Full Length Research Paper

Role of first-trimester free beta subunit of human chorionic gonadotropin (β-HCG) and pregnancy-associated plasma protein-A (PAPP-A) as markers for intrauterine fetal growth restriction

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Intrauterine growth restriction (IUGR) is a major determinant of perinatal morbidity and mortality. Standard diagnostic methods like sonography are accurate, but they can not be employed as the screening tools. Lately, it has been proposed that there might be an association between IUGR and placental dysfunction; hormones such as human chorionic gonadotropin-beta subunit (β-HCG) and pregnancy-associated plasma protein-A (PAPP-A) may be good early indicators of unwanted outcomes. This study is aimed at evaluating the possible association of serum free β-HCG and PAPP-A levels in the first trimester with IUGR in chromosomaly normal pregnancies. In this cohort study, 1440 normal singleton pregnancies were evaluated in Tabriz Alzahra Teaching Hospital during a 17-month period. Serum free β-HCG and PAPP-A levels were measured in women at 10 to 14 weeks of pregnancy by enzyme linked immunosorbent assay (ELISA). All the women were followed up to determine the time of delivery and to be categorized as with or without IUGR. Serum free β-HCG and PAPP-A levels were compared between the groups. The rate of IUGR was 4.4%. The mean serum levels of free β-HCG and PAPP-A were 2.6±2.7 (range: 0.1 to 10.8, median: 0.9) M.O.M (multiples of the median) 2.4±2.6 (range: 0.1 to 8.9, median: 0.9) MoM, respectively. The median serum β-HCG was 1 and 0.9 MoM in cases with normal and IUGR pregnancies, respectively (p=0.587). The median serum PAPP-A was significantly lower in patients with IUGR (0.7 vs. 0.9 MoM; p=0.044). The optimal cut-off point for PAPPA was 0.79 MoM with sensitivity and specificity of 51 and 54%, respectively. This study showed that low level of serum PAPP-A but not β-HCG during the first trimester is associated with IUGR.

Key words: Fetal growth restriction, human chorionic gonadotropin-beta subunit, pregnancy-associated plasma, protein-A.

INTRODUCTION

When a fetus fails to fulfill its expected growth potential, an intrauterine growth restriction (IUGR) will occur, which is a serious complication in pregnancy. It is one of the major leading causes of perinatal morbidity and mortality (Cowans and Spencer, 2007; Smith et al., 2002). Fetal growth is defined as an increase in the number and size of cells or mass of tissue and fetal development is the result of changes in the structure and function of cells and tissues (Wu et al., 2006). These are complex biological events that are influenced by the fetal genome in early fetal life; however, later in pregnancy, environmental, nutritional and hormonal factors become the major determinants of fetal growth and development.

First-trimester impaired placental function may be one of the potential underlying processes that result in IUGR

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(Wu et al., 2006; Cunningham et al., 2010). Early diagnosis of fetuses at increased risk of being growth-restricted enabled more appropriate surveillance, thereby optimizing management, which has been shown to reduce the risk of adverse fetal outcome. Effective screening for IUGR will become a possibility by having more understanding about its pathology. There is considerable evidence that throughout pregnancy, insulin and insulin like growth factors (IGF) I and Π have roles in fetal growth and development. These growth factors, produced by virtually all fetal organs, are proinsulin-like polypeptides that stimulate cell differentiation and division (Cowans and Spencer, 2007; Cunningham et al., 2010). Pregnancy-associated plasma protein-A (PAPP-A) and free ß subunits of human chorionic gonadotropin (β-HCG) are used for the screening of Down syndrome at 10 to 14 weeks of pregnancy (Cowans and Spencer, 2007; Cunningham et al., 2010). Recently, retrospective studies have shown that low levels of maternal serum PAPPA and free β-HCG are associated with development of pregnancy complications (Gagnon et al., 2008; Ong et al., 2000). PAPPA is known to be a protease for 1GF binding proteins (IGF BPS), so its level can affect the level of free 1GF by reversing the inhibitory effects of the 1GF BPs and therefore contribute to fetal growth (Smith et al., 2002). However, there have been little retrospective studies regarding this subject and the results of previous studies are controversial, and there is no general agreement about the relation between PAPPA and free ß subunits of H.C.G and IUGR.

The aim of this prospective cohort study was to evaluate any association between serum levels of free β-HCG and PAPPA at 10 to 14 weeks of pregnancy and intrauterine growth restriction in order for them to benefit from the increased surveillance of this condition.

MATERIALS AND METHODS

We conducted a cohort study on pregnant women who attended the prenatal screening program at Alzahra Teaching Center, affiliated with Tabriz University of Medical Sciences, Tabriz, Iran between May 1, 2009 and September 25, 2010. Inclusion criteria included: (a) Singleton pregnancies without considering cytogenetic or phenotypic status of fetus; (b) A gestational age of less than 14 weeks; (c) Being a resident of Tabriz; (d) Lack of maternal disease. The exclusion criteria included: (a) A history of maternal chronic disease; (b) Complications with the pregnancy such as hemorrhage, threatened abortion, ectopic pregnancy or incomplete abortion; (c) Advanced gestational age (more than 14 weeks); (d) Cytogenetic or phenotypic abnormalities of the fetus. All study patients gave informed consent to be included in this study, which had been approved by the Regional Research Ethics Committee. Blood samples were obtained from all cases that had the inclusion criteria, between 10 to 14 weeks of pregnancy. A total of 1498 pregnant women who visited the prenatal clinic of Alzahra hospital were qualified to enter this study and were followed during pregnancy until delivery. Pregnancies identified with chromosomal abnormalities through prenatal diagnosis (n=9) or major abnormalities at the second or third trimester (n=15) were excluded from the study. 38 cases missed due to lack of follow up. The final study population consisted of 1440 pregnancies without fetus or neonate abnormalities.

The free beta subunits of human chorionic gonadotropin and pregnancy associated plasma protein A were measured between 10 to 14 weeks of pregnancy using the Eliza method.

The maternal serum free beta subunit of H.C.G was measured in nanogram/mililiter (ng/ml) and PAPPA was measured in nanogram/mililiter (ng/ml). Both measurements were converted into multiples of the median (M.O.M) by dividing each result by the expected median marker level in normal pregnancies at the same gestational age calculated from ultrasound crown-ramp (CRL) (Cowans and Spencer, 2007; Cunningham et al., 2010). Maternal weight in kilograms and gestational age at the time of blood sampling were taken into consideration for calculating of free β-HCG and PAPPA M.O.M. We used Alfa software and the persica technique for calculating MoM. All of the participants were evaluated at 28 weeks using sonographic fetal biometric measurement for detection of IUGR.

Detection of intra uterine growth restriction was done by calculating the ratio of head circumference (HC) to abdominal circumference (AC) as revealed by ultrasound examination (Cunningham et al., 2010). We followed the individuals every two weeks until the end of the pregnancy. IUGR was determined to have occurred when birth weight was below the 10th percentile for gestational age. The level of maternal serum of free beta subunit of H.C.G and PAPPA at 10 to 14 weeks of pregnancy was compared between two groups, with and without IUGR. Data are presented as mean ± standard deviation. Significance levels were set at the P<0.05. Data management and analysis was performed using SPSS15.

Quantitative variables were compared using independent samples t-test or Mann-Whitney u-test and qualitative variables using contingency tables and chi-square test. The optimal cutoff point was determined by receiver operating characteristic (ROC) curve, and the sensitivity and specificity of optimal cutoff point of PAPPA and free Beta HCG in prediction of IUGR were estimated.

RESULTS

In this study, the rate of IUGR was 4.4% (63 cases). As Table 1 shows, there were no significant differences in demographic characteristics of patients with and without IUGR.

Table 2 compares maternal past history between two groups with and without IUGR.

The mean serum levels of free β-HCG ubunit and PAPPA were 2.6±2.7 (range: 0.1 to 10.8, median: 0.9 MoM) and 2.4±2.6 (range: 0.1 to 8.9, median: 0.9 MoM), respectively.

As shown in Table 1, the median serum level of free β-HCG in cases with IUGR was lower than that in normal pregnancy cases, but the difference was not significant. The median serum level of PAPPA was significantly lower in patients with IUGR than those who had normal pregnancies (0.7 MoM vs. 0.9 MoM (p=0.044).

The rate of preterm labor in the IUGR group was significantly higher than that of the normal group (20.6% versus 6.6%, p<0.001) (Table 1).

The difference in mean birth weight for newborns of the two groups was significant (1850 g vs. 3350 g, p<0.001) (Figure 1). The best cutoff point of PAPPA for prediction of IUGR was ≥0.79 MoM, which was estimated using

Table 1. Demographic characteristic of mothers with and without IUGR and significance (p) from controls.

Variable	IUGR-	IUGR+	p-Value
Maternal age (year)	26.2±4.7 (17-34)	26.1±5.1 (17-34)	0.933
Maternal weight (kg)	65.7±9.2 (49-87)	62.3±6.5 (49-75)	0.085
Maternal height (cm)	158.7±5.9 (149-170)	157.5±7.5 (149-176)	0.125
Parity(N)	1.3±1.3 (0-6)	1±1 (0-4)	0.083
Gravid (N)	1.2±1.1 (1-5)	1.2±0.8 (1-3)	0.856
Preterm birth(before 37 weeks)	6.6 (91)	20.6 (13)	<0.001*
Free β-HCG (MoM)(ng/ml)	2.6±2.8 (0.1-10.8)	1.8±1.7 (0.3-5.6)	0.587
PAPPA (MoM)(ng/ml)	2.5±2.7 (0.1-8.9)	1.4±1.2 (0.1-3.9)	0.044*
Birth weight (g)	2500±550 (2850-4300)	1850±450 (1250-2450)	<0.001*
Gestational age at screen (week)	12±1.3 (10-14)	12.2±1.3 (10-14)	-
Fetal gender(male)(N)	718	722	0.933
Smoking(N)	6	8	0.933

*, Statistical significance, significance level were set at the $p < 0.05$. Data are presented as mean±S.D.

Table 2. Maternal past history of patients undergoing first trimester screening test.

Variable	IUGR-	IUGR+	P-Value
Diabetes	1.5 (20)	0 (0)	0.308
Hypertension	5.8 (78)	3.8 (4)	0.405
Abortion	9.7 (130)	7.7 (8)	0.496
Preeclampsia	1.1 (15)	0 (0)	0.323
Fetal abnormality	1.2 (16)	0 (0)	0.299
IUGR	4.7 (63)	3.8 (4)	0.460

Significance level were set at the $p < 0.05$.

a ROC curve. The sensitivity and specificity of this test was 51 and 54%, respectively (Figure 2).

DISCUSSION

In this prospective cohort study on 1440 pregnant women, the mean serum levels of free β-HCG and PAPPA between 10 to 14 weeks gestation were 2.6±2.7 (0.9 MoM) and 2.4±2.6 (0.9 MoM), respectively. This is the same as the results of other studies. For example, Morssink et al. (1998) in a study on 800 cases of singleton pregnancies found a median level of 1 and 0.9 MoM for free β-HCG and PAPPA, respectively, in the first trimester of pregnancy. The median level of free β-HCG and PAPPA were 1 and 1 MoM respectively in Spencer et al.'s (2005) study of 4300 singleton pregnancies between 10 to 14 weeks gestational age.

In a study by Ardawi et al. (2007) of 1616 singleton pregnancies, the median level of free β-HCG was 1.03 MoM and it was 1.01 MoM for PAPPA.

Our study was a prospective cohort study, with a large number of pregnant women with no significant difference in demographic characteristic and smoking status (Table 1) and this is the advantage of our study because some factors such as sample size, complications of pregnancy, gestational age, fetal gender, smoking, ethnicity, maternal weight, gravidity and parity may influence the maternal serum levels of free β-HCG and PAPPA (Ardawi et al., 2007; Cole, 1994). Previous retrospective studies provided conflicting results about the relationship between maternal serum H.C.G. and PAPPA in the first trimester and the subsequent development of pregnancy complications. Spencer et al. (2005) carried out a study on 4390 women with singleton pregnancies with 172 cases of IUGR. The comparison of maternal serum PAPPA between IUGR and normal outcome group showed significantly low levels for those in the IUGR group, but the serum level of free β-HCG was not significantly different between the two groups. Their results match the results of our study. Pedersen et al. (1995) measured maternal serum PAPPA at 8 to 14 weeks in 93 pregnancies and found a positive correlation with birth weight. In contrast, in a case control study on 800 patients by Morssink et al. (1998) there was no significant association between first trimester free β-HCG and PAPPA levels and subsequent intra uterine growth restriction.
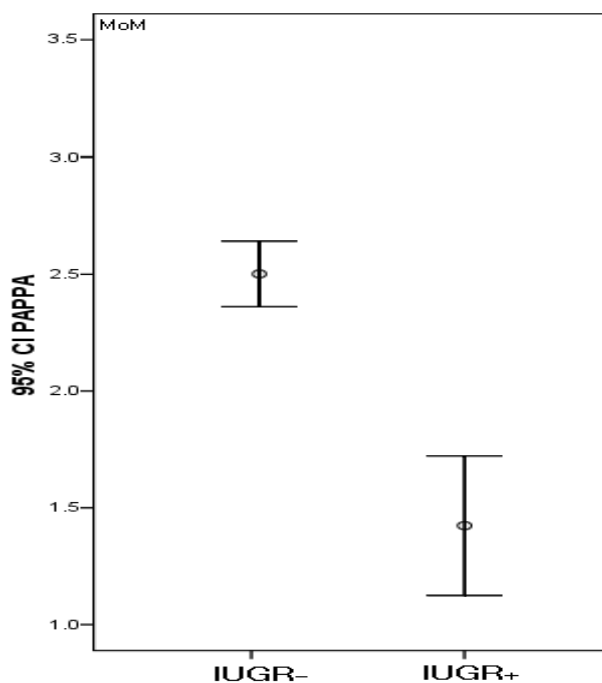
Figure 1. Error bar of maternal serum PAPPA in patients undergoing first trimester screening test.
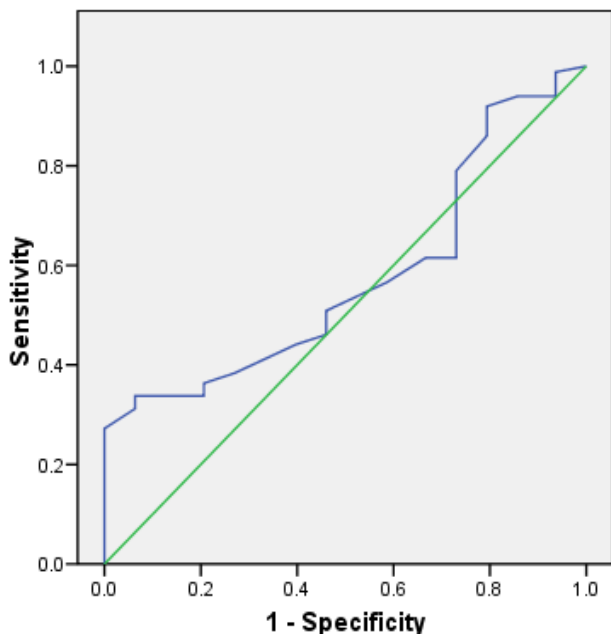

Figure 2. ROC curve for cut off point of maternal serum PAPPA in determining of IUGR. The best cut off point of PAPPA was >0.79 MoM with 51% sensitivity and 54% specificity in prediction of IUGR.

Johnson et al. (1993) measured maternal serum PAPPA and intact human chorionic gonadotrophin levels at 7 to

13 weeks gestational age in 62 pregnancies achieved by *in vitro* fertilization and embryo transfer and found a weak association between a low level of PAPPA and low birth weight.

Ranta et al. (2011) in a cohort study on 2844 pregnant women found that the concentration of first trimester screening serum markers were lower in pregnancies with preeclampsia, small of gestational age and preterm delivery. Montanari et al. (2009) in a study on 2178 women who underwent first-trimester measurement of serum concentration of PAPPA and free β-HCG , found that low first trimester maternal serum PAPPA is significantly associated with reduced fetal size and increased risk of IUGR. Goetzinger et al. (2009) in a retrospective cohort study found low PAPPA and high free β-HCG levels in the first trimester of pregnancy are associated with small for gestatinal age growth pattern; these results are in contrary with our results. In our study, the median serum level of PAPPA was significantly lower in patients with IUGR and median serum level of free β-HCG in cases with IUGR was lower than normal pregnancies, but the difference was not significant. Spencer et al. (2007) in a study found low levels of maternal serum PAPPA in the absence of an abnormal karotype,are associated with an increased risk for IUGR and this is the same as the results of our study.

Yaron et al. (2002) in a study on 1622 singleton pregnancies showed that decreased levels of first trimester maternal serum PAPPA is predictive not only for chromosomal anomalies but also for adverse pregnancy outcomes. In their study, there was a significant relationship between a low level of PAPPA and IUGR; they could not determine a cutoff point, but they showed the rate of IUGR significantly increased when the median level of PAPPA was lower than 0.5 MoM (Yaron et al., 2002).

In our study, by determining the cut off level of 0.79 MoM for PAPPA by the ROC curve, the specificity and sensitivity of PAPPA between 10 to 14 weeks of pregnancy for prediction of IUGR was 54 and 51%, respectively.

Smith et al. (2002) in a study on 8839 singleton pregnancies showed serum concentrations of PAPPA; a trophoblast-specific protein regulating IGF function in first trimester is highly predictive of subsequent adverse pregnancy outcomes such as IUGR, but there is not a relationship between serum concentration of free β-HCG and IUGR. Kirkegaard et al. (2011) in a study on 9450 singlton pregnant women found that low PAPPA at the first trimester of pregnancy is associated with small for gestational age.

Adverse outcomes in late pregnancy may be determined in the first trimester of pregnancy by measurement of PAPPA which regulate IGF function. The role of the IGF system in early pregnancy may be critical in normal placental development. Krantz et al. (2004) in a cohort study on 8012 pregnant women showed

an association between serum levels of PAPPA and free β-HCG in the first trimester of pregnancy and adverse pregnancy outcomes, especially a high predictive value of PAPPA levels below 1st percentile (Krantz et al., 2004).

Although both PAPPA and free β-HCG are produced by the syncytiotrophoblast, different patterns of association with complications of pregnancy may reflect different pathophysiological mechanisms relating first–trimester trophoblast function and later adverse outcomes. PAPPA has been identified as a protease for IGF binding protein. IGFBPS bind to IGR- 1 and II, inhibiting their interaction with cell surface receptors and have a key role in the regulation of IGF function. PAPPA breaks IGFBP, and a low level of PAPPA is associated with high level of IGFBP and low level of free IGF. IGF has a key role in fetal growth and in the autocrine and paracrine control of trophoblast invasion to deciduas and uptake of glucose and amino acids (Smith et al., 2002; Lawrence et al., 1999).

So, first-trimester serum concentrations of PAPPA is highly predictive of IUGR and control of the IGF system in early pregnancy may be critical in normal development of placenta, and we can identify adverse pregnancy outcomes in the first trimester of pregnancy by measurement the level of PAPPA between 10 to 14 weeks gestational age. However, detection of exact mechanisms requires further studies (Smith et al., 2002; Gagnon et al., 2008).

We conclude that a low level of maternal serum PAPPA at 10 to 14 weeks of pregnancy can predict IUGR, but we could not determine the exact cut off level. Further studies with a larger sample size may be helpful.

In the cases with a low level of PAPAA between 10 to 14 weeks gestation, increased frequency of antenatal visits and frequent monitoring of fetal growth for detection of IUGR was shown to be useful. And this group may benefit from increased surveillance for this condition.

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