

Predictive value of pregnancy-associated plasma protein-A (PAPP-A) and free beta-hCG on fetal growth restriction: results of a prospective study

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Abstract

Purpose Low levels of plasmatic pregnancy-associated plasma protein-A (PAPP-A) and high levels of free-beta human chorionic gonadotropin (beta-hCG) could influence the outcome of pregnancy. The objective of this study is to assess the correlation between PAPP-A and free beta-hCG and birth weight.

Materials and methods Prospective follow-up study performed on 3332 patients in the first trimester of pregnancy who were subjected to a screening test focused on evaluation of fetal aneuploidy (SCA-TEST). The values of PAPP-A and free beta-hCG were both analyzed as raw values and subsequently converted to a multiple of the median (MoM). Statistical analysis was performed using SPSS version 17.0.1 (SPSS Inc., Chicago, USA).

Results The incidence of “small for gestational age” in patients with PAPP-A MoM <1st and <5th %₀ was statistically significant (12 and 9.8 %; $p < 0.0001$). Also statistically significant data have been highlighted about free beta MoM > 95th %₀ (7 %; $p = 0.03$). The values of PAPP-A MoM > 99th %₀ are significantly correlated with

an increased risk of “large for gestational age” (16.7 %; $p < 0.0001$).

Conclusion Our study demonstrates that specific values of PAPP-A and free beta-hCG could identify the risk of low or high birth weight since the first trimester of pregnancy.

Keywords Pregnancy-associated plasma protein-A · Free-beta human chorionic gonadotropin · Intra-uterine growth restriction · Fetal aneuploidy · Birth weight · Preterm labor

Introduction

In the last few years, a particular attention has been placed on fetal growth restriction (FGR), in order to detect early predictors and to reduce the high morbidity and mortality associated to this complication. Today the evaluation of fetal growth could take advantage of prenatal imaging techniques, which allow a more careful study using accurate curves of estimated fetal weight, built and individualized on unselected populations. Based on various studies conducted over the past decade, it is clear that in the first trimester we could suspect the onset of some obstetric complications, such as FGR or premature labor/birth [1–4]. Particularly, some authors have given special emphasis to the evaluation of some proteins examined during the screening for fetal aneuploidy in the first trimester of pregnancy [5–7].

The analysis of the literature showed how different clinical and biological factors, including the findings of low levels of plasmatic pregnancy-associated plasma protein-A (PAPP-A) and high levels of free-beta human chorionic gonadotropin (beta-hCG), could influence the outcome of

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pregnancy. PAPP-A is a glycoprotein produced by trophoblast syncytium; it appears in maternal blood in early pregnancy and its value increases during the gestation until the end of the same [8].

Conserva et al. [8] have shown that a value of PAPP-A < 5th % for gestational age is associated with obstetric complications such as preterm labor and low birth weight (<10th %). A low value of this glycoprotein was detected only in cases of vascular insufficiency, such as fetuses with intra-uterine growth restriction (FGR) or preterm birth [9, 10]. The same studies demonstrated how glycoprotein PAPP-A has a key role in the development of the placenta in early pregnancy [10, 11].

The combined test (SCA-TEST) also provides the evaluation of free beta-hCG, whose values have been studied in the first trimester of pregnancy to confirm if it has any influence on birth weight. As environments with low oxygen levels have higher production of free beta-hCG by chorionic villus sampling, it is possible that this condition occurs in a pregnancy with gestational hypertensive syndrome or FGR. The study performed by Goetzinger et al. [10] confirmed that levels of free beta-hCG > 95th % for gestational age are associated with a low birth weight.

In view to reduce perinatal mortality and morbidity in fetuses with diagnosis of FGR, some authors have focused their attention on the study of the placenta and placental vascularization, as there is a reduced supplementation of oxygen by mother to fetus. The results have shown that low levels of PAPP-A are associated with an increased incidence of obstetric complications. The volume of the placenta, as previously demonstrated by Metzenbauer et al. [12, 13], is reduced in pregnant women with low levels of this glycoprotein, even if it, as only predictive value, would be insufficient to have a clinical role to identify FGR [14].

The objective of the present study is to assess the correlation between PAPP-A and free beta-hCG in the first trimester of pregnancy and birth weight.

Materials and methods

Population study

We performed a prospective follow-up study on 3332 patients in the first trimester of pregnancy (between 11 + 0 and 13 + 6 gestational weeks), who were subjected to a screening test focused on the evaluation of fetal aneuploidy (SCA-TEST) at the Center for Prenatal Diagnosis and Fetal Medicine Maternal Artemisia Main Center of Rome, in the period between January 2007 and December 2008. The study protocol was approved by the Ethics Committee and conformed to the ethical guidelines of the Helsinki

Declaration (as revised in Tokyo, 2004). Each woman signed an informed consent before entering the study, and her anonymity was preserved.

Study design

The patients who required the execution of SCA-TEST to evaluate the combined risk of Down syndrome from a population of unselected women were considered eligible for the study. The social and medical history of the mother (age, ethnicity, BMI, smoking, diabetes) and the pregnancy history (gestational age, spontaneous or IVF pregnancy, treatment with progesterone) were obtained during the execution of the screening test, and included in a database. The effective gestational age was calculated by last menstrual period (LMP) and measurement of CRL (between 42 and 85 mm). We included only Caucasian women with single pregnancies; and live fetuses, without any fetal defects observed during the ultrasound exam to evaluate NT and CRL. All enrolled patients were recorded and identified by a personal number. The ultrasound used to perform scans were “© Voluson 730 Pro” and “Voluson E8 ©” General Electrics. The probes used were 2D transabdominal probes (4.5–16.5 MHz).

NT measurement

The scan of the fetus should be median sagittal (to make sure that you have got a proper scan, you have to display the fetal profile) and it has to be done by an adequate zoom showing fetus that fills three-quarters of the screen image, displaying profile and upper chest. An essential element is to differentiate fetal skin and amniotic membrane. We have to wait so that the fetus moves slightly away from the membrane itself. The fetus has to be in a “neutral” position (Fig. 1), not too extended and not particularly flexed; the same scan has to be used to measure CRL. In a hyper-extended position it may overestimate the detection of NT.



Fig. 1 Fetus in a “neutral” position

The caliper should be sited on the point of maximum thickness of NT and the inner edge of the two-internal hyperechoic lines. In the examination, several measurements should be performed and, the measurement which has better followed the above requirements has to be used in the calculation of risk. In 95 % of cases, the measurement of NT could be obtained by transabdominal scan; in the remaining 5 % of the cases the trans-vaginal scan is used, when the NT is borderline, or when there is a low echogenicity, due to the low acoustic impedance of pregnancy tissues.

CRL measurement

CRL should be measured with the fetus in a sagittal, neutral position (neither hyper nor overflexed), excluding the yolk sac. The measurement is obtained by maximum length of fetal echoes, by calipers sited at the cephalic extreme and at the end of the fetal trunk, with a straight line between the two poles; it should be between 42 and 85 mm.

Dosage of biochemical markers

The kit used to evaluate the levels of free beta-hCG and PAPP-A were the BRAHMS free beta-hCG and the PAPP-A KRIPTOR CAL., two automatic system based on immunofluorescence. According to previous scientific studies [8], normal range of the two markers was processed in relation to the different gestational age (Manual SCATEST-vers 2.0 SIDI.P.). For the free beta-hCG, see Table 1. For the PAPP-A see Table 2.

Uterine artery velocimetry

When the patients underwent to our center for the screening of fetal aneuploidy during the first trimester of pregnancy, we analyzed the pulsatility index of both uterine arteries. As each arterial fluximetric wave also the uterine artery has an increased flow's phase during ventricular contraction (systolic flow) and a reduced speed flow' step during ventricular relaxation (diastolic flow). PI and RI express the correlation between systolic flow and diastolic flow. The reference values indicate when there is a mean value of PI more than 1.45 the flowmetry of the uterine

arteries is called “inadequate”. The sonographer has to perform doppler ultrasound when the patient is placed in a slightly lateral decubitus, holds her breath and has not any uterine contraction. Then we evaluated the mean value of the measurements on the arteries of the two sides (left and right). Not respecting these conditions could lead to an increased vascular impedance for the compression of myometrial vessels (arcuate, radial and spiral arteries).

Follow-up

Patients were contacted by a telephone interview by a doctor of the center, who was not aware of the combined test screening results. The following information was recorded: date of birth, mode of delivery (spontaneous vaginal delivery/operative delivery or caesarean section), birth weight, maternal diseases complicating pregnancy (gestational diabetes and hypertension), maternal and/or fetus complications at delivery, and presence of neonatal abnormalities and/or disease.

Statistical analysis

Assuming an incidence of SGA in the order of 4 %, with a test power of 80 % and a significance level of 5 %, we needed 2400 fetuses to verify the differences in the incidence of the two groups determined by percentile equal to 5 %; 3332 patients were considered eligible for the study. The considered variables, such as BMI, gestational age at delivery and maternal age were expressed as mean \pm standard deviation ($M \pm SD$), while qualitative variables were expressed as a percentage (%). A linear model of logistic regression has been used for statistical analysis of the data.

The values of PAPP-A and free beta-hCG, studied at 1st, 5th, 95th and 99th %, were both analyzed as raw values and subsequently converted to a multiple of the median (MoM) in order to assess the association with small for gestational age (SGA) babies and large for gestational age (LGA) babies through the univariate analysis.

Statistical analysis was performed using SPSS version 17.0.1 (SPSS Inc., Chicago, USA). The test used to calculate the p value related to the smoke and to velocimetry of uterine arteries was the Fisher's Exact Test for Count Data. A p value <0.05 was considered statistically significant.

Results

From January 2007 to December 2008, altogether 3332 women came to our center for the evaluation of nuchal translucency (NT) and enrolled in the study; 113 were

Table 1 Normal range of free beta-hCG

Week gestation	5 % (ng/mL)	Median (ng/mL)	95 % (ng/mL)
11	16.68	44.83	120.47
12	14.63	39.33	105.69
13	11.28	30.32	81.49
14	8.29	22.29	59.89

Table 2 Normal range of PAPP-A

Week gestation	5th % ₀ (mIU/mL)	Median (mIU/mL)	95th % ₀ (mIU/mL)
11	0.632	1.587	3.986
12	1.040	2.612	6.560
13	1.543	3.874	9.730
14	2.062	5.178	13.005

excluded (2.7 %) for spontaneous miscarriage, 67 (1.6 %) for major fetal abnormalities, 279 (6.7 %) for twin pregnancies, 69 (2 %) were not Caucasian women, and 4 (0.01 %) women had diabetes. In the evaluation of PAPP-A and free beta-hCG, 250 patients were ruled out due to a computer problem; 72 patients were lost to follow-up. Overall a total of 2478 women were identified and analysed.

The SGA patients (<5th %₀) were 50 (2.0 %), the SGA (<10th %₀) were 74 (2.7 %) and LGA patients (>95th %₀) were finally 1418 (55.6 %).

The association between incidence of SGA (<5th and <10th %₀) and LGA (>95th %₀) and the dosage of serum PAPP-A MoM and free beta MoM was assessed by univariate analysis. The incidence of SGA in patients with PAPP-A MoM < 1st %₀ was statistically significant (12 %; $p < 0.0001$), as well as in patients with PAPP-A MoM < 5th %₀ (9.8 %; $p < 0.0001$). Also statistically significant data have been highlighted regarding values of free beta MoM > 95th %₀ (7 %; $p = 0.03$); on the opposite way, however, it does not show any statistical significance analysis of the raw values of PAPP-A > 99th %₀ ($p = 0.13$) (Tables 3, 4, 5).

By analysis of the association between the values of PAPP-A MoM and free Beta MoM with LGA, it was shown that: the values of PAPP-A MoM > 99th %₀ are correlated with an increased risk of LGA with high statistical significance (16.7 %; $p < 0.0001$), however no statistical significance was found between the values of free Beta MoM and risk of LGA. The results are shown in Tables 6 and 7. We have analyzed other variables, which could affect the birth of infants SGA or LGA such as BMI, gestational age at delivery (GAD), maternal age at birth, sex, smoking and preterm delivery (PP). Regarding the BMI, this factor seems to affect fetal growth only in the case of a SGA < 5th %₀ and a BMI of the mother significant (22.7 ± 2.8 vs 21.7 ± 6.0 SD; $p = 0.02$); considering

instead a SGA < 10th %₀ maternal BMI does not affect fetal growth (21.7 ± 6.1 vs 21.7 ± 5.9 SD). However a difference, extremely significant from a statistical point of view, is evident among patients who gave LGA birth: these patients, in fact, have a higher BMI (22.2 ± 5.6 vs 21.2 ± 6.3 SD). Low GAD and high maternal age at delivery statistically significantly correlate with the risk of SGA births. Particularly the GAD has a high statistical significance in both the case of SGA < 5th %₀ (34.4 ± 2.6 vs 39.2 ± 1.7 DS) that in the case of SGA < 10th %₀ (34.9 ± 2.7 vs 39.3 ± 1.7 DS). Maternal age would seem instead to have a more important role in the incidence of SGA < 5th %₀ compared to SGA < 10th %₀ (34.6 ± 4.9 vs 32.9 ± 4.0 SD; 34.3 ± 5.3 vs 32.9 ± 4.0 SD). Some considered variables (BMI, GAD and maternal age at delivery) correlate statistically significantly with LGA babies.

The smoke instead seems to not affect statistically on the birth of SGA infants <5th %₀ ($p = 0.62$) nor SGA infants <10th %₀ ($p = 0.78$); it does not affect born LGA babies ($p = 0.67$). As for the sex of the baby, it is more likely that an SGA infant is female, and conversely, LGA infants are male.

Another variable that we analyzed was the incidence of preterm deliveries (<37 weeks) in patients with a low value of plasmatic PAPP-A. In this sense, our study showed a high rate of correlation between values of PAPP-A MoM > 95th %₀ (19.8 %; $p < 0.0001$) or even values of PAPP-A MoM > 99th %₀ (37.5 %; $p < 0.0001$) and preterm birth, with a low incidence and statistical significance for values of PAPP-A MoM <1stand <5th %₀ ($p = 0.002$; $p = 0.003$). The values of free Beta MoM on the other side seem to affect the risk of preterm birth only if >95th %₀ ($p = 0.01$).

In multivariable analysis, considering the four subdivisions of PAPP-A MoM, free Beta hCG MoM, maternal age and preterm birth, shows that a value of PAPP-A

Table 3 Correlation between free beta-hCG and SGA

	SGA < 5th % ₀	<i>p</i> value	SGA < 10th % ₀	<i>p</i> value
FreeBMoM < 1st % ₀	0/26 (0 %)	0.47	0/26 (0 %)	0.38
FreeBMoM < 5th % ₀	2/128 (1.6 %)	0.74	3/128 (2.3 %)	0.76
FreeBMoM > 95th % ₀	8/127 (6.3 %)	<0.0001	9/127 (7 %)	0.003
FreeBMoM > 99th % ₀	2/25 (8.0 %)	0.03	2/25 (8 %)	0.15

Table 4 Correlation between PAPP-A and SGA

	SGA < 5th %	<i>p</i> value	SGA < 10th %	<i>p</i> value
PAPPMoM < 1st %	3/25 (12.0 %)	<0.0001	3/25 (12 %)	0.005
PAPPMoM < 5th %	10/132 (7.6 %)	<0.0001	13/132 (9.8 %)	<0.0001
PAPPMoM > 95th %	9/116 (7.8 %)	<0.0001	12/116 (10.3 %)	<0.0001
PAPPMoM > 99th %	3/24 (12.5 %)	<0.0001	4/24 (16.6 %)	<0.0001

Table 5 Correlation between row values of PAPP-A and free beta-hCG in SGA and LGA

	SGA < 5th %	<i>p</i> value	SGA < 10th %	<i>p</i> value	LGA	<i>p</i> value
FreeB < 1st %	0/27 (0 %)	0.47	0/27 (0 %)	0.38	18/27 (66.7 %)	0.25
FreeB < 5th %	2/139 (1.4 %)	0.67	3/139 (2.1 %)	0.67	60/139 (56.8 %)	0.79
FreeB > 95th %	8/135 (5.9 %)	<0.0001	9/135 (6.6 %)	0.004	69/135 (51.1 %)	0.26
FreeB > 99th %	2/26 (7.7 %)	0.03	2/26 (7.6 %)	0.12	11/26 (42.3 %)	0.17
PAPP < 1st %	3/27 (11.1 %)	<0.0001	3/27 (11.1 %)	0.007	10/27 (37.0 %)	0.05
PAPP < 5th %	11/139 (7.9 %)	<0.0001	14/139 (10 %)	<0.0001	61/139 (43.9 %)	0.004
PAPP > 95th %	10/135 (7.4 %)	<0.0001	12/135 (8.8 %)	<0.0001	61/135 (45.2 %)	0.01
PAPP > 99th %	1/27 (3.7 %)	0.49	2/27 (7.4 %)	0.13	9/27 (33.3 %)	0.02

Table 6 Correlation between free beta-hCG and LGA

	LGA	<i>p</i> value
FreeBMoM < 1st %	16/26 (61.5 %)	0.54
FreeBMoM < 5th %	70/128 (54.7 %)	0.83
FreeBMoM > 95th %	68/127 (53.5 %)	0.63
FreeBMoM > 99th %	12/25 (48.0 %)	0.44

Table 7 Correlation between PAPP-A and LGA

	LGA	<i>p</i> value
PAPPMoM < 1st %	10/25 (40.0 %)	0.11
PAPPMoM < 5th %	56/132 (42.4 %)	0.002
PAPPMoM > 95th %	47/116 (40.5 %)	0.001
PAPPMoM > 99th %	4/24 (16.7 %)	<0.0001

MoM < 5th %, a value of free Beta hCG MoM > 95th %, maternal age and preterm delivery correlate, independently from each other with the risk of SGA and could therefore be considered predictors.

Considering all these variables studied, because the analysis of the literature we have found that several authors have also evaluated the level of uterine artery Doppler flow in the first trimester of gestation, our ultimate goal was to evaluate our population results. Then we divided our cohort of patients into two groups. From 132 patients with PAPP-A < 5th %, the first group were women with Uta PI values >95th % and the second group of women with Uta PI values <95th %.

The analysis obtained also confirmed in our data a significant incidence of risk of SGA infants (<10th %) ($p < 0.0001$) (Table 8).

Discussion

Several authors have focused their attention to find biomarkers or predictors of obstetric complications, such as preterm birth, pre-eclampsia or FGR [3–8]. In particular, relatively to fetal growth restriction, it was demonstrated by scientific literature that a low value of plasmatic PAPP-A, dosed during the first trimester of pregnancy is associated with low birth weight [8]. Since the first trimester is therefore possible, according to these studies, to identify risk conditions of SGA or FGR, through the combination of demographic characteristics and maternal and fetal parameters using biochemical markers that are part of screening for fetal aneuploidy [8, 10, 15–17].

Our study in fact, in line with what has already been written in the literature, confirms that a low level of PAPP-A MOM (<1 and <5th %) as well as a high value of free BetaMOM (>95th %) are associated with SGA babies [10, 15]. It was also highlighted as a value of PAPP-A MOM > 99th % is highly correlated with the incidence of LGA births (16.7 %; $p < 0.001$).

By adding maternal characteristics such as BMI, gestational age at birth, maternal age and smoking, we wanted to analyze whether these factors could have an effect on birth weight. Univariate analysis in which were introduced the above variables, BMI does not affect SGA infants, if we consider a value <10th %, on the contrary, instead, a high value of maternal BMI, in agreement with what has already

Table 8 Correlation between PAPP-A and uterine artery Dopplers in SGA, non-SGA and LGA births

	SGA	Non-SGA	LGA
PAPP-A MoM < 5th % Ut A PI > 95th % $p < 0.0001$	12/22 (54.5 %)	10/22 (45.4 %)	0/22 (0 %)
PAPP-A MoM < 5th % Ut A PI < 95th %	1/110 (0.9 %)	53/110 (48 %)	56/110 (50.9 %)

been described by Goetzinger [10], influence the birth of an infant with weight >95th % with a significant statistical difference ($p < 0.0001$). A statistically significant difference could be detected in our study evaluating the gestational age at delivery with a significant statistic both in the case of a value <5th % in the case of a value <10th % ($p < 0.0001$). In the same way we could highlight that higher is the maternal age higher will be the risk to have a child with SGA < 5th and <10th % (respectively: $p < 0.002$, $p < 0.02$).

About a high birth weight (LGA), the three just considered variables affect our sample; then it is more likely that in this case women have a higher BMI, gestational age at delivery and a greater age ($p < 0.0001$, $p < 0.0001$; $p = 0.002$). Our data are in line with what is written in the literature [15, 18]. Considering also other variable such as the smoke we saw that it does not affect in any way the birth weight considering the SGA as LGA.

The women who smoke have the same risk to have a child SGA compared to non-smoking women, since it is in contrast with the literature [19]. This could be linked to several factors: as one of more frequent obstetric complications is preterm delivery (<37 weeks) we wanted to assess whether the value of PAPP-A and free beta-hCG could in any way affect the incidence. Actually there is a big difference between the two plasma proteins, as the free Beta hCG MoM seem to have no role in the prediction of preterm delivery. On the other side PAPP-A MoM value, if either <1st and the 5th % is that if >95th and >99th %, has a statistically significant impact on the risk of this complication (respectively $p = 0.002$ and $p = 0.003$, $p < 0.0001$ and $p < 0.0001$). Such value is in line with what has been described in the literature by Conserva et al. [8], who found that an association between preterm birth and low birth weight was probably linked to vascular insufficiency of the placenta.

In view of the fact that low birth weight is one of the most frequent obstetric complications, there are several studies, which were performed to recognize the possible causes. It is known that the placenta has a key role in growth and incidence of fetal complications related to this failure (pre-eclampsia, FGR, preterm birth). The impedance of blood flow in the uterine arteries decreases during pregnancy reflecting the invasion of trophoblast into the spiral arteries and converting them into low-resistance vessels. The value of uterine artery Doppler flow has been

shown to have utility in identifying diseases such as pre-eclampsia with placental insufficiency and FGR [20–22]. A value of UTA PI > 95th % is considered pathological.

Based on what was also analyzed by other authors in the scientific literature [20, 23], we added another important variable: the measurement of uterine artery velocimetry (UTA PI). In the first trimester, the study of these arteries has a diagnostic sensitivity for low birth weight of 25 %; however, it has a number of false positives by 5–10 %.

The analysis of these two groups highlights that the risk of FGR in the second group of pregnant women is higher with a statistically significant difference. Then it seems likely that the combination of two very important variables may increase the risk of having a baby with a low birth weight; this fact confirms once again that the study of the uterine circulation plays an important role in the identification of pregnant women at risk of obstetric complications.

In conclusion, our study demonstrates, in agreement with what was observed in other works, the possibility, since the first trimester of pregnancy, of identifying women with risk of developing infants with low birth weight.

The strength of our study is characterized not only by the size of the sample but also by the fact that it is a prospective case–control and that the study population is predominantly low-risk. It proposes several questions, based mainly on the doctor–patient relationship, which mainly concern the kind of approach we have to keep with the pregnant woman, who, after identifying the above risk factors, needs explanations about complications that could take over.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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