Original Article

The relationship between unexplained elevated serum markers in triple test, uterine artery doppler measurements and adverse pregnancy outcome

Ayse Yasemin Karageyim Karsidag, Esra Esim Buyukbayrak, Bulent Kars,
Ugur Suyugul, Orhan Unal, Mehmet Cem Turan
Obstetric and Gynecology Department, Dr. Lutfi Kirdar Kartal Education and Research Hospital, Istanbul, Turkey.

Abstract

Objective: To investigate the relationship between adverse pregnancy outcomes and unexplained elevations of second trimester maternal serum Human Chorionic Gonadotropin (hCG), Alpha Fetoprotein (AFP) levels and uterine artery Doppler measurements.

Methods: A total of 144 women between 16-20 weeks of gestation that applied to our clinic for triple test were enrolled into the study. Study group consisted of 84 pregnant women with hCG and/or AFP levels ≥ 2 MoM. Control group comprised of 60 pregnant women with hCG and AFP levels < 2 MoM. Study group was further subdivided into 3 subgroups: Subgroup I; only AFP ≥ 2 MoM (n=30), subgroup II; only hCG ≥ 2 MoM (n=64) and subgroup III; both AFP and hCG ≥ 2 MoM (n=10).

Result: Operative delivery rate (p=0.0017), overall complication rate (p=0.0002), bilateral early diastolic notch presence rate (p=0.015) were high and mean birth weight was low (p=0.045) in the study group. In subgroup I patients, low birth weight [LBW] (p=0.0008), preterm delivery (p=0.0001), preeclampsia (p=0.003) and preterm premature rupture of membranes [PPROM] (p=0.012) rates were high. In subgroup II patients, only small for gestational age baby [SGA] (p=0.016) rate was high. In subgroup III patients LBW (p=0.009), preterm delivery (p=0.0001) and PPROM (p=0.01) rates were high.

According to Doppler velocimetry studies, bilateral early diastolic notch presence rate was high (p= 0.015) in the study group.

Conclusion: Patients with high AFP levels and bilateral uterine artery diastolic notch presence, are candidates for pregnancy complications and these groups of patients should be followed up more intensively (JPMA 60:181; 2010).

Introduction

Second trimester maternal serum screening (MSS) test has become a routine obstetric practice in many countries. Low concentrations of maternal serum AFP (MSAFP) and unconjugated estriol with high human chorionic gonadotropin (hCG) levels are associated with an increased risk for Down syndrome.[1,2] Although maternal serum hCG is often elevated when a specific foetal chromosome abnormality or more than one foetus is present, other reasons for increased maternal serum hCG are not well established.[2] Several studies have noted that elevated hCG is associated with foetal growth restriction, preterm birth and maternal hypertension.[1-4] Elevated hCG was found in a high proportion of patients with obstetric complications and may be associated with increased risk for foetal death.[1-4]

Elevated MSAFP levels are seen in association with structural foetal anomalies, including open neural tube defects, abdominal wall defects, and placental anomalies.[1] Unexplained second trimester elevations in MSAFP have been associated with adverse obstetric outcomes, including preeclampsia, low birth weight, preterm birth, intrauterine growth restriction, abruption placenta and foetal death.[5-8]

Approximately 1% of patients have elevated MSAFP and/ or HCG levels which were not a cause of incorrect dates, structural or chromosomal abnormalities, or multiple gestations.[1] The unexplained elevated levels of hCG and/or AFP are thought to reflect early placental pathology that may be associated with the problem later in pregnancy.[1,4-8] These complications are preeclampsia, low birth weight (LBW), preterm delivery, intrauterine growth restriction, abruption placenta and foetal death.[1,4-8] In these high risk pregnancies, Doppler velocity waveforms of the uterine arteries possess a high predictive value regarding adverse prenatal outcome.[5] In the literature according to Campbell et al, elevated uterine artery resistance index at 16 to 18 weeks' gestation is associated with an increased risk for preeclampsia and intrauterine growth restriction (IUGR).[9] According to Bower et al including early diastolic notch in definition of an abnormal waveform improves the sensitivity for predicting preeclampsia and IUGR.[10]

The purpose of this study was to further examine the relationship between unexplained elevated hCG and/or AFP levels, Doppler velocity waveforms of the uterine arteries and adverse pregnancy events.

Methods

We designed a COHORT study to test the hypothesis that the presence of unexplained elevated hCG and/or AFP levels and abnormal Doppler waveforms of uterine artery might be associated with an elevated risk of obstetric complications. The study was approved by the Kartal Education and Research Hospital ethics committee and informed consent was obtained from all patients. This study was conducted between January 2004-2007 at perinatology clinic of our hospital. Structurally normal 144 singleton pregnancies who underwent triple serum screening test at 16-20 gestational weeks and who agreed to obtain further diagnostic evaluation were enrolled into the study. Patients with poor previous obstetric history, incorrect dating, placenta previa and gestational diabetes were excluded. The study group consisted of 84 consecutive patients who applied to our perinatology clinic with unexplained elevated hCG and/or AFP levels equal to or higher than 2 MoM (Multiples of the Median). The control group (n=60) was composed of randomly chosen patients with hCG and AFP levels less than 2 MoM.

The study group was further subdivided into three subgroups. Subgroup I; only maternal serum AFP $\geq$ 2 MoM (n=30), subgroup II; only maternal serum hCG $\geq$ 2 MoM (n=64) and subgroup III; both AFP and hCG $\geq$ 2 MoM (n=10). During calculations we compared each subgroups with all of the other patients [for example; subgroup I (MSAFP $\geq$ 2 MoM, n=30) with all of the other patients who have MSAFP<2 MoM (n=114)].

All patients had second trimester ultrasound evaluations and Doppler velocimetry studies at 22-24 weeks of gestation. Levels of hCG and AFP were reported in MoM for each gestational age and corrected for race, diabetes and maternal weight. The serum assays of triple screening test were analyzed in our hospital biochemistry laboratory and the risk was calculated using Immulite 2000 equipment with immunochemiluminometric method and PRISCA. Each sonogram was performed by a single experienced ultrasonographer with a Diasonic Synergy ultrasound machine (General Electric, Norway) using a color Doppler probe. Uterine artery velocimetry was performed in all patients. The waveform was visually analyzed for the presence or absence of a diastolic notch. The presence of bilateral diastolic notch or resistance index equal to or greater than 0.7 (above the 95 % percentile) and pulsatility index equal to or greater than1.63 (above the 95 % percentile) were considered abnormal. All pregnant women were followed up until delivery. Mode of delivery, gestational age, birth weight and complications were recorded. Pregnancy outcome was defined as adverse if one of the following outcomes had been observed: foetal death, preeclampsia (blood pressure greater than 140/90 mmHg after 20 weeks of gestation with generalized oedema and proteinuria), preterm delivery (delivery before 34 weeks of gestation), delivery of a small infant for gestational age [SGA] (baby's weight <10th percentile according to gestational age), low birth weight [LBW] (baby's weight <2500 gram) and preterm premature rupture of membranes (PPROM).

Statistical calculations were performed with Graph Pad Prisma V.3 programme for Windows. Besides standard

descriptive statistical calculations (mean and standard deviations), unpaired t test was used in the comparison of groups. Chi square test and Relative Risk (RR) were performed during the evaluation of qualitative data. The results were evaluated within 95 % confidence interval. Statistical significance level was established at $p<0.05$.

Results

Mean maternal age of study group (29.15 ± 4.53) and control group (28.37 ± 4.70) was similar. Birth week, number of performed amniocentesis and foetal sex were similar in both study and control groups (Table-1). But operative delivery rate (p=0.0017) and complication rate (p=0.0002) were higher in the study group than in the control group (Table-1). As a result birth weight was significantly lower in the study group (p=0.045) (Table-1).

When subgroup I (MSAFP ≥ 2 MoM, n=30) was compared with all of the other patients who had MSAFP<2 MoM (n=114), LBW (p=0.0008), preterm delivery (p= 0.0001), preeclampsia (p= 0.003) and PPROM (p=0.012) rates were significantly higher. The results of elevated MSAFP and the risk of adverse outcomes are summarized in Table-2. Unexplained elevated MSAFP predicted LBW (RR= 2.35, CI 95 %= 1.34-4.12), preterm delivery (RR=3.8, CI 95 %= 1.9-7.58), preeclampsia (RR= 2.53, CI 95 %= 1.26-5.05) and PPROM (RR=3.25, CI 95 %=1.18-8.97).

In subgroup II (hCG ≥ 2 MoM, n=64) patients only SGA baby rate (p=0.016) was higher than all of the other

Table-1: Comparison of two groups' characteristics and Doppler study results.

		Study Group (n*= 84)	Control Group (n*= 60)	
Birth Week		36.38 ± 4.72	37.50 ± 4.09	NS**
Birth Weight (Grams)		2807.62 ± 926.92	3211.50 ± 926.23	0.045
Foetal Sex	Female	40 (48)	29 (48)	
	Male	44 (52)	31 (52)	NS**
Type of delivery	Vaginal delivery	37 (44)	36 (60)	
	Caesarean delivery	47 (56)	24 (40)	0.0017
Obstetric complications	Low birth weight	25(30)	9(15)	
	Preterm delivery	17(20)	7(12)	
	Preeclampsia	19(23)	6(10)	
	Preterm premature rupture of membrane	9(11)	4(7)	
	Small for gestational age	26(31)	8(13)	
	In utero mort foetalis	3 (4)	3 (5)	0.0002
Amniocentesis	Performed	13 (16)	6 (10)	
Doppler study results	Bilateral positive early diastolic notch	17 (20)	4 (7)	0.015
	Resistance Index ≥ 0.7	38 (45)	18 (30)	NS**
	Pulsatility Index ≥ 1.63	16 (19)	5 (8)	NS**

n*: Number of Patients (%). NS**: Not Significant.

Table-2: Comparison of subgroup I, II, III patients with all the other patients regarding adverse obstetric outcome.

AFP†	hCG††	Both AFP and hCG								
		≥2MoM* (n**=30)	<2MoM (n=114)	RR*** (95% CI)	≥2MoM (n=64)	<2MoM (n=80)	RR (95%CI)	≥2MoM (n=10)	<2MoM (n=134)	RR (95%CI)
Low Birth Weight		13	21	2.35	19	15	1.58	7	27	3.47
		-43	-18	(1.34-4.12)	-30	-19	(0.87-2.86)	-70	-20	(2.04-5.88)
Preterm delivery		12	12	3.8	13	11	1.47	8	16	6.7
		-40	-11	(1.9-7.58)	-20	-14	(0.71-3.07)	-80	-12	(3.84-11.66)
Preeclampsia		10	15	2.53	12	13	1.15	3	22	1.82
		-33	-13	(1.26-5.05)	-19	-16	(0.56-2.35)	-30	-16	(0.65-5.07)
Preterm Premature Rupture of Membranes		6	7	3.25	7	6	1.45	4	9	5.95
		-20	-6	(1.18-8.97)	-11	-8	(0.51-4.12)	-40	-7	(2.21-15.98)
Small for Gestational Age		7	27	0.98	22	12	2.29	3	31	1.29
		-23	-24	(0.47-2.03)	-34	-15	(1.23-4.26)	-30	-23	(0.47-3.51)
In Utero Mort Foetalis		3	3	3.8	1	5	0.25	1	5	2.68
		-10	-3	(0.8-17.8)	-2	-6	(0.02-2.08)	-10	-4	(0.34-20.79)

†: Alpha fetoprotein
††: Human chorionic gonadotrophin
*: Multiples of median
n** : number of patients, ( ) shows percentage of the patients
RR (95 % CI)***: Relative Risk (95 % Confidence interval)

patients who have hCG <2 MoM (n=80). Unexplained elevated hCG predicted only SGA (RR=2.29, CI 95 %= 1.23-4.26) (Table-2).

When subgroup III patients (AFP and hCG ≥ 2 MoM, n=10) were compared with all of the other patients (AFP and/or hCG < 2 MoM, n=134) preterm delivery rate (p=0.0001), LBW (p=0.009) and PPROM (p=0.01) rates were significantly high. Unexplained elevated hCG and MSAFP predicted LBW (RR= 3.47, CI 95 %= 2.04-5.88), preterm delivery (RR=6.7, CI 95 %= 3.84-11.66) and PPROM (RR=5.95, CI 95 %=2.21-15.98) (Table-2).

Looking at the Doppler study results bilateral early diastolic notch presence rate was higher in the study group (n=17, 20 %) than in the control group (n=4, 7 %), (p= 0.015) (Table-1). Among these 17 patients in the study group, 12 patients (14 %) developed obstetric complications [preterm delivery n=3 (4 %), preeclampsia n= 3 (4 %), HELLP syndrome n=1 (1 %), SGA n=3 (4 %), IUMF n=2 (2 %)].

When we compared the three subgroups according to pathologic Doppler study results (bilateral early diastolic notch presence, RI ≥ 0.7, PI ≥ 1.63) there was no statistically significant difference except subgroup II patient's PI values (p=0.028) (Table-3).

Table-3: Comparison of three subgroups according to Doppler study results.

	Early diastolic notch bilateral positive	p	Resistance index ≥0.7	p	Pulsatility index ≥1.63	p
AFP† ≥ 2 MoM (n=30)	5	NS**	15	NS**	4	NS**
hCG†† ≥ 2 MoM (n=64)	12	NS**	29	NS**	15	0.028
BothAFP and hCG ≥ 2 MoM (n=10)	1	NS**	6	NS**	3	NS**

†: Alpha fetoprotein
††: Human chorionic gonadotrophin
n* : number of patients
NS**: Not significant.

Discussion

In modern supervision of pregnancy first trimester screening is more preferable than second trimester screening. But, especially in developing countries first trimester screening is still not routine or cannot be done easily because first trimester screening is relatively a newer modality than second trimester screening and learning curve is not complete. Also, patients especially in rural areas don't come to antenatal control visits in first trimester so automatically first trimester screening is not possible in all patients. In these group of patients second trimester screening is still a very important method of biochemical screening.

MSAFP and hCG are glycoproteins produced by foetal yolk sac and syncytiotrophoblast, respectively.[4] Increased MSAFP levels can be seen under some conditions like neural tube defects, ventral wall defects, teratoma, cystic hygroma and congenital viral infections and perinatal loss.[4] Maternal serum hCG is often elevated in some specific foetal chromosomal abnormalities, multiple gestation and prenatal loss.[4]

Unexplained MSAFP elevations in such cases are most likely the result of transplacental leakage of AFP from the foetal to the maternal circulation. This may be due to functional or structural abnormalities of the placenta providing an increased area of transport or due to a defective endothelial barrier.[6] Indeed, placental and cord anomalies have been found in association with increased MSAFP level in several studies.[7,8] This may suggest that early placental pathology permits a more rapid diffusion of AFP from the feto-placental compartment to the maternal compartment.

Abnormally increased levels of hCG may be due to decreased placental perfusion with subsequent reduced oxygenation of the cytotrophoblast, leading to increased hCG production.[11] This hypoxia induced cytotrophoblast proliferation has also been demonstrated in histological studies.[11] Placental pathology at delivery (such as infarction, ischaemic changes, villitis and intervillous thrombosis), confined placental mosaicism and velamentous cord insertion have been found to be associated with hCG overproduction.[7,8,11,12] Another possible explanation may be inadequate trophoblastic remodeling of the maternal uterine vasculature, with an absence of normal physiologic changes in the spiral arteries leading to placental hypoxia and hCG overproduction.[13]

Given that the underlying pathophysiologic reason of many obstetric complications involves placental dysfunction on some level, it would be logical to claim that pregnancies complicated by elevated MSAFP and/or hCG levels could have higher rates of adverse events.[14]

Katz et al found that elevated maternal serum AFP (MSAFP) has been found to be associated with a 2- to 4-fold increase in low birth weight resulting from both preterm

delivery and intrauterine growth retardation. Unexplained MSAFP elevations are also associated with up to 10-fold increase of placental abruption and a 10-fold increase in perinatal mortality.[15]

Gonen et al said that patients with elevated levels of hCG had a significantly higher risk for hypertension and foetal growth restriction.[16]

Hurley et al found that pregnant women with an unexplained elevation in MSAFP, who also had an abnormal MShCG (< or = 0.5 MoM > or = 2.5) were at significantly greater risk of delivering a low-birth-weight infant compared to women with a normal hCG.[17]

The result of our study confirms previously reported observations of increased maternal and perinatal complications in patients with unexplained elevated MSAFP and/or hCG levels. In our study, we found that patients with unexplained elevated MSAFP levels had high risks of preterm delivery, LBW, preeclampsia and PPROM. Patients with unexplained elevated hCG levels had higher risk of having a SGA baby. Patients with both elevated unexplained MSAFP and hCG levels had high risk of preterm delivery, LBW and PPROM. These results are compatible with the literature.[15-17]

Women with elevated MSAFP and/or hCG levels are usually referred for second trimester amniocentesis. This procedure involves a small risk of iatrogenic loss (0.5%). Several studies have shown that the association between elevated marker levels and foetal death is independent of amniocentesis.[18,19]

In the literature the finding of biateral early diastolic notch in the uterine artery Doppler velocimetry waveforms is a sensitive method for predicting unfavourable pregnancy outcomes.[20,21] The Doppler velocimetry study results of our study were similar with the literature. Bilateral early diastolic notch presence was seen in 17 patients of study group and 70 % of these patients developed obstetric complications. But bilateral early diastolic notch presence was seen in only 4 patients of control group and 1 patient among them (25 %) developed obstetric complication.

We considered the mean resistance index (RI) greater than or equal to 0.7 (above the 95th percentile) and the mean pulsatility index (PI) greater than or equal to1.63 (above the 95th percentile) as abnormal according to the literature.[22,23] Complications that are characterized by spiral artery vasculopathy and resultant increased uterine artery resistance are suitable for detection with Doppler studies.[3,23-25] But in our study pathologic Doppler study results (RI $\geq$ 0.7, PI $\geq$ 1.63) were not statistically significant. This is may be the result of a small study population.

In conclusion second trimester triple screening test is an important and beneficial test to triage patients with high risk of adverse pregnancy outcome, because high MSAFP and/or hCG levels are strongly related with preterm delivery, LBW, preeclampsia, PPROM and SGA baby. Early diastolic notch presence in uterine artery is also an important predictive method of adverse pregnancy outcome too.

In conclusion, if a patient has MSAFP and/or hCG $\geq$ 2 MoM and early diastolic notch in uterine artery it is advisable to consider the patient as a high risk patient and follow up accordingly.

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