Pregnancy-Associated Plasma Protein A and Pregnancy Outcomes

Viorica Radoi, MD, L.C. Bohiltea, MD
Genetic Department, UMF Carol Davila

Correspondence:
Viorica Radoi
e-mail: viorica.radoi@yahoo.com

Abstract

Objectives. *The purpose of this study is to assess the relationship between the first-trimester maternal serum pregnancy-associated plasma protein-A (PAPP-A) levels and pregnancy complications.*
Materials and methods: *The risk of adverse perinatal outcome among 484 women recruited to Life Memorial Hospital, in a prospective nonintervention cohort study was related to maternal circulating concentrations of trophoblast-derived proteins at 10-13+6 wk gestation.*
Results: *In this study, we demonstrate that maternal circulating concentrations of PAPP-A at 10-13+6 wk gestation are significantly predictive of adverse perinatal outcome in later pregnancy. Women with a pregnancy-associated plasma protein A (PAPP-A) in the lowest fifth percentile at 10-13+6 wk gestation had an increased risk of intrauterine growth restriction, premature delivery, preeclampsia and stillbirth. In contrast, levels of free ß-human CG, another circulating protein synthesized by the syncytiotrophoblast, were not predictive of later outcome. PAPP-A has been identified as a protease specific for IGF binding proteins.*
Conclusions: *Control of the IGF system in the first and early second trimester trophoblast may have a key role in determining subsequent pregnancy outcome.*
Keywords: *Pregnancy-Associated Plasma Protein A, Insulin-like growth factor, adverse pregnancy outcomes*

PAPPA is a member of the metzincin family of metalloproteases, known as a sensitive marker of adverse pregnancy outcomes. Metalloproteinase pregnancy-associated plasma protein A is a critical growth regulatory factor during fetal development. PAPPA increases IGF bioavailability and mitogenic effectiveness in vitro through regulated cleavage of IGF-binding protein 4 (IGFBP4).

IGF-1 consists of 79 amino acids in a single chain with three intramolecular disulfide bridges. IGF-1 has a molecular weight of 7649 Daltons. IGF-1 is produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion. Production is stimulated by growth hormone and can be retarded by undernutrition, growth hormone insensitivity, lack of growth hormone receptors, or failures of the downstream signaling pathway post GH receptor including SHP2 and STAT5b. Approximately 98% of IGF-1 is always bound to one of 6 binding proteins (IGF-BP).

Its primary action is mediated by binding to specific IGF receptors present on many cell types in many tissues. The signal is transduced by intracellular events. IGF-1 is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication and a potent inhibitor of programmed cell death. Almost every cell in the human body is affected by IGF-1, especially cells in muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs. In addition to the insulin-like effects, IGF-1 can also regulate cell growth and development, especially in nerve cells, as well as cellular DNA synthesis. IGF-1 is closely related to a second protein called "IGF-2". IGF-2 also binds the IGF-1 Receptor. However, IGF-2 alone binds a receptor called the "IGF II Receptor" (also called the Mannose-6 phosphate receptor). The insulin growth factor-II receptor (IGF2R) lacks signal transduction capacity, and its main role

is to act as a sink for IGF-2 and make less IGF-2 available for binding with IGF-1R. As the name "insulin-like growth factor 1" implies, IGF-1 is structurally related to insulin, and is even capable of binding the insulin receptor, albeit at lower affinity than insulin.

The insulin-like growth factors (IGF1 and IGF2) are important determinants of fetal growth and postnatal development (Baker et al., 1993; Stewart and Rotwein, 1996). IGF bioactivity is modulated by a family of six IGF-binding proteins (IGFBPs) (Firth et al., 2002), the structure and function of which can be regulated by specific IGFBP proteases (Wetterau et al., 1999; Bunn and Fowlkes, 2003). Recently, pregnancy-associated plasma protein A (PAPPA) was identified as a novel zinc-binding metalloproteinase secreted by normal human fibroblasts with IGFBP4 as its substrate (Lawrence et al., 1999). IGFBP4 is an inhibitor of IGF action and, in this capacity, may serve as a pericellular reservoir for IGFs (Mohan et al., 1989; Pintar et al., 1998). Cleavage of IGFBP4 by PAPPA results in increased bioavailability and mitogenic effectiveness of IGFs in vitro (Conover et al., 1995; Byun et al., 2001; Ortiz et al., 2003). Along with fibroblasts, PAPPA proteolytic activity has been identified in cultured osteoblasts (Conover et al., 1995; Qin et al., 2000; Ortiz et al., 2000), vascular smooth muscle cells (Bayes-Genis et al., 2001a) and ovarian granulosa cells (Conover et al., 2001).

The maternal serum level of PAPPA increases exponentially until term. PAPPA is found in the ovarian follicles, follicular fluid, luteal cells, and fallopian tubes of non-pregnant women and in the seminal vesicles and seminal fluid of males. Because low serum levels of PAPPA have been demonstrated in first-trimester pregnancies associated with chromosomally abnormal fetuses, PAPPA has been suggested as a potential biochemical marker for such pregnancies. Westergaard et al. (1983) found complete absence of PAPPA in both maternal serum and placental tissue from a pregnancy with Cornelia de Lange syndrome.

Furthermore, increased PAPPA expression in vivo has been shown to be associated with conditions of heightened IGF activity, such as neointimal hyperplasia following balloon angioplasty of pig coronary arteries (Bayes-Genis et al., 2001a), active atherosclerotic plaques in human coronary arteries (Bayes-Genis et al., 2001b), and healing human skin (Chen et al., 2003). Although PAPPA was originally described as a protein of placental origin circulating in human pregnancy, these data indicate additional roles for PAPPA, outside of pregnancy, in localized and finely controlled growth states.

The IGF family is believed to be important in implantation and placental physiology. PAPP-A recently has been identified as an IGFBP-4 protease, and the IGFBP-4 is the second most abundant IGFBP in the placental bed. At the maternal-fetal interface, PAPP-A proteolyze IGFBP-4 and thus increase IGF bioavailability locally in the placenta to promote IGF-2-mediated trophoblast invasion into maternal deciduas and to modulate IGF regulation of steroidogenesis and glucose and amino acid transport in the villus. In human pregnancy, the elevated levels of PAPP-A and IGFBP-4 proteolysis are normally detected in the maternal circulation in early gestation.

Therefore, low maternal PAPP-A serum levels in the first trimester are supposed to be an early sign of placentation defect during the implantation processes.

The 1547-residue PAPP-A polypeptide is secreted as a disulfide-bound dimer of 400 kDa. PAPP-A contains the elongated zinc-binding motif (HEXXHXXGXXH) and belongs to the metzincin superfamily of metalloproteinases, also including the astacins, the reprolysins, the serralysins, and the matrix metalloproteinases. As it cannot be grouped into any of these families, PAPP-A is the founding member of a fifth metzincin family, the pappalysins, which also include the recently discovered PAPP-A2. In addition to a proteolytic domain, the PAPP-A subunit contains three lin-notch repeats (LNR-1-3, each of 26-27 residues) and five complement control protein modules (CCP-1-5, each of 57-77 residues). The LNR module is known from the Notch-related receptors, which play important roles in the regulation of developmental programs. Of particular interest, the pappalysins are the only known proteins, besides the Notch receptors, in which LNR modules are present. The CCP modules, also known as short consensus repeats, are found in several other proteins, in particular proteins of the complement system. It has been recently found that CCP-3 and CCP-4 mediate cell surface adhesion of PAPP-A.

PAPP-A was first isolated from the serum of pregnant women, where its concentration rises throughout gestation. Interestingly, depressed circulating levels of PAPP-A antigen correlate with Down's syndrome and low birth weight. In pregnancy serum, the vast majority of PAPP-A (>99%) is covalently bound in a 2:2 complex to the 206-residue proform of eosinophil major basic protein, pro-MBP . The subunits of the 500-kDa PAPP-A·pro-MBP complex can be separated from each other only after denaturation and reduction. The mature 117-residue MBP polypeptide is well known from granules of the eosinophil leukocyte, where it functions as a cytotoxic effector molecule, but no evidence suggests that MBP is generated from pro-MBP of the PAPP-A·pro-MBP complex. Rather, pro-MBP functions as a proteinase inhibitor of PAPP-A, whose mechanism of inhibition is currently unknown.

The PAPP-A subunit contains 82 cysteine residues, but their pairing cannot be predicted by comparison to other proteins with known disulfide structure, as PAPP-A shows global similarity only to the recently discovered PAPP-A2; the disulfide structure of PAPP-A protein modules (LNRs and CCPs) cannot be inferred by similarity, because other similar modules have unknown cysteine pairing or differ from the modules of PAPP-A. The pro-MBP subunit contains a total of 12 cysteines

First-trimester maternal serum pregnancy-associated plasma protein-A (PAPP-A) has been reported to be an important biochemical marker in prenatal screening of fetal Down syndrome.

Intrauterine growth restriction and preterm birth are major determinants of perinatal morbidity and mortality. Much of routine prenatal care involves detecting women at increased risk of these adverse events and targeting intensive monitoring and interventions. Standard reviews of fetal physiology suggested that variation in human fetal growth was largely a phenomenon of the second half of pregnancy, and it is during this phase of pregnancy when women receive the bulk of prenatal care. However, a previous study showed that embryos and fetuses that were smaller than expected in the first trimester of pregnancy were more likely to have pregnancy complications, including intrauterine growth restriction and preterm birth. In addition to the fetal growth restriction, several reports demonstrated that low maternal serum PAPP-A levels were associated with adverse pregnancy outcomes, such as miscarriage, preeclampsia, and stillbirth. According to the most recent study by Smith et al, low concentrations of PAPP-A during the first trimester were associated with an earlier onset of spontaneous labor at full term.

Objectives

The purpose of this study is to assess the relationship between the first-trimester maternal serum pregnancy-associated plasma protein-A (PAPP-A) levels and pregnancy complications.

Materials and methods

Blood samples were obtained from women between 10 and 13+6 wk gestation, attending Life Memorial Hospital, as part of a prospective, nonintervention study on combined ultrasound and biochemical screening for Down's syndrome.

Data collection

We used data from the Combined Ultrasound and Biochemical Screening.

The study evaluated the use of ultrasound measurement of fetal nuchal translucency in combination with analysis of maternal serum PAPP-A and the free subunit of human chorionic gonadotrophin as a first trimester screening test for Down syndrome in a routine prenatal clinic setting.

Participation in the study involved measuring nuchal translucency at the time of the first ultrasound and obtaining additional blood at the time of phlebotomy for routine prenatal investigations. PAPP-A was assayed between 10 and 13w+6d weeks gestation. Gestational age at the time of recruitment was assessed by crown-rump length (CRL).

Definitions and denominators

Small for gestational age (SGA) was defined on the basis of centiles derived from the cohort, and preterm birth was defined as delivery before 37 completed weeks of gestation.

Nulliparous women were defined as women either having their first pregnancy or women whose births were preceded only by pregnancies that resulted in abortion before 24 wk gestation. Gestational age was defined as the number of completed weeks of gestation. A small-for-gestational-age baby was defined as a live-born baby that was less than the fifth percentile of BW for the given week of gestation, using percentiles derived from 409,541 live births in Scotland between 1992 and 1998 (G.C.S. Smith). The denominator was all live births. Very preterm delivery was defined as birth of a live-born baby between 24 and 32 wk gestation inclusive and the denominator was all live births at or after 24 wk gestation. Moderately preterm delivery was defined as live births between 33 and 36 wk gestation inclusive and the denominator was all live-births at or after 33 wk gestation. Spontaneous preterm birth was defined as vaginal delivery of a live-born baby between 24-36 wk where labor had not been induced. The denominator was spontaneous preterm births plus term births. Stillbirth was defined as delivery of a dead baby at or after 24 wk gestational age and the denominator was all births at or after 24 wk gestational age. Preeclampsia was defined as pregnancy-induced hypertension with proteinuria. Maternal age was defined as the age of the mother, in completed years,

at term. Maternal height was measured in centimeters, maternal weight was measured in kilograms at the time of blood sampling, and body mass index (BMI) was calculated using weight divided by height squared. Nonsmoking was defined as never having smoked, at the time of first attendance for prenatal care; ex-smokers were defined as women who stopped smoking either before or during pregnancy; and smokers were defined as women who smoked throughout pregnancy.

Maternal serum levels of PAPP-A were expressed as multiples of the median (MoM) for gestational age, as is conventional for biochemical indices in pregnancy that vary with week of gestation. Because PAPP-A levels vary inversely with maternal weight, MoM were corrected for maternal weight using reciprocal-linear regression. Low PAPP-A was defined as the lowest 5% of values for gestational age (0.4 MoM).

Selection of study cohort

The database included 484 records of singleton pregnancies where values for PAPP-A, FßhCG, and gestational age at the time of sampling were documented and outcome data were available. We excluded 13 (2,68%) records with missing values for birth weight (BW), 3 (0.61%) with missing values for perinatal outcome (i.e. stillbirth or live birth), 5 (1.03%) records where the gestational age at delivery was outside the range of 24-43 wk, and 7 (1,44%) records

Table 1

Characteristics and outcomes of study group

Study group characteristics		
Age (yr)	Median (IQR)	29,83 (21,1-39.3)
	Age > 35 yr	27 (10,15.2%)
Ethnicity	Non-Caucasian	1 (0,37%)
Smoking status	Nonsmokers	407 (89,25%)
	Ex-smokers	29 (6.35%)
	Current smokers	20 (4,38%)
Weight (kg)	Median (IQR)	64.9 (49.5-82.4)
Gestational age at sampling	Median (IQR) (weeks)	11,6 (8,1-13,6)
Outcome data		
BW (g)*	Median (IQR)	3295 (2810-3780)
Less than 5th percentile[1]		28 (6,14%)
Gestational age at delivery[1]	24-32 wk	5 (1.09%)
	33-36 wk	23 (5,04 %)
	37-43 wk	426 (93.42%)
Stillbirths		2 (0.43%)

Data are number (%) unless stated otherwise. IQR, Interquartile range.
[1]Excludes stillbirths

Table 2

Univariate analysis of first-trimester biochemistry and perinatal outcomes

	PAPP-A		FßhCG	
	Smallest 5th percentile (n = 198)	>5th Percentile (n = 258)	Smallest 5th percentile (n = 183)	>5th Percentile (n = 273)
BW < 5th percentile for gestational age	22 (11.11%)	6 (2,32%)	12 (6.55%)	16 (5.86%)
Delivery, 24–32 wk	4 (3,4%)	2 (0.77%)	1 (0.54%)	5 (1.83%)
Delivery, 33–36 wk	9 (7.69%)	9 (3.48%)	5 (2.73%)	13 (4.76%)
Preeclampsia	11 (5.55%)	8 (3.1%)	10 (5,46%)	9 (3,29%)
Stillbirth	1 (0,50%)	1 (0.38%)	1 (0.54%)	1 (0.36%)

where the karyotype was abnormal or missing. This left a study group of 456.

Results

The basic demographic and outcome data are given in Table 1. Values of PAPP-A measured in the study ranged from 0.05-13.20 IU/liter, and values of FßhCG ranged from 3.7-283 IU/liter.

We then determined the ability of the lowest 5% of MOMs for each serum marker to identify women at increased risk of adverse outcomes in later pregnancy (table 2). Women with the lowest 5% of PAPP-A MOMs were at increased risk of intrauterine growth restriction, moderately and extremely premature birth, preeclampsia, and stillbirth. Women with the lowest 5% of FßhCG MOMs were at increased risk of intrauterine growth restriction but none of the other outcomes.

Discussion

In this study, we demonstrate that maternal circulating concentrations of PAPP-A at 10-13+6 wk gestation are significantly predictive of adverse perinatal outcome in later pregnancy. These data indicate that, in a proportion of women, adverse pregnancy outcome is determined in the first trimester of pregnancy.

We had previously studied ultrasonic measurement of the CRL of the fetus and found that embryos and fetuses that were smaller than expected in the first trimester were more likely to be low BW, low BW at term, in the smallest fifth percentile of weight for gestational age, and born extremely prematurely. That study had certain weaknesses. First, the fetus might be smaller than expected because of variation in the assumed day of ovulation. Second, we were able to obtain an ideal menstrual history and early ultrasound in only approximately 10% of women, which meant that the technique could not be used as a screening test. Our current findings of an association between low PAPP-A and adverse outcome provide additional weight to our hypothesis that adverse perinatal outcome may be determined in early pregnancy. These observations suggest that measurement of specific circulating trophoblast-derived proteins in the first trimester of pregnancy may provide a potential screening tool to identify women at increased risk of subsequent adverse pregnancy outcome.

The pattern of the association varied for different outcomes. In the case of preeclampsia, stillbirth, and extremely premature birth, the association was only with the smallest decile of PAPP-A. In the case of growth restriction and moderately premature delivery, the association was observed across the whole range of PAPP-A. Moreover, low FßhCG was also associated with growth restriction, although this was lost after adjusting for PAPP-A, whereas low FßhCG was not significantly associated with the other outcomes, even in univariate analysis (tables 2). Both PAPP-A and FßhCG are produced by the syncytiotrophoblast. It seems likely that these different patterns of association may reflect different pathophysiological mechanisms relating first-trimester trophoblast function and later adverse perinatal outcome. The fact that the strength and pattern of the association differed for the two trophoblast-derived proteins suggests that PAPP-A is not acting as a simple marker of the volume or health of the trophoblast but that the association reflects a specific property of PAPP-A in the physiological regulation of trophoblast function.

The precise mechanisms linking first-trimester levels of trophoblast-derived proteins and adverse outcomes are not known yet. PAPP-A has been identified as a protease for IGF binding protein (IGFBP)-4. IGFBPs bind IGF-I and IGF-II, inhibiting their interaction with cell surface receptors and have, therefore, a key role in modulating IGF activity. Because PAPP-A breaks down IGFBP, low levels of PAPP-A would be expected to be associated with high levels of IGFBP and, therefore, low levels of free IGF. The IGFs have a key role in regulating fetal growth. The current observation that PAPP-A was predictive of a range of adverse obstetric outcomes implies a fundamental role of this system in development of the placenta in early pregnancy, and the observed association between low PAPP-A and poor perinatal outcome is clearly biologically plausible.

We observed reduced levels of PAPPA in smokers. Smoking inhibits apoptosis of the syncytiotrophoblasts and may weaken feto - placental exchange. Smoking adversely affects the placental vessels and nutrient supply to the fetus, affecting PAPPA productions and lowering IGFs which may have synergistic adverse effects on the fetal growth (Tul et al., 2003).

Conclusions

In summary, first-trimester serum concentrations of PAPP-A, a trophoblast-specific protein regulating IGF function, is highly predictive of a range of subsequent adverse pregnancy outcomes. These observations imply that adverse outcome in late pregnancy may be determined in the first trimester of pregnancy, that control of the IGF system in early pregnancy may be critical in normal placental development, and that women at high risk of adverse pregnancy outcome may be identified in very early pregnancy. ■

References

1. Aldrich, C.L., Stephenson, M.D., Karrison, T., Odem, R.R., Branch, D.W., Scott, J.R.,Schreiber, J.R.,Ober, C., 2001, HLA genotypes and pregnancy outcome in couples with unexplained recurrent miscarriage. Mol.Hum.Reprod. 7, 1167-1172
2. Conover, C.A., Bale, L.K., Overgaard, M.T., Johnstone, E.W.,Laursen, U.H., Fuchtbauer, E.M., Oxvig, C., van Deursen, J., 2004. Metalloproteinase pregnancy associated plasma protein A is a critical growth regulatory factor during fetal development . Development 131, 1187-1194
3. Johnson, M.R., Riddle, A.F., Grudzinskas,, J.G., Sharma, V., Collins, W.P., Nicolaides, K.H., 1993. Reduced circulating placental protein concentrations during the first trimester are associated with preterm labour and low birth weight. Hum. Reprod. , 1942-1947.
4. Jones, J.I.,Clemmons, D.R., 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocr.Rev. 16,3-34.
5. Lawrence, J.B., Oxvig, C., Overgaard, M.T., Sottrup-Jensen,L., Gleich, G.J., Hays, L.G., Yates III, J.R. Conover, C.A., 1999.The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. Proc. Natl. Acad. Sci. U.S.A. 96,3149-3153.
6. Lissak, A., Sharon,A., Fruchter,O., Kassel,A., Sanderovitz, J., Abramovici, H., 1999. Polymorphism for mutation of cytosine to thymine at location 677 in the methylenetetrahydrofolate reductase gene is associated with recurrent early fetal loss. Am. J. Obstet. Gynecol. 181, 126-130.
7. Nayak, N.R., Giudice, L.C., 2003. Comparative biology of the IGF system in endometrium, deciduas, and placenta,and clinical implications for foetal growth and implantation disorders.Placenta 24,281-296
8. Gordon C. S. Smith, Emily J. Stenhouse, Jennifer A. Crossley, David A. Aitken, Alan D. Cameron and J. Michael Connor., 2002. Early Pregnancy Levels of Pregnancy-Associated Plasma Protein A and the Risk of Intrauterine Growth Restriction, Premature Birth, Preeclampsia, and Stillbirth. The Journal of Clinical Endocrinology & Metabolism Vol. 87, No. 4 1762-1767
9. Suzuki, K., Sata, F., Yamada, H., Saijo, Y., Tsuruga, N., Minakami, H., Kishi, R., 2006 Pregnancy associated plasma protein A polymorphism and the risk of recurrent pregnancy loss. Journal of Reproductive Immunology 70 (2006) 99-108.