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Evaluation of the impact of triple test results on perinatal outcomes

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Abstract

Objective: This study aims to determine the relationship between the poor perinatal outcomes and maternal serum alpha-fetoprotein (AFP), beta human chorionic gonadotropin (β-hCG) and unconjugated estriol (uE3) MoM values during second trimester.

Methods: Maternal serum AFP, ,–hCG and uE3 MoM values and perinatal outcomes of 1111 pregnant women who admitted to our clinic for second trimester screening test were analyzed. The patients with abnormal MoM values and the patients with normal values were compared in terms of poor perinatal outcomes.

Results: In our study, it was found that there was a significant relationship between elevated maternal serum AFP levels (≥2 MoM) and the hypertension development induced by pregnancy, and that the risk increased 4 times in these cases. Significant relationship was observed between elevated β-hCG levels (≥2 MoM) and pregnancy-induced hypertension and isolated fetal growth retardation development, and it was found that the risk of pregnancy-induced hypertension increased 3 times while the risk of isolated fetal growth retardation increased 2 times. There was no significant relationship between decreased uE3 levels (<0.5 MoM) and poor perinatal outcomes. In pregnant women whose maternal serum AFP and β-hCG levels elevated together (≥2 MoM), there was a significant increase in the incidence rate of pregnancy-induced hypertension and it was found that the risk increased 16 times. The incidence of perinatal complication was found significantly low in cases with normal levels of maternal serum AFP, β-hCG and uE3 values.

Conclusion: Serum markers evaluated in second trimester screening test may be used to identify aneuploidy and congenital anomalies as well as high risk pregnancies. In our study, we found association between poor perinatal outcomes with elevated maternal serum AFP and β-hCG levels.

Keywords: AFP, β-hCG, perinatal outcome, triple test, uE3.

Özet: Üçlü test sonuçlarının perinatal sonuçlar üzerine etkisinin değerlendirilmesi

Amaç: Bu çalışma ikinci trimesterde maternal serum alfa fetoprotein (AFP), beta human koryonik gonadotropin (β-hCG) ve unkonjuge estriol (uE3) MoM değerleri ile olumsuz perinatal sonuçlar arasındaki ilişkinin belirlenmesi amacıyla yapılmıştır.

Yöntem: Kliniğimize ikinci trimester tarama testi için başvuran 1111 gebenin maternal serum AFP, β-hCG ve uE3 MoM değerleri ve perinatal sonuçları incelendi. Anormal MoM değerlerine sahip hastalar, normal değerleri olan hastalarla olumsuz perinatal sonuçlar açısından karşılaştırıldı.

Bulgular: Çalışmamızda artmış maternal serum AFP seviyeleri (≥2 MoM) ile gebeliğin indüklediği hipertansiyon gelişimi arasında anlamlı ilişki görüldü ve bu olgularda riskin 4 kat arttığı saptandı. Artmış β-hCG seviyeleri (≥2 MoM) ile gebeliğin indüklediği hipertansiyon ve izole fetal büyüme kısıtlılığı gelişimi arasında anlamlı ilişki görüldü ve gebeliğin indüklediği hipertansiyon riskinin 3 kat, izole fetal büyüme kısıtlılığı riskinin ise 2 kat artmış olduğu saptandı. Azalmış uE3 seviyeleri (<0.5 MoM) ile olumsuz perinatal sonuçlar arasında anlamlı ilişki görülmedi. Maternal serum AFP ve β-hCG seviyelerinin beraber yükseldiği (≥2 MoM) gebelerde gebeliğin indüklediği hipertansiyon görülme oranında anlamlı artış saptandı ve riskin 16 kat arttığı görüldü. Maternal serum AFP, β-hCG ve uE3 ölçüm değerleri normal olan olgularda perinatal komplikasyon görülme oranı anlamlı olarak düşük saptandı.

Sonuç: İkinci trimester tarama testinde değerlendirilen serum belirteçleri, anöploidi ve konjenital anomalileri ve beraberinde yüksek riskli gebelikleri saptamada kullanılabilir. Çalışmamızda açıklanamayan artmış maternal serum AFP ve β-hCG seviyeleri kötü perinatal sonuçlarla ilişkili bulundu.

Anahtar sözcükler: Üçlü test, perinatal sonuç, AFP, β-hCG, uE3.

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Introduction

Biochemical markers of maternal serum alpha-fetoprotein (AFP), beta human chorionic gonadotropin (β-hCG) and unconjugated estriol (eE3) serum checked in the second trimester screening test are used to screen aneuploidies such as trisomy 21 and trisomy 18, and congenital anomalies such as neural tube defects.[1,2]

Positive prenatal screening tests may be associated with aneuploidies such as trisomy 21 and trisomy 18, and congenital anomalies such as abdominal anterior wall defects and neural tube defects.[3,4] It was also found that there is a relationship between poor perinatal outcomes and abnormal results after aneuploidy and congenital anomalies such as neural tube defects are excluded.[5,6]

This study was conducted to investigate the relationship between high AFP and/or β-hCG and low uE3 levels and poor prenatal outcomes including perinatal complications such as gestational diabetes mellitus (GDM), preterm labor, isolated fetal growth retardation, pregnancy-induced hypertension, preterm premature rupture of membrane, stillbirth, ablatio placentae, cholestasis, placenta previa etc.

Methods

This is a retrospective study carried out on 1111 pregnant women between July 2012 and March 2013. The study was started with 2240 women with singleton pregnancies who referred for routine antenatal second trimester screening test between 16 and 20 weeks of gestation. A total of 1079 pregnant women who did not undergo their routine antenatal follow-ups until delivery in our hospital and 50 pregnant women that we could not access to their triple test results were not included. Multiple pregnancies and those with congenital anomalies such as known chromosomal anomaly, neural tube defect and abdominal anterior wall defect were excluded from the study. Perinatal records of 1111 pregnant women before and after delivery who underwent their follow-ups and deliveries in our clinic and included to our study were reviewed. Of all patients, demographic information, delivery age, week of gestation, delivery type, newborn gender, birth weight, Apgar scores, the values of β-hCG, AFP and uE3 MoM found by triple screening test were screened and recorded. Gestational age was calculated according to the last menstrual period and according to biparietal diameter measured during triple test in those with unknown last menstrual period. AFP and uE3 values were calculated as nanogram/milliliter, and β-hCG values as international unit/milliliter. AFP values were adapted according to the maternal weight, but β-hCG and uE3 values were not adapted. All three values were arranged for gestational age by using multiples of the median.

First, cut-off values of triple test serum markers were determined on the basis of the study carried out by Dugoff et al.[7] The cut-off value for AFP and β-hCG was 2 MoM while it was 0.5 MoM for uE3. In the triple screening test results, the biochemistry values found to be below 2 MoM for AFP and β-hCG and above 0.5 MoM for uE3 were considered to be normal. Patients with abnormal values for AFP, β-hCG and uE3 were compared with patients having normal values for each analysis both separately and together in terms of obstetric complications.

The incidence rates of pregnancy-induced hypertension, isolated fetal growth retardation, ablatio placentae, preterm labor, preterm premature rupture of membrane, oligohydramnios, GDM, and stillbirth were found to conform to the criteria determined clinically.

Preeclampsia was considered to be as proteinuria (≥0.3 g proteinuria in 24-h urine or, if quantitative measurement is not possible, 1+ and above proteinuria in spot urine) or target organ dysfunction (platelet count <100.000/microliter, serum creatinine >1.1 mg/dl or double amount of serum creatinine without any other renal disease, at least 2 times of normal concentrations of hepatic transaminases, pulmonary edema, cerebral visual symptoms) accompanying to the new-onset hypertension (systolic blood pressure being ≥140 mmHg or diastolic blood pressure being ≥90 mmHg two times with at least 4-hour interval, systolic blood pressure being ≥160 mmHg or diastolic blood pressure ≥90 mmHg two times with a few minute of intervals) after 20 weeks of gestation in women who were normotensive previously. Two or more of the values (fasting as ≥95 mg/dl, first hour as ≥180 mg/dl, second hour as ≥155 mg/dl, third hour as ≥140 mg/dl) of 100g oral glucose tolerance test being high according to ACOG 1994 criteria were considered as gestational diabetes mellitus. Severe vaginal bleeding which could not be explained with extrauterine reasons was defined as placenta previa in a pregnant woman with normal localized placenta. ACOG (American Congress of Obstetricians and Gynecologists) only defines intrauterine fetal death above 500g as fetal death. Deaths above 28 weeks of gestation (late fetal deaths) and within first week of birth were considered as perinatal mortality. According to the growth curves based on predicted fetal weight measured with ultrasonography, pregnancies in which those below 10th

percentile but constitutional were excluded and those exhibiting progressive deviation from growth curve during 3-week follow-up and having concurrent oligohydramnios and pathologic fetal Doppler findings were defined as fetal growth retardation. Amniotic fluid index being ≤5 cm was considered as oligohydramnios. Preterm labor was defined as the delivery carried out with cervical dilatation and effacement accompanying to uterine contraction before 37 weeks of gestation. Preterm premature rupture of membrane was considered as the rupture of amniotic membrane and breaking of amniotic fluid before the labor and 37 weeks of gestation.

NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and Sample Size) 2008 Statistical Software (Kaysville, UT, USA) was used for statistical analyses. For comparing descriptive statistical methods (mean, standard deviation, median, frequency, rate, minimum, maximum) with continuous variables when evaluating study data, Student t test was used in the two-group comparison of variables displaying normal distribution, and Mann-Whitney U test in the two-group comparison of parameters not displaying normal distribution. In the comparison of discontinuous variables, Pearson's chi-square test, Fisher's exact test and Yates' continuity correction test were used. Significance level was considered as $p<0.05$.

Results

When demographic data of the cases were evaluated, mean maternal age was found as 30.7±6.12 years and age range was found as 18–49, birth weight of babies was between 330 and 4860g, and mean weight was 3218.12±650.16 g (**Table 1**).

In 45 cases included in the study, AFP MoM value was found as 2 and above (**Table 2**). Relationship was found between elevated maternal serum AFP levels and pregnancy-induced hypertension ($p<0.01$) and it was seen that the risk increased 4 times (OR=4.299, 95% CI 1.904–9.705) (**Table 3**).

In 124 cases included in the study, β-hCG MoM value was found as 2 and above (**Table 2**). It was found that there was a relationship between elevated β-hCG MoM values and pregnancy induced hypertension ($p<0.01$) and isolated fetal growth retardation ($p<0.05$) and it was seen in these cases that the risk increased 3 times in pregnancy-induced hypertension (OR=3.245, 95% CI 1.768–5.958) and 2 times in isolated fetal growth retardation (OR=2.3, 95% CI 1.148–4.609) (**Table 3**).

Table 1. Distribution of demographic characteristics.

		Min – Max	Mean±SD
Maternal age		18–49	30.07±6.12
Birth weight of baby		330–4860 g	3218.12±650.16 g
1-minute Apgar score		0–10	7.99±1.36
5-minute Apgar score		0–10	9.12±1.25
		N	%
Week of gestation	>37 weeks	966	86.9
	<37 weeks	144	13.0
Delivery type	Normal	638	57.4
	Cesarean section	473	42.6
	Emergency	348	73.6
	Elective	125	26.4
Gender of baby	Female	522	47.0
	Male	589	53.0
Number of smoking pregnant women		79	71.1
Intense care need	Baby	79	7.1
	Mother	1	0.1

In 40 cases included in the study, uE3 MoM value was found as 0.5 and below (**Table 2**). In these cases, there was no significant difference in terms of the development of poor perinatal outcomes (**Table 3**).

In cases where maternal serum AFP and β-hCG levels elevated together, a significant relationship was found in the pregnancy-induced hypertension ($p<0.01$) and it was seen that the risk increased 16 times (OR=16.11, 95% CI 4.766–54.458).

In cases in which these markers were normal, poor perinatal outcomes were lower.

The number of pregnant women whose perinatal outcomes could not be found was 839. It was found that 50 cases had GDM, 59 cases had hypertensive diseases induced by pregnancy, 70 cases had preterm

Table 2. Distribution of AFP, β-hCG and uE3 MoM variables.

	Min – Max	Mean ±SD	
AAFP MoM	0.23–5.58	1.05±0.48	
β–hCG MoM	0.12–10.40	1.16±0.77	
uE3 MoM	0.08–2.74	1.07±0.37	
		N	%
AFP MoM	<2 MoM	1065	95.9
	≥2 MoM	45	4.1
β-hCG MoM	<2 MoM	986	88.8
	≥2 MoM	124	11.2
uE3 MoM	>0.5 MoM	1070	96.4
	≤0.5 MoM	40	3.6

Alpha fetoprotein (AFP) values were given as ng/ml, beta human chorionic gonadotropin (β-hCG) values as mIU/ml, and unconjugated estriol (uE3) values as ng/ml.

Table 3. Evaluations according to AFP, β-hCG and uE3 MoM levels.

		AFP MoM			β-hCG MoM			uE3 MoM		
		<2 MoM (n=1065)	≥2 MoM (n=45)	p	<2 MoM (n=985)	≥2 MoM (n=124)	p	>0.5 MoM (n=1070)	<0.5 MoM (n=40)	p
		Mean±SD (Median)	Mean±SD (Median)		Mean±SD (Median)	Mean±SD (Median)		Mean±SD (Median)	Mean±SD (Median)	
		n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
maternal age		30.02±6.11	31.11±6.29	[a]**0.242**	29.99±6.07	30.67±6.49	[a]**0.243**	30.11±6.11	28.98±6,20	[a]**0.251**
Birth weight of baby		3242.68± 621.41	2667.78± 978.62	[b]**0.001****	3235.82± 611.47	3088.55± 886.20	[b]**0.456**	3229.19± 638.99	2956.75± 846.11	[b]**0.106**
1-minute Apgar score		8.02±1.30	7.38±2.22	[b]**0.067**	8.01±1.32	7.80±1.58	[b]**0.088**	8.00±1.34	7.68±1.64	[b]**0.116**
5-minute Apgar score		9.15±1.19	8.47±2.24	[b]**0.025***	9.15±1.24	8.95±1.39	[b]**0.084**	9.14±1.24	8.83±1.65	[b]**0.101**
Perinatal complication	Complication is present	249 (23.4)	22 (48.9)	[c]**0.001****	229 (23.2)	41 (33.1)	[e]**0.016***	256 (23.9)	15 (37.5)	[c]**0.076**
	Gestational diabetes mellitus	47 (4.4)	3 (6.7)	[d]**0.451**	47 (4.8)	3 (2.4)	[c]**0.337**	49 (4.6)	1 (2.5)	[d]**1.000**
	Gestational hypertensive diseases	51 (4.8)	8 (17.8)	[d]**0.002****	43 (4.4)	16 (12.9)	[c]**0.001****	55 (5.1)	4 (10.0)	[d]**0.158**
	Preterm labor	64 (6.0)	6 (13.3)	[d]**0.058**	63 (6.4)	7 (5.6)	[c]**0.898**	65 (6.1)	5 (12.5)	[d]**0.101**
	Premature rupture of membrane	8 (0.8)	1 (2.2)	[d]**0.312**	8 (0.8)	1 (0.8)	[d]**1.000**	9 (0.8)	0 (0.0)	[d]**1.000**
	Placentae previa	6 (0.6)	0 (0.0)	[d]**1.000**	4 (0.4)	2 (1.6)	[d]**0.138**	6 (0.6)	0 (0.0)	[d]**1.000**
	Cholestasis	2 (0.2)	0 (0.0)	[d]**1.000**	2 (0.2)	0 (0.0)	[d]**1.000**	2 (0.2)	0 (0.0)	[d]**1.000**
	Ablatio placentae	4 (0.4)	0 (0.0)	[d]**1.000**	4 (0.4)	0 (0.0)	[d]**1.000**	4 (0.4)	0 (0.0)	[d]**1.000**
	Fetal growth retardation	48 (4.5)	3 (6.7)	[d]**0.457**	40 (4.1)	11 (8.9)	[c]**0.029***	47 (4.4)	4 (10.0)	[d]**0.107**
	Oligohydramnios	11 (1.0)	0 (0.0)	[d]**1.000**	10 (1.0)	1 (0.8)	[d]**1.000**	11 (1.0)	0 (0.0)	[d]**1.000**
	Stillbirth	7 (0.7)	1 (2.2)	[d]**0.283**	8 (0.8)	0 (0.0)	[d]**0.608**	7 (0.7)	1 (2.5)	[d]**0.255**

[a]Student-t test, [b]Mann-Whitney U test, [c]Yates continuity correction test, [d]Fisher's exact test, [e]Pearson Chi-Square, *p<0.05, **p<0.01

labor, 10 cases had premature rupture of membrane, 6 cases had placenta previa, 2 cases had cholestasis, 4 cases had ablatio placentae, 11 cases had oligohydramnios and 8 cases had stillbirth (**Fig. 1**).

Discussion

It was found in the studies performed that the risk for developing poor perinatal outcomes during advanced weeks of pregnancy increased in normal fetuses with

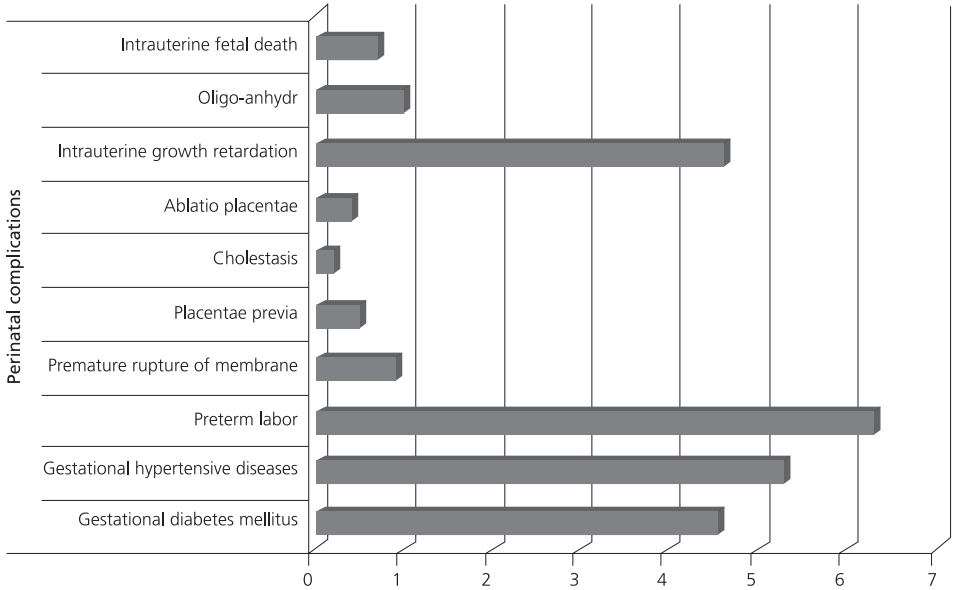

Fig. 1. Distribution of perinatal complications.

unexplained elevated maternal serum AFP and β-hCG levels and decreased uE3 levels.[8] It was shown in the study of Sagol et al. that the risk for developing obstetric complication in pregnant women below 35-year-old with false positivity in triple test results increased for 3.6 times.[9]

The relationship between elevated maternal serum AFP levels and preterm labor, preeclampsia and fetal loss before 24 weeks of gestation is known.[10] Tikkanen et al. showed the relationship between elevated second trimester maternal serum AFP and ablation placenta.[11] In our case, we observed ablatio placenta in 4 cases but found no significant relationship with AFP levels.

Proctor et al. found that fetal growth retardation, preterm labor and still birth risk before 32 weeks of gestation and elevated second trimester maternal serum AFP were associated.[12] Spaggiari et al. also reported same findings.[13] In our study, we found statistically significant increase in the incidence rate of pregnancy-induced hypertension in elevated AFP (>2 MoM) levels and 4 times increased risk, but no statistically significant difference in other complications (GDM, preterm labor, premature rupture of membrane, fetal growth retardation and stillbirth) although there was increase.

Dugoff et al. analyzed serum screening results of 33,145 patients included in the multicentered FASTER study and they found significant increase in the risk of developing poor perinatal outcome in patients with two or more abnormal markers.[7] Baer et al. found in the consecutive integrated screening test that poor perinatal outcomes including also fetal loss and preterm labor increased in false positive results and when all poor perinatal outcomes were analyzed, they found the risk higher in cases with more than one marker was abnormal compared to the cases with high level of AFP only.[14] In pregnant women whose maternal serum AFP and β-hCG levels elevated together (≥2 MoM), there was an increase in the incidence rate of pregnancy-induced hypertension and it was found that the risk increased 16 times in our study. In our study, we found statistically significant low levels for perinatal complication rate in cases with normal AFP, β-hCG and uE3 values compared to the cases with abnormal values.

The relationship between abnormal β-hCG and placental pathologies is known.[15] Physiological changes do not occur in spiral arterioles associated with insufficient trophoblast invasion developing during the first weeks of gestation, and therefore uteroplacental circulation is degenerated. Hyperplasia occurs in syncytiotrophoblasts as a result of hypoxia developing in placental tissue and β-hCG production increases.[16,17] According to another study, there was a relationship between elevated β-hCG (>2.5 MoM) and preterm labor, low birth weight and fetal growth retardation.[18] In the study of Bakır et al., cases with β-hCG >2.5 MoM were found to be associated with poor perinatal outcomes.[19] In our study, we found the rate of pregnancy-induced hypertension and isolated fetal growth retardation in cases whose β-hCG levels were ≥2 MoM was significantly higher, and that the risk was 3 times higher in pregnancy-induced hypertension and 2 times higher in isolated fetal growth retardation.

In some studies in the literature, it was reported that low pregnancy associated plasma protein A (PAPP-A) and uE3 values were associated with poor perinatal outcomes[9,13,20,21] and that low uE3 levels (<0.5 MoM) were associated with poor perinatal outcomes such as pregnancy-induced hypertension, fetal growth retardation and oligohydramnios.[22–25] We did not find any statistically significant difference between low levels of uE3 MOM and perinatal complications in our study.

It is known that serum parameters predict gestational complications at a certain rate. Also, it is not clear if these relationships are independent from other factors or not. In addition, presence of a relationship does not show that these parameters can be used in clinic safely. On both regards, this study has limitations. The relationships found were not tested by using regression analyses if they have an impact independent from other factors. Also, positive and negative predictive values were not calculated. Despite all these deficiencies, the research results may be of vital importance for arousing the attention of obstetricians and gynecologists in Turkey towards considering also the outputs of triple test MoM values except trisomy.

Conclusion

According to the results of our study, unexplained elevated maternal serum AFP and β-hCG levels at second trimester are associated with poor fetomaternal outcomes. Therefore, it is required to inform such cases in detail, to explain possible complications and to follow up and evaluate these cases more carefully and in detail during their pregnancies.

Conflicts of Interest: No conflicts declared.

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