levels.

All miscarriages and stillbirths were excluded

during subgroup analysis of NICU admission, low Apgar scores, and preterm delivery as these did not provide clinical outcomes. Only miscarriages were excluded from the stillbirth data and vice versa for the miscarriage data.

Neonatal Intensive Care Unit Admission

There were 128 (2.9%) NICU admissions. An increased rate of NICU admissions was associated with low PAPP-A level (adjusted OR, 3.1; 95% CI, 1.8-5.3) [Table 1], but not with low beta-hCG level. The difference in PAPP-A level was also significant when applied only to those delivered at gestational week 37 or later (adjusted OR, 3.5; 95% CI, 1.6-7.9).

Low Apgar Score

A total of 27 (0.6%) infants had low Apgar scores. A significant increased rate of low Apgar score was associated with low beta-hCG level when applied to infants delivered at or after 37 weeks of gestation (adjusted OR, 8.8; 95% CI, 1.7-45.8). Low PAPP-A level was not found to be associated with a low Apgar score of <7 at 5 minutes (adjusted OR, 2.6; 95% CI, 0.8-8.6) [Table 1].

Small for Gestational Age

Using the 10th centile as a cut-off measurement, there were 100 (2.3%) SGA newborns. A significant increased rate of SGA newborns was associated with low PAPP-A level (adjusted OR, 4.8; 95% CI, 2.8-8.2)

Table 1. Neonatal intensive care unit admissions, low Apgar scores, and small-for-gestational-age infants after delivery of women with different levels of PAPP-A and beta-hCG*

	PAPP-A				beta-hCG			
	All deliveries (n=4367)		≥37 Weeks (n=4108)		All deliveries (n=4367)		≥37 Weeks (n=4108)	
	<0.4 MoM (n=204)	≥0.4 MoM (n=4163)	<0.4 MoM (n=182)	≥0.4 MoM (n=3926)	<0.4 MoM (n=190)	≥0.4 MoM (n=4177)	<0.4 MoM (n=180)	≥0.4 MoM (n=3928)
NICU admissions	16 (7.8%); 3.1 (1.8-5.3) p<0.001	112 (2.7%)	7 (3.8%); 3.5 (1.6-7.9) p=0.002	44 (1.1%)	7 (3.7%); 1.3 (0.6-2.8) p=0.53	121 (2.9%)	4 (2.2%); 1.9 (0.7-5.3) p=0.23	47 (1.2%)
Low Apgar scores	3 (1.5%); 2.6 (0.8-8.6) p=0.13	24 (0.6%)	1 (0.5%); 2.8 (0.4-30.1) p=0.24	6 (0.2%)	3 (1.6%); 2.8 (0.8-9.3) p=0.10	24 (0.6%)	2 (1.1%); 8.8 (1.7-45.8) p=0.01	5 (0.1%)
SGA infants	18 (8.8%); 4.8 (2.8-8.2) p<0.001	82 (2.0%)	14 (7.7%); 4.7 (2.6-8.6) p<0.001	68 (1.7%)	7 (3.7%); 1.7 (0.8-3.7) p=0.19	93 (2.2%)	7 (3.9%); 2.1 (0.9-4.6) p=0.07	75 (1.9%)
Preterm deliveries	22 (10.8%); 2.0 (1.3-3.2) p=0.003	243 (5.8%)	-	-	10 (5.3%); 0.9 (0.5-1.7) p=0.69	255 (6.1%)	-	-

Abbreviations: beta-hCG = beta-human chorionic gonadotrophin; MoM = multiples of the expected normal median; NICU = neonatal intensive care unit; PAPP-A = pregnancy-associated plasma protein A; SGA = small for gestational age

* Data are shown as No. (%), odds ratios (interquartile range), and p values

[Table 1]. The beta-hCG level was not shown to have any statistical correlation with SGA newborns.

Preterm Delivery

A total of 265 (6.1%) preterm deliveries was found. Significant increased rate of preterm deliveries was associated with low PAPP-A level (adjusted OR, 2.0; 95% CI, 1.3-3.2), but not with low beta-hCG level (Table 1).

On further analysis of these 265 individual case notes, eight (3.0%) had intrauterine growth restriction (IUGR), of which only one was born by iatrogenic preterm delivery, i.e. induction of labour at 34 weeks for a fetus with IUGR with abnormal Doppler ultrasound results. The other seven deliveries were due to spontaneous preterm labour or preterm premature rupture of membrane.

Miscarriage

A total of 148 (out of 4515; 3.3%) deliveries were due to miscarriage. Low PAPP-A (adjusted OR, 1.3; 95% CI, 0.7-2.6) or beta-hCG levels (adjusted OR, 0.8; 95% CI, 0.3-1.9) were not associated with increased risk of miscarriage [Table 2].

Stillbirth

There were 11 (out of 4378; 0.3%) stillbirths. A significant increased rate of stillbirth was associated with low PAPP-A level (adjusted OR, 7.7, 95% CI, 2.0-29.1) [Table 2]. The beta-hCG level was not statistically correlated with stillbirth.

Discussion

In this retrospective study of 4367 pregnancies, we found that low PAPP-A level was significantly associated with an increased rate (adjusted OR) of SGA infants

(4.8), preterm deliveries (2.0), NICU admissions (3.1), and stillbirths (7.7), but not with low Apgar scores or miscarriages. Low beta-hCG level was not associated with any of these adverse outcomes except in a small subgroup of infants with low Apgar scores delivered after 37 weeks of gestation (8.8).

Our study results regarding PAPP-A level are comparable to studies in western populations¹⁻¹⁵. However, the non-significant results for low Apgar scores and miscarriage might be due to the small number of cases with abnormal outcomes in this study causing statistical inadequacy. A larger study with more participants might help in eliminating this error.

It was found that the risk of newborns being admitted to the NICU was increased if the placental biochemical marker PAPP-A level was <0.4 MoM. The same association was established in deliveries at or after 37 weeks of gestation, which could further support that the increased rate of NICU admissions was not solely due to the effects of prematurity. On reviewing individual case records, it was noted that only one of the 265 preterm deliveries was iatrogenic due to intervention for IUGR, and all the others were due to spontaneous preterm labour.

In contrast to the results for PAPP-A level, beta-HCG level was not shown to be significantly associated with adverse pregnancy outcomes in our study, except in a small subgroup analysis of infants with low Apgar scores delivered at or after 37 weeks of gestation. Indeed, the same observation has been made in other reports, and hypotheses have been postulated to explain these discrepancies¹⁻⁵.

Both PAPP-A and beta-hCG are produced by syncytiotrophoblasts. It seems likely that these

Table 2. Miscarriages and stillbirths in women with different levels of PAPP-A and beta-hCG*

Variable [†]	PAPP-A		beta-hCG	
	<0.4 MoM	≥0.4 MoM	<0.4 MoM	≥0.4 MoM
Miscarriage	9/213 (4.2%); 1.3 (0.7-2.6) p=0.43	139/4302 (3.2%)	5/195 (2.6%); 0.8 (0.3-1.9) p=0.57	143/4320 (3.3%)
Stillbirth	3/207 (1.4%); 7.7 (2.0-29.1) p=0.003	8/4171 (0.2%)	0/190 Odds ratio NA p=1.0	11/4188 (0.3%)

Abbreviations: beta-hCG = beta-human chorionic gonadotrophin; MoM = multiples of the expected normal median; NA = not applicable; PAPP-A = pregnancy-associated plasma protein A

* Data are shown as No. (%), odds ratios (interquartile range), and p values

† Stillbirth data were excluded from the miscarriage data and miscarriage data were excluded from the stillbirth data

different patterns of association may reflect different pathophysiological mechanisms relating to first trimester trophoblast function and later adverse pregnancy outcomes. The fact that the strength and pattern of the associations differed for the two trophoblast-derived proteins suggests that PAPP-A is not acting as a simple marker of the volume of the trophoblast, but that the association reflects a specific property of PAPP-A in the physiological regulation of trophoblastic function. Pregnancy-associated plasma protein A has been identified as a glycoprotein protease that acts on insulin-like growth factor (IGF)-binding protein (IGFBP), specifically IGFBP-4. Once IGF-1 and IGF-2 are released from their IGFBPs, they promote fetal growth and development through metabolic and differentiation pathways. This provides a biological rationale for PAPP-A influencing fetal-placental growth and development, particularly for an association between low PAPP-A level and poor pregnancy outcome¹⁻¹⁶.

The strength of our study lies in the fact that our prenatal screening programme covers approximately 90% of the population booked at our unit and is free of charge. Accordingly, our study population is highly representative without an oversampling of high-risk pregnancies. However, this is a retrospective study and like all retrospective reviews, it could be subjected to collection and interpretation errors, and bias. The size of our study population made it difficult to study rare, but important, neonatal outcomes such as neonatal death or hypoxic ischaemic encephalopathy. We chose a cut-off of 0.4

MoM for PAPP-A and free beta-hCG levels because this corresponds roughly to the fifth centile, and this cut-off has most often been used in previous studies¹⁻⁸. A lower cut-off could have been relevant, and more clinically applicable, because of a lower screen-positive rate, but the size of our population did not allow us to investigate this cut-off properly.

From previous studies¹⁻¹⁶ and this present study, an unexplained low PAPP-A level (<0.4 MoM) in the first trimester is associated with an increased frequency of adverse obstetrical outcomes but, at present, no specific protocols for monitoring and intervention are available. In our department, we have been performing universal first trimester combined screening for Down syndrome. The proportion of women with low PAPP-A or beta-hCG levels was around 9.0% (394/4367). At present, if the PAPP-A or beta-hCG level is very low (<0.2 MoM), a mid-trimester detailed scan followed by fetal assessment (growth scan plus Doppler ultrasound studies of the umbilical artery and middle cerebral artery) at 28 to 30 weeks will be scheduled after counselling the women. These measures might help in detecting intrauterine growth restriction in the at-risk population.

Conclusion

Low PAPP-A level was significantly associated with adverse neonatal outcomes. These results may be crucial in counselling women and consideration of increased fetal surveillance in such cases.

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