Iran Red Crescent Med J. 2018 April; 20(4):e64310. doi: 10.5812/ircmj.64310.

Published online 2018 April 30. **Research Article**

Clinical Significance of Abnormal Results of Second Trimester Hormones in the Absence of Aneuploidy

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Received 2017 November 22; **Revised** 2018 January 26; **Accepted** 2018 February 24.

Abstract

Background: Abnormal levels of hormones during the second trimester of pregnancy may predict genetic disorders and complications of pregnancy.

Objectives: This study was performed to evaluate the clinical significance of abnormal results in second-trimester markers in the absence of aneuploidy.

Methods: This case-control study was conducted between May 2014 and December 2015 in the maternal-fetal unit, Trakya University Faculty of Medicine in Turkey. Overall, 108 Turkish pregnant females were included in this study. This research recruited patients (n = 46) with normal karyotype, who underwent invasive prenatal tests because of abnormal levels of second-trimester hormones, along with a cohort of controls (n = 31) with hormonal results within normal ranges. For each patient, the researchers recorded the mode of delivery, gestational age at delivery, birth weight, complications, and adverse outcome of the pregnancy. Data were analyzed using Fisher's exact tests and Yates continuity correction tests for qualitative variables, and t- test and Mann-Whitney U test for quantitative variables.

Results: Maternal age (mean ± SD) of the entire group was 31.77 ± 5.68 years (study group: 31.23 ± 4.39; controls: 32.13 ± 6.43, $P > 0.05$). Preterm delivery and preeclampsia were significantly higher in the study group ($P = 0.02$). In the study group, Alpha Fetoprotein (AFP) levels were significantly higher in patients with preeclampsia yet not in the controls. The AFP values under 0.77 multiple of the median in patients with elevated test results in the absence of aneuploidy appeared to be associated with the development of preeclampsia later in pregnancy.

Conclusions: Although the significance of higher AFP values have been discussed in the literature in terms of the development of adverse outcomes, the present study suggests that lower values must also be taken into account during patient follow-up.

Keywords: Aneuploidy, Disorders, Genetic, Hormone, Preeclampsia, Pregnancy

1. Background

The association between mid-trimester maternal serum markers and pregnancy complications, including fetal loss, gestational hypertension, preeclampsia, intrauterine growth restriction (IUGR), preterm delivery, placental abruption, and intrauterine fetal death has been reported before (1-3). Furthermore, AFP has been reported as an important predictor of pregnancy complications.

Alpha fetoprotein is a glycoprotein and a member of the albuminoid gene family (4, 5). It is produced in early pregnancy by fetal liver and yolk sac (6). Synthesis of AFP in the fetal liver increases through the 20th week of gestation and remains fairly constant after that until the 32nd week (6).

There is a well-known association between elevated AFP and Preeclampsia (PE) prediction (7). Bredaki et al. (8) showed that Multiple of the Median (MoM) of AFP was 108826 in pregnant females, who developed PE and nearly 1.0 MoM in those, who did not develop PE.

Serum AFP concentration may be affected by gestational age and maternal characteristics, including maternal weight, racial origin, and cigarette smoking (9)

It has also been reported that low Estriol (E3) levels in the second trimester may predict fetal growth restriction and low birth weight (10). Abnormally high or low levels of human chorionic gonadotropin (HCG) are generally associated with an increased risk of adverse pregnancy outcomes (11).

Abnormal second-trimester hormone levels showing high risk for genetic disorders in the absence of aneuploidy are a common problem in clinical practice. However, the clinical significance of this situation remains unknown. In particular, the principal outstanding questions are concerned with patient counseling and the frequency of antenatal follow-up. This case-control study was per-

formed to evaluate the importance of abnormal results of second-trimester hormones, especially in the absence of a chromosomal disorder.

2. Methods

2.1. Study Participants

The present study was approved by the ethics committee of Trakya University Medical Faculty, Edirne, Turkey (Registration number: TUTF/BAEK 12016-156).

This trial was conducted between May 2014 and December 2015 at the maternal-fetal unit, Trakya University Faculty of Medicine, Edirne, Turkey.

2.2. Setting

In this cross-sectional, case-control study, 108 sonographically normal singleton pregnancies with a triple serum screening test, which had been performed at 16 to 20 gestational weeks, were divided into two groups. A cut-off of 1/270 for Down's syndrome was defined as high risk for Down syndrome.

Group I consisted of 77 sonographically normal singleton pregnancies with abnormal triple serum screening test (high risk for trisomy 21) and normal karyotype, which was revealed by amniocentesis.

Group II consisted of 31 sonographically normal singleton pregnancies with normal triple serum screening test.

2.3. Exclusion Criteria

The exclusion criteria were as follow:

1- Patients with poor previous obstetric histories, incorrect dating, obesity, gestational diabetes, smoking and multiple pregnancies.

2- Females with a high risk for other aneuploidies (trisomy 13 or 18, defined as a risk > 1/100), extremely low AFP [< 0.4 multiple of the median (MoM)], or E3 (< 0.2 MoM)

3- Patients with any sonographic findings (minor markers, oligohydramnios, polyhydramnios), structural malformations, and placental pathologies.

2.4. Measurements

Maternal serum alfa-fetoprotein (AFP), human chorionic gonadotrophin (HCG), and unconjugated estriol (uE3) levels were measured using a fluorescent immunoassay and then converted to MoM. Demographic characters, mode of delivery, gestational age at delivery, birth weight, and complications, such as macrosomia, were recorded. Fetal death, IUGR, and PE were defined as adverse outcomes. Adverse perinatal outcomes were compared in patients, according to the test results. Threshold values were calculated for complications, which were found to be significant. Receiver operating characteristic (ROC) curve analysis was conducted to determine the optimal cut-off values.

2.5. Sample Size

The sample size was calculated by the G power (v3.1.7) program. The power of the work was expressed as 1-β (β = probability of type II error), and in general, researches should have at least 80% power. According to Cohen's effect size coefficients, it was decided that there should be at least 26 people in the groups according to the calculation made assuming that the evaluations to be made between the two independent groups would have a large effect size (d = 0.80).

2.6. Statistical Analyses

Statistical analyses were performed using the number cruncher statistical system (NCSS 2007) (Kaysville, Utah, USA). Data were analyzed using descriptive statistical procedures (mean, median, frequency, standard deviation, minimum, and maximum). The Student's t-test was used to compare normally distributed variables, while the Mann–Whitney U test was used to compare variables, which were not normally distributed. Fisher's exact test and Yates' continuity correction test were used to compare data. Furthermore, $P < 0.01$ and $P < 0.05$ was considered to be statistically significant.

3. Results

Due to insufficient clinical history, 31 out of the 77 patients in group I were excluded. Consequently, the final study cohort included a total of 46 patients. The control group (Group II, n = 31) consisted of randomly selected patients with a normal test result.

A total of 77 patients (study group: 46, control group: 31 females) were investigated. Maternal age (mean ± SD) of the entire group was 31.77 ± 5.68 years (study group: 31.23 ± 4.39; controls: 32.13 ± 6.43, $P > 0.05$). Demographic characteristics of the patients (height, body mass index (BMI), blood pressure, and medical history) were not different between the groups ($P > 0.05$). Birth weight, intrauterine growth retardation, gestational weight, and route of delivery were similar between the two groups (Table 1).

In total, 18 out of the 77 patients (23.4%) delivered before 37 weeks, and preterm delivery was significantly higher in the control group ($P = 0.02$). However, PE was higher in the study group (Table 1, $P = 0.022$).

E3, HCG, and AFP values are shown in Table 2. In the high-risk study group, AFP levels (mean ± SD) were significantly higher in patients with PE. Estriol and HCG values

Table 1. Comparison of the Characteristics of the Patients and Adverse Outcomes of Pregnancy Between the Study and The Control Group

	Study Group (n = 46)	Control Group (n = 31)	P Value
Age (year)			0.465[a]
Min - Max (Median)	21 - 46 (33)	21 - 41 (30)	
Mean ± SD	32.13 ± 6.43	31.23 ± 4.39	
Gestational age at delivery			0.001[b]
Min - Max (Median)	34 - 42 (39)	27 - 41 (38)	
Mean ± SD	38.57 ± 1.68	36.9 ± 3.07	
Gestational weight (gr)			0.071[a]
Min - Max (Median)	2000 - 4240 (3275)	1360 - 4440 (3120)	
Mean ± SD	3230.54 ± 521.17	2971.29 ± 720.93	
Delivery			0.590[c]
Vaginal	11 (23.9)	5 (16.1)	
S/C	35 (76.1)	26 (83.9)	
IUGR			0.430[d]
No	43 (93.5)	27 (87.1)	
Yes	3 (6.5)	4 (12.9)	
Preeclampsia			0.022[d,e]
No	35 (76.1)	30 (96.8)	
Yes	11 (23.9)	1 (3.2)	

[a] Student t-test.
[b] Mann Whitney U Test, P < 0.01.
[c] Yates' Continuity Correction Test.
[d] Fisher's Exact Test.
[e] P < 0.05.

were not significantly different among patients, who developed PE.

According to ROC analysis, the most effective threshold for AFP was 0.77 MoM, resulting in an odds ratio of 9.6 (95% CI: 1.726 to 53.405). The sensitivity and specificity of this threshold for preeclampsia was 80% and 70.5%, respectively. The positive and negative predictive values were 44.4% and 92.3%, respectively.

4. Discussion

Maternal serum HCG and AFP have been used for the detection of Trisomy 21 and neural tube defects during the second trimester of pregnancy for many years. In the absence of these conditions, the meaning of an unexplained elevation in maternal serum HCG and maternal serum AFP during the second trimester has become the focus of much interest. Many studies have indicated an association between the elevation of mid-trimester markers and adverse pregnancy outcomes (2, 12, 13) and such adverse outcomes are usually the result of placental insufficiency, including preeclampsia, intrauterine growth restriction, preterm birth, fetal loss, and placenta accreta. In addition, oligohydramnios, gestational diabetes, and macrosomia have been identified as representing other complications associated with abnormal serum E3, AFP, and HCG values (14).

These adverse outcomes of pregnancy have been evaluated in pregnant patients exhibiting an elevated second trimester markers in a variety of studies, and the detection rate of markers for such complications has been analyzed. A recent Cochrane review of these studies showed that an unexplained elevation of maternal serum AFP (> 2.5 MoM), HCG (> 3 MoM) and/or inhibin-A (> or = 2 MoM) or reduced maternal serum AFP (< 0.25 MoM) and/or E3 (< 0.5 MoM) in the second trimester are associated with adverse obstetric outcomes (15).

In the present study, the researchers compared adverse pregnancy outcomes in patients with a positive test for trisomy 21 (the study group-Group I) with the pregnant patients having a normal test (control-Group II). The current analysis showed that preeclampsia was significantly higher in patients with a positive screening test than in the control group. Fetal death, gestational weight, and in-

Table 2. Median (Minimum-Maximum) Maternal Serum AFP, Hcg, And E3 Levels In Women Who Developed Preeclampsia With High-Risk Test Results

	Preeclampsia (n = 11)	No. (n = 35)	P
Afp			0.024[a]
Min - Max (Median)	0.3 - 1.7 (1.0)	0.3 - 1.6 (0.7)	
Mean ± SD	1.05 ± 0.44	0.73 ± 0.32	
Hcg			0.534
Min - Max (Median)	0.5 - 3.7 (1.3)	0.2 - 6.7 (1.4)	
Mean ± SD	1.44 ± 1.02	1.69 ± 1.30	
E3			0.354
Min - Max (Median)	0.4 - 0.9 (0.5)	0.2 - 9.3 (0.5)	
Mean ± SD	0.53 ± 0.15	0.86 ± 1.51	

[a] Mann Whitney U Test, P < 0.05 was considered significant.

trauterine growth retardation were not significantly different between the two groups.

The association between second-trimester serum markers and PE was reported previously. A systematic meta-analysis reported threshold values for a range of important markers. The most significant thresholds were 2.0 MoM for AFP, resulting in a positive likelihood ratio (LR) of 2.36 and a negative likelihood ratio of 0.96 (16). In contrast, Kang et al. failed to identify an association between AFP, uE3, and PE (17). In a recently reported study, the median value of AFP was 1088 MoM in patients with PE (8).

In the present study, the researchers investigated patients, who tested positive in the second-trimester screening test for genetic disorders. The association between low maternal AFP and chromosomal trisomies was established over 30 years ago (18). In contrast, high levels of AFP have been associated with PE. The current data indicated that the most effective threshold for AFP was 0.77 MoM with sensitivity and specificity of 80% and 70.5% for preeclampsia, respectively. The most effective threshold for AFP in the present study was significantly lower than that reported previously. This particular result could not be attributed to ethnicity alone because the threshold values arising from the Turkish cohort were similar to those reported in the existing literature (19). To determine the effect of increased HCG and decreased E3 together for prediction of PE, larger studies are required.

Preterm delivery was also significantly higher in the study group. Mean gestational age of the patients in Group I and II were 36.9 ± 3.07 and 38.57 ± 1.68, respectively. The researchers did not detect any significant difference in the hormonal levels of patients, who delivered before 37 weeks of gestation. Larger studies are needed to clarify the association between second-trimester hormonal levels with preterm delivery.

4.1. Limitations

In the study time interval, there were only 77 patients, who met the criteria for group I. This research included both the patients with abnormal hormonal results and patients with normal karyotype results, which was proved by invasive tests. The indication for an invasive test was only abnormal second-trimester hormonal results. The patients with any sonographic findings were excluded from the study. Thus, the study group (n = 46) was meticulously selected, and the sample size was small.

4.2. Conclusion

According to the results, AFP values during the second-trimester may be more important in predicting preeclampsia. Adverse pregnancy outcomes, such as preeclampsia appear to be associated with much lower AFP values than that considered previously. It should, therefore, be considered that second trimester hormonal test results may predict certain adverse outcomes of pregnancy. Patients with a normal karyotype and abnormal results in the triple test are therefore good candidates for close follow-up during pregnancy.

Acknowledgments

The authors state that patient anonymity and ethical approval were considered.

Footnotes

Financial Disclosure: All authors state that there were no conflict of interests.

Funding/Support: The authors state that there was no funding.

References

1. Ananth CV, Wapner RJ, Ananth S, D'Alton ME, Vintzileos AM. First-Trimester and Second-Trimester Maternal Serum Biomarkers as Predictors of Placental Abruption. *Obstet Gynecol*. 2017;**129**(3):465–72. doi: 10.1097/AOG.0000000000001889. [PubMed: 28178056]. [PubMed Central: PMC5367463].
2. Katz VL, Chescheir NC, Cefalo RC. Unexplained elevations of maternal serum alpha-fetoprotein. *Obstet Gynecol Surv*. 1990;**45**(11):719–26.
3. Huang T, Hoffman B, Meschino W, Kingdom J, Okun N. Prediction of adverse pregnancy outcomes by combinations of first and second trimester biochemistry markers used in the routine prenatal screening of Down syndrome. *Prenat Diagn*. 2010;**30**(5):471–7. doi: 10.1002/pd.2505. [PubMed: 20440736].
4. Gitlin D, Boesman M. Serum alpha-fetoprotein, albumin, and gamma-G-globulin in the human conceptus. *J Clin Invest*. 1966;**45**(11):1826–38. doi: 10.1172/JCI105486. [PubMed: 4162738]. [PubMed Central: PMC292864].
5. Mizejewski GJ. Alpha-fetoprotein Structure and Function: Relevance to Isoforms, Epitopes, and Conformational Variants. *Exp Biol M*. 2016;**226**(5):377–408. doi: 10.1177/153537020122600503.
6. Gitlin D, Perricelli A, Gitlin GM. Synthesis of α-fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. *Cancer Res*. 1972;**32**(5):979–82.
7. Morris RK, Cnossen JS, Langejans M, Robson SC, Kleijnen J, Ter Riet G, et al. Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis. *BMC Pregnancy Childbirth*. 2008;**8**:33. doi: 10.1186/1471-2393-8-33. [PubMed: 18680570]. [PubMed Central: PMC2533288].
8. Bredaki FE, Sciorio C, Wright A, Wright D, Nicolaides KH. Serum alpha-fetoprotein in the three trimesters of pregnancy: effects of maternal characteristics and medical history. *Ultrasound Obstet Gynecol*. 2015;**46**(1):34–41. doi: 10.1002/uog.14809. [PubMed: 25652769].
9. Bredaki FE, Wright D, Akolekar R, Cruz G, Nicolaides KH. Maternal serum alpha-fetoprotein in normal pregnancy at 11-13 weeks' gestation. *Fetal Diagn Ther*. 2011;**30**(4):274–9. doi: 10.1159/000330200. [PubMed: 22156386].
10. Settiyanan T, Wanapirak C, Sirichotiyakul S, Tongprasert F, Srisupundit K, Luewan S, et al. Association between isolated abnormal levels of maternal serum unconjugated estriol in the second trimester and adverse pregnancy outcomes. *J Matern Fetal Neonatal Med*. 2016;**29**(13):2093–7. doi: 10.3109/14767058.2015.1075503. [PubMed: 27480207].
11. Sirikunalai P, Wanapirak C, Sirichotiyakul S, Tongprasert F, Srisupundit K, Luewan S, et al. Associations between maternal serum free beta human chorionic gonadotropin (beta-hCG) levels and adverse pregnancy outcomes. *J Obstet Gynaecol*. 2016;**36**(2):178–82. doi: 10.3109/01443615.2015.1036400. [PubMed: 26368010].
12. Gross SJ, Phillips OP, Shulman LP, Bright NL, Dungan JS, Simpson JL, et al. Adverse perinatal outcome in patients screen-positive for neural tube defects and fetal Down syndrome. *Prenat Diagn*. 1994;**14**(7):609–13. [PubMed: 7526367].
13. Spencer K. Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome. *Prenatal Diag*. 2000;**20**(8):652–6. doi: 10.1002/1097-0223(200008)20:8<652::aid-pd882>3.0.co;2-6.
14. Sayin NC, Canda MT, Ahmet N, Arda S, Sut N, Varol FG. The association of triple-marker test results with adverse pregnancy outcomes in low-risk pregnancies with healthy newborns. *Arch Gynecol Obstet*. 2008;**277**(1):47–53. doi: 10.1007/s00404-007-0421-6. [PubMed: 17653738].
15. Gagnon A, Wilson RD, Society Of O, Gynaecologists Of Canada Genetics C. Obstetrical complications associated with abnormal maternal serum markers analytes. *J Obstet Gynaecol Can*. 2008;**30**(10):918–32. doi: 10.1016/S1701-2163(16)32973-5. [PubMed: 19038077].
16. Morris RK, Cnossen JS, Langejans M, Robson SC, Kleijnen J, ter Riet G, et al. Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: Systematic review and meta-analysis. *BMC Pregnancy Childb*. 2008;**8**(1). doi: 10.1186/1471-2393-8-33.
17. Kang JH, Farina A, Park JH, Kim SH, Kim JY, Rizzo N, et al. Down syndrome biochemical markers and screening for preeclampsia at first and second trimester: correlation with the week of onset and the severity. *Prenat Diagn*. 2008;**28**(8):704–9. doi: 10.1002/pd.1997. [PubMed: 18655226].
18. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal Serum Alpha-Fetoprotein Measurement: A Screening Test for down Syndrome. *Lancet*. 1984;**323**(8383):926–9. doi: 10.1016/s0140-6736(84)92389-4.
19. Karsidag AY, Buyukbayrak EE, Kars B, Suyugul U, Unal O, Turan MC. The relationship between unexplained elevated serum markers in triple test, uterine artery Doppler measurements and adverse pregnancy outcome. *J Pak Med Assoc*. 2010;**60**(3):181–6. [PubMed: 20225773].