

## THE ROLE OF SECOND TRIMESTER MATERNAL SERUM MARKERS; PREDICTING TIME OF DELIVERY IN HEALTHY PREGNANCIES

### İKİNCİ TRİMESTER MATERNAL SERUM BELİRTEÇLERİNİN SAĞLIKLI GEBELERDE DOĞUM ZAMANINI ÖNGÖRMEDEKİ ROLÜ

Aysegül GULBAHAR Seda AKGUN KAVURMACI

İzmir Katip Celebi University, Ataturk Training and Research Hospital Department of Obstetrics and Gynecology, İzmir

**Keywords:** Alpha-fetoprotein, human chorionic gonadotropin, gestational age, doğum ağırlığı

**Anahtar Sözcükler:** Alfa-fetoprotein, insan koryonik gonadotropini, gestasyonel yaş, birth weight

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## SUMMARY

**Introduction:** Markers used in second-trimester anomaly screening are also believed to play a role in fetal growth and development. While these markers were initially used only to investigate pregnant women at risk for neural tube defects, they were later used to examine other anatomical malformations, aneuploidy, and third-trimester complications. This study aims to examine these serum markers in managing healthy pregnancies without risk factors and to investigate the effectiveness of these markers in predicting the birth week and planning the birth time.

**Material and Method:** The data of the patients who were admitted to Gynecology and Obstetrics Clinic of our hospital and who had given birth in our hospital were retrospectively examined and recorded.

Demographic data of patients, such as age, weight at birth, obstetric history, delivery method, gestational week during delivery and newborn weight, serum markers of triple screening test were retrospectively obtained from the hospital records.

**Results:** The study included 100 patients. Sixty-six of the patients delivered their babies through cesarean section and 34 by vaginally. A comparison was made between groups according to gestational age, weight of pregnant women, newborn weight and triple screening test parameters. No significant difference was observed between the groups according to screening test serum markers ( $p > 0,05$ ). According to regression analysis, positive statistical significance was obtained between gestational age and newborn weight in predicting the birth probability at 37 weeks and before ( $p = 0.005$ ,  $p < 0.0001$ ).

**Conclusion:** As a result, it has been observed that second-trimester screening markers were not significant on predicting the timing of birth in normal pregnant women. We believe that prospective studies in which only patients who had a vaginal delivery and had a history of cesarean section in the pregnant women with started labor were included in the study group may change the results.

## ÖZ

**Giriş:** Fetal büyüme ve gelişme fetusun genetik yapısının, çevresel faktörlerin, annenin ve plasentanın etkili olduğu multi-fonksiyonel bir durumdur. Bu mekanizmada anne, plasenta ve fetus tarafından salgılanan hormonlar belirleyici rol oynamaktadır.

*İkinci trimester anomali taramasında kullanılan belirteçlerinde bu mekanizmada rol aldığı düşünülmektedir. Bu belirteçler başlangıçta sadece risk altındaki gebeyi nöral tüp defektleri açısından araştırmak amacıyla kullanılırken, daha sonra diğer anatomik malformasyonlar, anöploidi ve üçüncü trimester komplikasyonlarının araştırılmasında da kullanılmaya başlanmıştır. Mevcut çalışmalar çoğunlukla kötü prognozu öngörmeye belirteçlerin etkinliğini araştırmaktadır. Bizim bu çalışmadaki amacımız ise risk faktörü olmayan sağlıklı gebeliklerin yönetiminde bu belirteçleri incelemek ve doğum haftasını öngörmeye, buna bağlı olarak da doğum zamanını planlamada etkinliğini araştırmaktır.*

**Gereç ve Yöntem:** 2017-2020 yıllarında hastanemiz Kadın Hastalıkları ve Doğum Kliniğine başvuran üçlü tarama sonucu olan ve hastanemizde doğum yapmış hastaların verileri retrospektif olarak incelendi. Çalışmaya dahil edilen gebelerin yaş, doğum kilosu, obstetrik öyküsü, doğum şekli, bebek doğum kilosu gibi demografik bilgileri ve 3'lü tarama testi belirteçlerinin sonuçları hastane kayıtlarından öğrenildi.  $p < 0.05$  istatistiksel olarak anlamlı kabul edildi.

**Bulgular:** Çalışmaya üçlü tarama testi olan ve hastanemizde doğum yapan 100 hasta dahil edildi. Hastalar gebelik doğum haftasına göre 37 hafta öncesi ( $n=38$ ) ve 37 hafta sonrası doğum ( $n=62$ ) yapanlar olarak gruplara ayrıldı. Gruplar arasında gebelik yaşı, gebelerin kilosu, yenidoğan kilosu ve 3'lü tarama testi parametrelerine göre karşılaştırma yapıldı. 3'lü tarama testi parametrelerine göre gruplar arasında anlamlı bir fark izlenmedi ( $p > 0,05$ ). 37 hafta ve öncesi doğum olasılığını öngörmeye regresyon analizine göre gebelik yaşı, bebek doğum kilosu ile pozitif yönde istatistiksel olarak anlamlılık gözlemlendi ( $p=0,005$ ,  $p < 0,0001$ ).

**Sonuç:** Çalışmamızda normal gebelerde doğum zamanlamasının öngörmeye ikinci trimester tarama belirteçlerinin anlamlı olmadığı gözlemlenmiştir. Çoğu hastanın sezaryan öyküsü olması ve elektif sezaryan günlerinin planlandığı bilinen bir gerçek olup sadece normal doğum yapan ve doğum eylemi başlamış gebelerde sezaryan öyküsü olan hastaların çalışma grubuna dahil edildiği, prospektif araştırmaların sonuçları değiştirebileceğini düşünmekteyiz.

## INTRODUCTION

Fetal growth and development is a multi-functional condition in which environmental factors, mother and placenta, as well as the genetic structure of the fetus, play an effective role. This condition is essentially under the influence of molecular mechanisms at the cellular level. Hormones secreted by the mother, placenta, and fetus play a decisive role in this mechanism.

For pregnant women who missed the combined test in the first trimester; triple or, preferably, quadruple screening test is recommended between 16th and 19th weeks. The triple screening test consists of alpha-fetoprotein (AFP), unconjugated estriol (uE3), and human chorionic gonadotropin (hCG) markers, whereas adding Inhibin-A forms the quadruple screening test.

Markers used in second-trimester anomaly screening, are also believed to play a role in this mechanism. While these markers were initially used only to investigate pregnant women at risk for neural tube defects, they were later used to examine other anatomical malformations, aneuploidy, and third-trimester complications (1,2).

Recent studies have shown that good nutrition of the fetus, which can be achieved with the fetoplacental unit's well-functioning, is the primary and necessary condition for fetal growth. It has been proven that AFP, uE3 and hCG are significant as markers in determining pregnancy prognosis (intrauterine growth retardation [IUGR], preeclampsia, abruptio placentae, and miscarriage risk) in cases where the fetoplacental unit is impaired (3-5).

Apart from the screening test, these conditions have not only brought forward the possibility that placental AFP and maternal-derived hCG and UE3 can be markers for predicting fetal development and course of pregnancy but also have given a fresh impetus to studies on this issue.

Current studies mostly examine the effectiveness of these markers in predicting poor prognosis. While one study states that an increase in AFP helps predict preterm labor, IUGR, and risk of fetal loss, another study notes that hCG values are essential in predicting preeclampsia (6,7).

This study aims to examine these serum markers in managing healthy pregnancies without risk factors and to investigate the effectiveness of

these markers in predicting the birth week and, accordingly, in planning the birth time.

## **MATERIALS AND METHODS**

The data of the patients who were admitted to Gynecology and Obstetrics Clinic of our hospital between 2017 and 2020 and who had given birth in this hospital were retrospectively examined and recorded.

Pregnant women with an obstetrics history of gestational diabetes, preeclampsia, intrauterine growth retardation, children with chromosomal and congenital anomalies were excluded from the study. Additionally women having multiple pregnancies, pregnancy with assisted reproductive techniques, chromosomal and congenital anomalies, having risks associated with neural tube defects, abdominal wall anomalies or other anomalies in their current pregnancy were also excluded from the study. Lastly, pregnant women using cigarettes, alcohol, and drugs (antiepileptic, antihypertensive, insulin, etc.) and having a history of diseases (hypertension, diabetes, asthma, epilepsy, etc.), were also not included in the study.

Demographic data of patients, such as age, weight at birth, obstetric history, delivery method, gestational week during delivery and newborn weight, were retrospectively obtained from the hospital records.

The serum markers of triple screening test, AFP and hCG was measured by electrochemiluminescence immunoassay (ECLIA) method, and unconjugated estriol (uE3) was measured by solid-phase competitive chemiluminescence enzyme immunometric assay method. The results obtained were divided into median values corresponding to the gestational week and converted into MoM (Multiple of Median) units and MoM values were used in the study.

The study was approved by the relevant institutional review board (18.02.2021/0047) and conducted in accordance with the principles of the Helsinki Declaration. The requirement of informed consent was waived due to the retrospective nature of the study.

## **Statistical Analysis**

All the analyses were performed using SPSS V22.0 Software (IBM®, NY, USA). Categorical data were indicated by number and percentage (%), while numerical data were provided as arithmetic mean±standard deviation (minimum-maximum). The Chi-square test was used to compare categorical data. The compliance of variables with normal distribution was examined by analytical methods (Kolmogorov-Smirnov/ Shapiro-Wilk tests). In data with a normal distribution, the T-test was used, and if the data were not with the normal distribution, the Mann-Whitney U test was conducted. Pearson Correlation Analysis was used to determine the relationship between two continuous variables. A multiple regression analysis models was performed to analyze the relationships between independent variables. Through ROC analysis, the curve of the true positives to the false positives was drawn, and the area under the curve was calculated.  $p < 0.05$  was considered statistically significant.

## **RESULTS**

The study included 100 patients who had a triple screening test and gave birth in our hospital's gynecology and obstetrics clinic. Sixty-six of the patients delivered their babies through cesarean section and 34 by vaginally. Patients were divided into two groups according to their gestational weeks, as those who gave birth before and after 37 weeks; before 37 weeks of pregnancy, 25 of them delivered by cesarean and 13 by vaginal delivery; and after 37 weeks of pregnancy 41 of them delivered by cesarean and 21 by vaginal delivery.

The most common cesarean indication of the patients was past cesarean history 32 (48.4%). Others were fetal distress 21 (31,8%), prolonged labor 9 (13,6%) and malpresentation 4 (6,2%), respectively.

Patients' other demographic characteristics are given in Table 1.

Patients were divided into two groups according to their gestational weeks. The groups were compared according to the parameters of gestational age, gestation weight, newborn

weight, and the triple screening test markers (Table 2).

According to regression analysis, positive statistical significance was obtained between gestational age and newborn weight in predicting the birth probability at 37 weeks and before (P=0.005, p< 0.0001) (Table 3).

The gestational age and newborn weight were examined according to ROC analysis to predict

the probability of birth at the 37 weeks and before, and the optimal cut-off values for predicting the birth at the 37 weeks and before were found to be 25.5 for gestational age and 3210 gr for the newborn weight (Table 4). The correlation of independent variables with newborn weight was examined, and it was observed that this was not statistically significant (Table 5).

**Table 1.** The patients' demographic characteristics and clinical laboratory results.

	Mean ± SD	Min-Max
Gestational Age	28,36 ± 5,05	19 - 42
Gravida	1,89 ± 1,13	1 - 5
Parity	0,59 ± 0,84	0 - 4
Weight (kg)	77,63 ± 9,42	58 - 105
Birth weight (gr)	3332,17 ± 483,79	2400 - 4970
Birth Week	38,2 ± 2,7	35 weeks-3 days / 40 weeks-5 days
AFP (MoM)	0,99 ± 0,32	0,43 - 2,42
hCG (MoM)	1,03 ± 0,57	0,2 - 0,78
uE3 (MoM)	1,38 ± 0,46	0,18 - 2,81

kg: kilogram, gr: gram, AFP: alpha-fetoprotein, hCG: human chorionic gonadotropin, uE3: unconjugated estriol

**Table 2.** Comparison of screening markers and demographic characteristics by gestational weeks

	≤ 37 weeks (n = 38)	> 37 weeks (n = 62)	p
Gestational Age (Year)	26,55 ± 4,9	29,59 ± 4,8	0,003
Weight (kg)	76,54 ± 10,22	78,14 ± 9,22	0,335
Birth weight (gr)	2838 ± 242,75	3612 ± 266-72	0,000
AFP (MoM)	0,98 ± 0,33	0,99 ± 0,32	0,684
hCG (MoM)	1,11 ± 0,81	0,98 ± 0,35	0,983
uE3 (MoM)	1,43 ± 0,50	1,34 ± 0,43	0,386

kg: kilogram, gr: gram, AFP: alpha-fetoprotein, hCG: human chorionic gonadotropin, uE3: unconjugated estriol, p< 0.05

**Table 3.** Logistic regression analysis results in predicting birth at 37 weeks and before.

	OR (95% CI)	p
Gestational Age (Year)	1,114 (1,042 - 1,257)	0,005
Weight (kg)	1,019 ( 0,975 - 1065)	0,412
Birth weight (gr)	1,011 (1,006 - 1016)	< 0,0001
AFP (MoM)	1,030 (0,288 - 3,684)	0,964
hCG (MoM)	0,660 (0,299 - 1,457)	0,640
uE3 (MoM)	0,667 (0,274 - 1,624)	0,372

kg: kilogram, gr: gram, AFP: alpha-fetoprotein, hCG: human chorionic gonadotropin, uE3: unconjugated estriol, p< 0.05

**Table 4.**ROC Analysis in predicting the probability of birth at the 37 week and before.

	Cut-off	Area under curve (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV(%) (95% CI)	NPV (%) (95% CI)	p
Birth Weight (gr)	3210	0,970 (0,929-1,000)	92,11 (78,62-98,34)	100 (94,13-100)	100 (97.1-100)	95,31 (87,28-98,37)	0.00
Gestational Age	25,5	0,676 (0,565-0,787)	52,63 (35,82-69,02)	83,61 (71,91-91,85)	66,67 (51,28-79,17)	73,91 (66,56-80,13)	0,003

**Table 5.** Correlation analysis of variables with newborn weight

	r	p
Age (year)	0,11	0,26
Weight (kg)	0,01	0,92
AFP (MoM)	0,14	0,16
hCG (MoM)	0,02	0,98
uE3 (MoM)	-0,05	0,57

kg: kilogram, gr: gram, AFP: alpha-fetoprotein, hCG: human chorionic gonadotropin, uE3: unconjugated estriol, p< 0.05

## DISCUSSION

Considering our findings, serum markers used for triple screening don't show any significance for determining gestational week in pregnant women with no risk factors. On the other hand, it has been observed that maternal age and newborn weight, which is a permanent factor for all pregnant women, are positively significant in predicting the birth week of pregnancy.

With the advancement of prenatal biochemical screening test programs, it is known that AFP, hCG, and UE3 have been studied by many researchers in predicting pregnancy outcomes. Considering the findings in recent studies, abnormally low (<0.5 MoM) or high (> 2.0 MoM) free hCG levels have often been associated with a risk of increased adverse pregnancy outcomes (8). Similarly, in another study, poor pregnancy outcomes such as fetal growth retardation and low birth weight have been reported to increase in pregnant women with high serum AFP levels (9). In studies conducted on serum UE3 values, however, it has been observed that it is inversely associated with poor pregnancy results (10). Insufficient uE3 has been associated with long-term pregnancies and difficulties in initiating labor due to sulfatase deficiency (11). As a result of all

these studies, increased AFP, hCG, and decreased UE3 levels have been reported to be stimulating in terms of adverse perinatal outcomes.

The findings of these studies were examined in the meta-analysis studies, and although it was observed that these serum markers were associated with poor pregnancy outcomes, it was also seen that most of the studies were retrospective and that their sensitivity rate and positive predictive values were relatively low. All of these observations have proven that using these markers as a standard for screening the poor pregnancy outcome prediction is not appropriate (1, 12). What makes our study distinctive from the others is that all pregnant women with risk factors and a poor obstetric history were excluded from the study. In this group of patients that we have identified, serum markers used for triple screening do not provide any significance for predicting the gestational week of pregnancy.

Similar to our study, studies conducted in uncomplicated normal pregnant women have already examined the role of markers in predicting birth weight and found that serum AFP levels are inversely correlated with newborn

weight, while serum UE3 levels positively correlate with newborn weight. It was also noted that the only significant factor independently associated with the newborn weight is the maternal serum UE3 levels. There was no difference between their hCG levels (13).

In another study that AFP was evaluated, it was found that there was an association between increased AFP and low birth weight. The recognition of AFP as an indicator of placental damage has confirmed the presence of this association. Nevertheless, some studies have not reported such an association; however, it has been reported that AFP is not a specific indicator for fetal growth in uncomplicated pregnancies with normal placenta (14, 15). This is also the case for uE3. uE3 is synthesized from the fetal liver, adrenal cortex, and placenta, and is an indicator of the fetoplacental condition in maternal serum. For this reason, uE3 is not a marker in predicting fetal growth in uncomplicated pregnancies (16).

In our study, these markers, general demographic characteristics, and newborn weight were also examined in predicting the gestational week in normal pregnant women; and as a result, no significant association was observed between triple screening markers and gestational week. This has shown us that triple screening serum markers cannot be used as markers in predicting pregnancy outcomes in uncomplicated pregnancies in which placentation is normal.

On the other hand, maternal age and newborn weight were positively significant on predicting the gestational week; and early maternal age and low birth weight in the ultrasonographies performed

were positively significant on predicting the risk of delivery before 37 weeks. Similar studies are also available in the literature, and the findings are alike (17-19).

Our primary limitations were; single-centered organization, retrospective nature, and relatively low number of patients. We believe that the results would be more accurate by conducting the studies in a multi-centered with a prospective nature and increased participation of patients. Another limitation of our study was the delivery methods between patient groups. Between and within groups, cesarean delivery rates were higher than vaginal deliveries. We believe, this distribution changes the outcome of our predictions on the gestational week of pregnancy.

## CONCLUSION

As a result, it has been observed that second-trimester screening markers were not significant on predicting the timing of birth in normal pregnant women. It is a known fact that most patients, who had a cesarean section in the past, schedules their elective cesarean dates. We believe that prospective studies in which only patients who had a vaginal delivery and had a history of cesarean section in the pregnant women with started labor were included in the study group may change the study results. In our study, the groups were divided in two groups with delivery before 37 weeks and after. We believe that it would be appropriate to conduct a study in which each week of birth constitutes separate groups, and post-term pregnancies are included as a separate group.

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## Sorumlu Yazar

Ayşegül GÜLBAHAR (Uzm. Dr.)  
İzmir Katip Çelebi Üniversitesi, Atatürk Eğitim ve Araştırma Hastanesi,  
Kadın Hastalıkları ve Doğum Kliniği, İZMİR  
Tel: 05053558340  
E mail: draysegulgulbahar@gmail.com  
ORCID: 0000-0001-6533-6195

Seda AKGÜN KAVURMACI ( Uzm. Dr.) ORCID: 0000-0001-9792-1786

