ORIGINAL ARTICLE

Eastern Journal of Medicine
East J Med 26(3): 418-425, 2021
DOI: 10.5505/ejm.2021.77775

Abnormal First and Second Trimester Maternal Serum Marker Levels For Aneuploidy Screening and Adverse Pregnancy Outcomes

Lale Susan Karakış[1*], Engin Turkgeldi[2], Huseyin Kiyak[3], Neşe Yücel[4]

[1]*Bahceci IVF Centre, Fulya, Istanbul, Turkey*
[2]*Koç University, Department of Obstetrics and Gynecology, İstanbul, Turkey*
[3]*Istanbul Health Sciences University, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul, Turkey*
[4]*Kahramanmaraş Sütcü İmam University School of Medicine, Kahramanmaraş, Turkey*

ABSTRACT

To investigate if there is an association between pregnancy complications and abnormal maternal serum analytes used for first trimester screening (FTS) and second trimester screening (STS) for aneuploidy.
More than two hundred three pregnant women who had FTS or STS for Down's syndrome who presented between July 2009 and January 2010 were included. First trimester maternal levels of PAPP-A and free hCG or 2nd trimester maternal levels of AFP, uE3 and hCG were compared between healthy pregnancies and those with preeclampsia, intrauterine growth retardation, intrauterine fetal death or oligohydramnios.
PAPP-A levels less than 0.4 MoMs were related with elevated preeclampsia and fetal growth retardation risk. AFP levels over 2.5 MoMs were related with all adverse outcomes evaluated in the study including preeclampsia, fetal growth retardation, intrauterine fetal demise and oligohydramnios. While uE3 levels below 0.5 MoM were found to be related with higher preeclampsia incidence, elevated 2nd trimester hCG levels over 3.0 MoMs were associated with fetal growth retardation. No significant relationship could be established between low 1st trimester free hCG levels (less than 0.5 MoMs) and any of the adverse outcomes.
First and 2nd trimester serum analytes for Down syndrome screening are significantly linked with pregnancy complications. However, because their sensitivity and positive predictive values are low, these analytes are not shown to be effective in the screening of pregnancy complications when used alone.

Keywords: AFP, hCG, PAPP-A, fetal growth retardation, oligohydramnios, preeclampsia, uE3

Introduction

Pregnancy complications including stillbirth, preeclampsia and small for gestational age(SGA) contribute considerably to perinatal mortality and morbidity (1). Recent evidence indicates to a common underlying pathophysiological mechanism involving abnormal placentation in early pregnancy for these pregnancy complications. Failure of trophoblast invasion into the maternal spiral arteries during the 1st and early 2nd trimesters is suggested to lead to reduced uteroplacental blood perfusion and placental insufficiency with subsequent intrauterine hypoxia and perinatal asphyxia (2). Early recognition of women with high risk for these obstetric complications will help in improving maternal and neonatal prognosis by closer monitoring and administration of prophylactic medical treatment when necessary.

The maternal serum levels of certain analytes produced by the fetal-placental unit are currently used for fetal aneuploidy screening during the 1st and 2nd trimesters. Maternal serum pregnancy associated plasma protein A (PAPP-A) and free βhCG levels with or without sonographic nuchal translucency (NT) measurement between 11 and 14 weeks of pregnancy are used for first trimester screening (FTS) for Down Syndrome and Trisomy 18. Measurement of alpha-fetoprotein (AFP), human chorionic gonadotrophin (HCG) and unconjugated estriol (uE3) with or without inhibin-A are used for the second trimester screening (STS) between 15 and 20 weeks of pregnancy. Besides aneuploidy, high or low levels of these serum analytes secreted from the fetal-

***Corresponding Author:** Lale Susan Karakış, Bahceci IVF Centre Hakkı Yeten Cad. 11/M3 Terrace Fulya, 34365, Sisli, Istanbul
E-mail: lalesusan@gmail.com, Phone: +90 (536) 341 44 22
ORCID ID: Lale Susan Karakış: 0000-0003-1870-5415, **Engin Turkgeldi:** 0000-0002-5008-3292, **Huseyin Kiyak:** 0000-0001-7580-9179, **Neşe Yücel:** 0000-0002-5689-1311
Received: 02.03.2021, **Accepted:** 03.07.2021

placental unit may be also reflective of placental insufficiency.

Few recent studies have reported abnormal 1st or 2nd trimester aneuploidy screening results to be linked to increased risk of adverse outcomes including preeclampsia, fetal growth restriction, and intrauterine fetal death (3-5). Nevertheless, the threshold levels indicating increased risk of complications and the type of adverse outcome associated with each abnormal maternal serum analyte varies between studies.

The present study aimed to investigate whether abnormal maternal serum marker levels utilized in the fetal screening of aneuploidy in the 1st or 2nd trimesters of pregnancy are associated with pregnancy complications including fetal growth retardation, preeclampsia, oligohydramnios and stillbirth.

Materials and Method

This is a retrospective case-control study involving all pregnant women between the ages of 18 and 45 who underwent antenatal surveillance and delivered between July 2009 and January 2010. This study was approved by the institutional review board of the hospital.

Two groups of patients were included into the study as those who underwent 1st trimester fetal aneuploidy screening at 11-14 weeks of pregnancy (PAPPA, free BhCG and NT measurements) together with AFP testing for neural tube defects at 16-18 weeks; or those who underwent 2nd trimester triple screening (AFP, total BhCG, uE3) at 16-18 weeks of pregnancy. All serum analyte measurements were reported as 'multiples of the median'(MoM). The median MoM values of maternal serum analytes of women who were diagnosed with preeclampsia, intrauterine fetal death, fetal growth retardation or oligohydramnios were compared with MoM values of women who delivered healthy full term neonates without any pregnancy complications. Women with chronic diseases, hypertension, thyroid disease, diabetes, antenatal hemorrhage, miscarriage, multiple gestation, preterm rupture of membranes, preterm labour, placenta previa, polihydramnios, fetal chromosomal or major structural anomalies were excluded from the study.

Preeclampsia was diagnosed as a new onset systolic blood pressure of at least 140mm Hg or a diastolic blood pressure of at least 90 mmHg measured at least two times 4 hours apart after 20 weeks of gestation, together with proteinuria of +2 on dipstick analysis or at least 300 mg protein in a 24 hours urine sample in the absence of urinary infections (6). Although this previous definition required the presence of proteinuria, more recent guidelines state that preeclampsia can be diagnosed in the absence of proteinuria if the new-onset hypertension accompanies signs or symptoms of end organ dysfunction (7). Women with estimated fetal weight measurements below the 10th percentile with a remarkably reduced growth rate on serial ultrasound examinations with at least two week intervals in the absence of preeclampsia were diagnosed to have fetal growth restriction (2). Intrauterine fetal death was defined as fetal death occuring after the 24th week of gestation. Oligohydramnios was defined as an amniotic fluid index (AFI) measurement of less than or equal to 5 cm or the deepest vertical amniotic fluid pocket measurement of less than or equal to 2 cm (8). Only patients with isolated oligohydramnios in the absence of chromosomal abnormalities or fetal malformations, growth retardation or materanal disease were included.

Normal threshold MoM levels for serum analytes suggested by Gagnon et al. were used. PAPP-A less than 0.4 MoM, 1st trimester hCG less than 0.5 MoM, AFP more than 2.5 MoM, 2nd trimester hCG more than 3 MoM and uE3 less than 0.5 MoM were considered to be pathological (4).

The SPSS package program for Windows 10.0 was used for stastistical analysis. Distributions of variables and normality were evaluated visually with histograms and Shapiro–Wilk test, when needed.

The student's t-test and Mann Whitney U tests were used for comparison of variables. Receiver operator characteristic (ROC) curve analysis was performed to evaluate the predictive value of 1st and 2nd trimester analytes in the prediction of adverse obstetric outcomes. A p value less than 0.05 was defined as statistically significant.

Results

A total of 213 patients were included to the study. Of the study patients, 128(60%) underwent FTS for Down syndrome (NT, PAPPA-A ve BhCG) together with 2nd trimester AFP testing for neural tube defects, and 85(%40) underwent 2nd trimester triple screening (AFP, hCG, uE3). The median age of women involved in the study was 28 years. The median week of gestation for screening was 12 weeks and 5 days for in the 1st

Table 1. Comparison of Maternal Serum Mom Values İn Patients With Preeclampsia and Normal Pregnancy Outcomes

	Normal		Preeclampsia		
	Mean	SD	Mean	SD	p
1. trim. hCG	1,17	0,78	0,79	0,37	0,078
PAPP-A	1,21	0,65	0,72	0,41	0,006
AFP	0,95	0,35	1,37	0,71	0,011
2. trim. hCG	1,42	0,97	1,32	0,51	0,914
uE3	1,06	0,40	0,90	0,41	0,017

MoM: multiple of the median; SD: standard deviation; trim: trimester, hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, PAPP-A: pregnancy associated plasma protein A, uE3: unconjugated estriol . All values were compared using Mann-Whitney U test

Table 2. Comparison of Maternal Serum Mom Values İn Patients With Fetal Growth Retardation and Normal Pregnancy Results

	Normal		FGR		
	Mean	SD	Mean	SD	P
1. trim. hCG	1,17	0,78	1,46	1,09	0,293
PAPP-A	1,21	0,65	0,54	0,24	<0,001
AFP	,95	0,35	1,65	0,65	<0,001
2. trim. hCG	1,42	0,97	3,00	1,55	0,001
UE3	1,06	0,40	0,94	0,37	0,360

MoM: multiple of the median; SD: standard deviation; trim: trimester; FGR: fetal growth restriction hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, PAPP-A: pregnancy associated plasma protein A, uE3: unconjugated estriol. All values were compared using Mann-Whitney U test

Table 3. Comparison of Maternal Serum Mom Values İn Patients With İntrauterine Fetal Death and Normal Pregnancy Outcomes

	Normal		IUFD		
	Mean	SD	Mean	SD	p
hCG	1,42	0,97	1,22	0,55	0,948
AFP	0,97	0,47	1,36	0,35	0,004
UE3	1,06	0,40	1,22	0,14	0,103

MoM: multiple of the median; SD: standard deviation; trim: trimester; IUFD: intrauterine fetal death hCG: human chorionic gonadotropin, uE3: unconjugated estriol, AFP: alpha-fetoprotein. All values were compared using Mann-Whitney U test

tirmester and 16 weeks 2 days in the 2nd trimester. The median week of gestation at delivery of women with healthy pregnancies was 39 weeks, which was statistically significanty higher than the median week of gestation at delivery of 34 weeks in women with pregnancy complications ($p<0.001$). Out of the 128 patients who underwent FTS, 86 patients had healthy deliveries, 15 had preeclampsia 12, had fetal growth restriction and 15 had oligohydramnios. Out of the 85 patients who underwent STS, 48 had normal pregnancy outcomes while 16 were diagnosed with preeclampsia, 12 with fetal growth restriction and 9 with stillbirth.

Comparison of median MoM values of maternal serum analytes in women with healthy pregnancies and women with preeclampsia is presented in Table 1. Statistically significant differences were found in the median MoMs of PAPP-A, uE3 and AFP levels between the two groups. No difference was detected in the median MoMs of 1st and 2nd trimester hCG levels among the two groups.

Comparison of median MoMs of maternal serum analytes in patients with fetal growth restriction and normal pregnancies is presented in Table 2.

Table 4. Comparison of Maternal Serum Mom Values İn Patients With Oligohydramnios and Normal Pregnancy Outcomes

	Normal		Oligohydroamnios		
	Mean	SD	Mean	SD	P
hCG	1,17	0,78	1,02	0,65	0,498
PAPP-A	1,21	0,65	1,19	0,33	0,483
AFP	0,95	0,35	1,17	0,32	0,013*

SD: standard deviation hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, PAPP-A: pregnancy associated plasma protein A. All values were compared using Mann-Whitney U test

Table 5. ROC analysis showing the performance of 1st trimester combined test parameters and second trimester AFP MoM levels in detecting pregnancy complications

	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
Test Result				Lower Bound	Upper Bound
1. trim. hCG	0,426	0,053	0,164	0,322	0,530
AFP	0,759	0,042	0,000***	0,677	0,842
PAPP-A	0,323	0,049	0,001***	0,228	0,418

Area Under the Curve

Table 6. ROC analysis showing the performance of 2. trimester test parameters in detecting pregnancy complications

	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
Test Result				Lower Bound	Upper Bound
hCG	0,599	0,062	0,119	0,477	0,721
AFP	0,698	0,058	0,002	0,585	0,812
uE3	0,427	0,064	0,253	0,301	0,553

Area Under the Curve

Only AFP has been found to be associated with poor prognosis in the ROC analysis, $p<0.01$. b-hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, uE3: unconjugated estriol

Statistically significant differences were found in the median MoM S of PAPP-A, AFP and 1st trimester hCG levels, while no difference was detected in uE3 levels or 2nd trimester hCG levels between the two groups.

Comparison of median MoMs of maternal serum analytes between women with intrauterine fetal death and normal pregnancy outcomes is presented in Table 3. Only AFP levels were statistically significantly different among the two groups ($p<0.01$).

Comparison of median MoM values of maternal serum markers in patients with oligohydramnios and normal pregnancies is presented in Table 4. Only AFP levels were statistically significantly higher in patients with oligohydramnios($p<0.01$).

Figures 1-5 show box-and-whisker plot graphics depicting the association between maternal serum markers and pregnancy complications. These graphs represent the median, 25th and 75th percentile values and distributions of maternal serum MoM values. No associations were found between 1st trimester abnormal hCG levels and any pregnancy complications. Lower than normal PAPP-A levels were related with preeclampsia and fetal growth retardation. High levels of AFP were found to be associated with all of the pregnancy complications. While high 2nd trimester levels of hCG were associated with fetal growth restriction, low uE3 levels were associated with preeclampsia.

Tables 5 and 6 show the ROC (receiver–operating characteristics) analysis results. Upon evaluation of the area under the ROC curve, the performance of FTS and STS parameters in the predicting adverse pregnancy outcomes were determined. In patients who underwent FTS and 2nd trimester

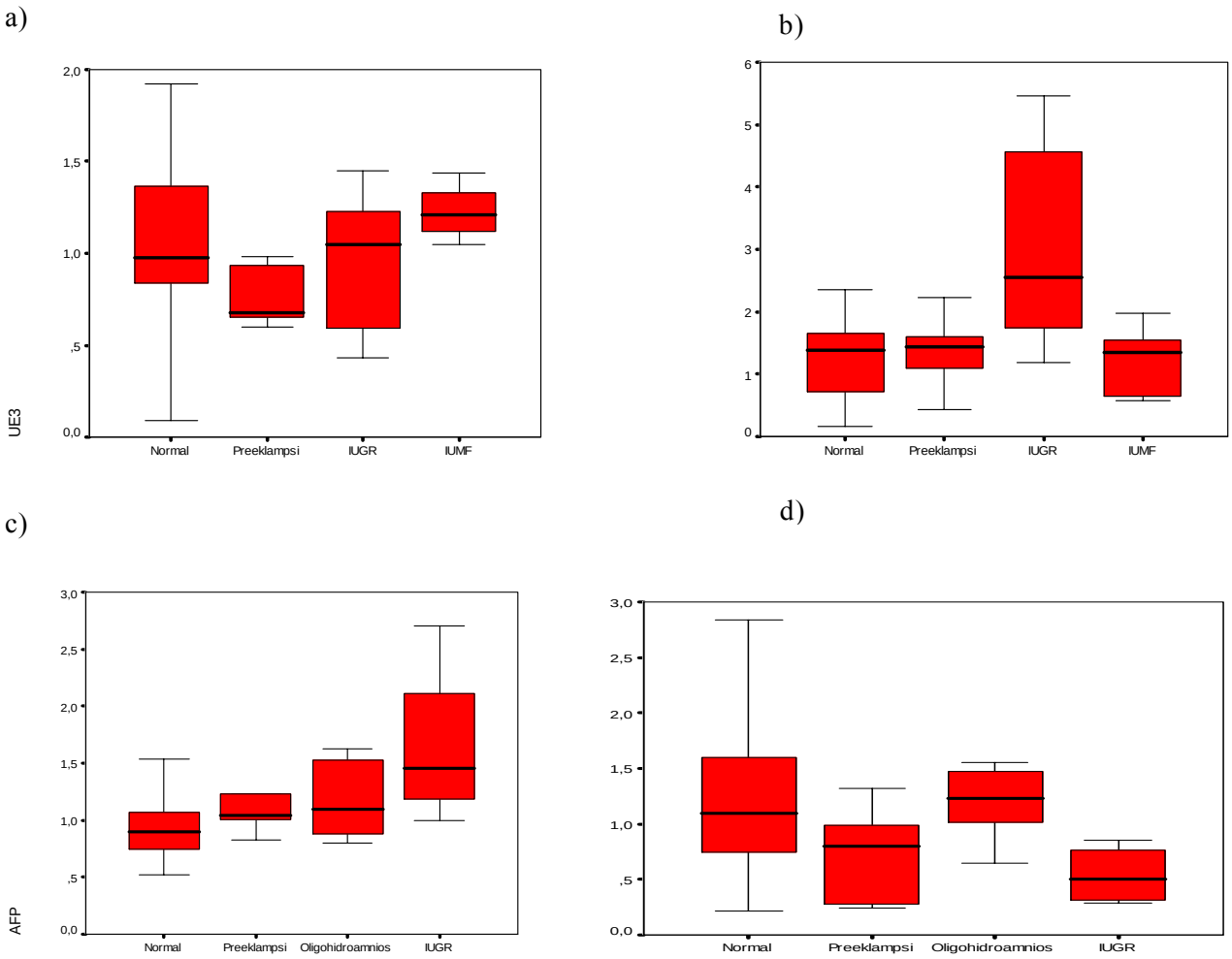

Fig. 1. Box-and-whisker plot graphics of serum analyte levels in normal pregancies and pregnancies complicated with preeclampsia, oligohydramnios, fetal growth restriction (median, 25th and 75th percentile values and distributions of maternal serum MoM values) 1a: comparison of uE3(unconjugated estriol) levels among complicated and healthy pregnancies; 1b: comparison of total hCG levels among complicated and healthy pregnancies; 1c: comparison of AFP levels among complicated and healthy pregnancies; 1d: comparison of PAPP-A levels among complicated and healthy pregnancies. hCG: human chorionic gonadotropin, PAPP-A: pregnancy associated plasma protein A, AFP: alpha-fetoprotein, uE3: unconjugated estriol, IUGR: intrauterine growth restriction

AFP and PAPP-A have been found to be associated with adverse pregnancy outcomes in the ROC analysis, $p<0.001$. b-hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, PAPP-A: pregnancy associated plasma protein A

AFP screening for neural tube defects, PAPP-A and AFP levels were predictive of pregnancy complications ($p<0.001$). In patients who underwent STS, AFP levels were found to be predictive of adverse pregnancy outcomes ($p<0.001$). ROC curves showing the sensitivity and specificity of FTS and STS parameters in detecting adverse pregnancy outcomes are presented in Figure 2 and Figure 3.

Discussion

In the present study, PAPP-A levels less than 0.4 MoMs were found to be related with an increased risk of preeclampsia and fetal growth retardation. No significant relationship could be established between 1st trimester free hCG levels (less than 0.5 MoMs) and any of the pregnancy complications. Of the 2nd trimester serum analytes, AFP levels over 2.5 MoMs were related with all of the adverse outcomes evaluated in the study including preeclampsia, fetal growth retardation, intrauterine fetal demise and oligohydramnios. While decreased levels of uE3 of less than 0.5 MoM were found to be associated

with an increased incidence of preeclampsia, elevated 2nd trimester hCG levels over 3.0 MoMs were associated with fetal growth retardation.

The multicenter FASTER (First and Second Trimester Evaluation of Risk) study including

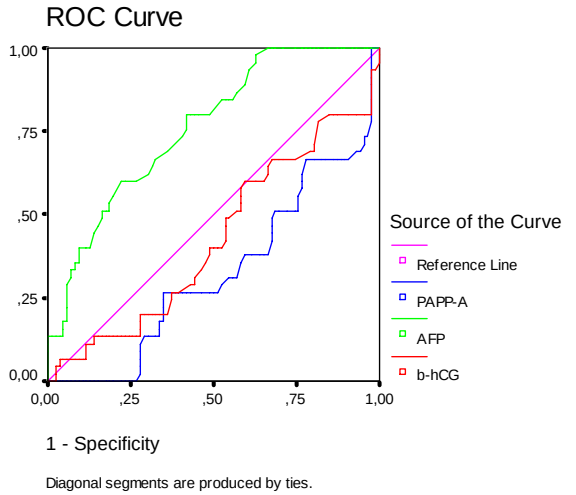

Fig. 2. ROC curve showing the sensitivity and specificities of 1st trimester PAPP-A, B-hCG and 2nd trimester AFP for the prediction of adverse pregnancy outcomes. b-hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, PAPP-A: pregnancy associated plasma protein

34,271 pregnant women found a statistically significant correlation between low PAPP-A levels and preeclampsia, spontaneous pregnancy loss, fetal birth weight lower than 5th percentile, gestational hypertention and preterm birth (9). Although this association was statistically significant, the positive predictive value and sensitivity of low PAPP-A levels for each of the pregnancy complications were relatively low. Two previous studies failed to show any association between high levels of PAPP-A and pregnancy complications (6, 10). In line with these studies, the present study found PAPP-A levels lower than 0.4 MoM to be related with preeclampsia and intrauterine growth restriction.

A fair number of studies have found low levels of 1st trimester free hCG to be associated with pregnancy loss. Krantz et al reported a significant correlation between levels of free hCG below the 1st persentile and fetal birth weight lower than the 10th percentile (adjusted OR 2.7, %95 CI, 1.3–1.9) (10). Huang et al found no relationsip between low levels of 1st trimester free hCG levels and any pregnancy complications (12). Similarly, the present study found no associations between low levels of hCG and pregnancy complications.

High levels of AFP have been found to be related with preeclampsia, hypertension, early pregnancy loss, preterm birth, intrauterine fetal death, fetal growth retardation and oligohydramnios (3, 12, 13). Yaron et al reported a statistically significiant association between AFP levels over 2.5 MoM and fetal growth restriction, pregnancy induced hypertention, intrauterine fetal death and

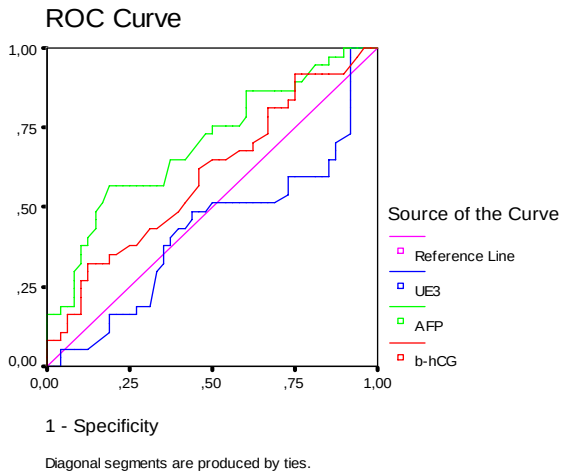

Fig. 3. ROC curve showing the sensitivity and specificities of 2nd trimester B-hCG, AFP and uE3 for the prediction of adverse pregnancy outcomes. b-hCG: human chorionic gonadotropin, AFP: alpha-fetoprotein, uE3: unconjugated estriol

oligohydramnios (8). Morris et al reported AFP levels over 2.0 MoMs to be the best parameter to predict small for getational age infants (13). Similar to previous studies, AFP levels over 2.5 MoMs were found to be related with all of the pregnancy complications evaluated in the present study including preeclampsia, fetal growth restriction, intrauterin fetal death and oligohydramnios.

Second trimester hCG levels have been reported to be related to increased incidence of preeclampsia, intrauterine fetal death, early pregnancy loss, and fetal growth restriction(12, 15, 16). In their study including 344 pregnant women, Towner et al found a statistically significant increase in the risk of preterm birth due to preeclampsia in patients with 2nd trimester hCG levels over 3 MoMs (OR 5.9, CI 1.5–23.2) (17). Önderoğlu et al reported high 2nd trimester hCG levels to reflect abnormal placentation and an increased risk of fetal growth restriction with levels bove 2-2.5 MoMs (18). The present study also found an association between fetal growth retardation and hCG levels above 2.5 MoMs, however no such association could be detected for preeclampsia or intrauterine fetal death.

Levels of unconjugated estriol have been found to be associated with several pregnancy complications (13, 19). Kowalczyk et al found an

increased incidence of fetal growth restriction and oligohydramnios in those with 2nd trimester uE3 levels below 0.75 MoMs (19). Sayın et al noted an increased risk of oligohydramnios when uE3 levels were below 1.26 MoMs (p:0.001), and gestational diabetes in those with levels below 0.88MoMs. However, no association was found between preeclampsia and low uE3 levels (20). Schleifer et al reported low uE3 levels to be associated with intrauterine fetal death (21). The present study found uE3 levels below 0.5 MoMs to be associated with preeclampsia, but not with fetal growth restriction or intrauterine fetal death.

Although clear associations have been shown between abnormal maternal analyte levels and adverse pregnancy events by many studies, currently used serum markers have low predictive performance for pregnancy complications as preeclampsia, intrauterine growth restriction and stillbirth with reported sensitivities varying from 5% to 43% (4, 14).

Recently, investigators have reported better outcomes for the screening of adverse events by combining serum markers with other maternal characteristics and various doppler indices measured during the antenatal follow up (22, 23, 24). Elsandabesee et al reported the addition of 18-22 gestational week uterine artery Doppler parameters to increase the specificity and sensitivity and positive and negative predictive values of high 2nd trimester hCG and AFP levels in predicting pregnancy complications (23). Poon et al reported that their algorithm for screening high risk pregnancies utilizing the combination of uterine artery PI (pulsatility index) at 11-13 weeks with maternal factors, mean arterial pressure, serum PAPP-A and PIGF (placental growth factor) could predict 90% of patients who would develop preeclampsia (25). In their guide for FTS and preeclampsia prevention published in 2019, FIGO (The International Federation of Gynecology and Obstetrics**)** recommended all pregnant women to be screened for preeclampsia using a risk calculator developed by the Fetal Medicine Foundation which is available at https://fetalmedicine.org/research/assess/preeclampsia (26). The algorithm has also been shown to be useful in the prediction of SGA deliveries (27). Low dose aspirin prophylaxis is recommended for patients identified to be at risk starting from 11-15 weeks until 36 gestational weeks.

Abnormal maternal serum markers used for the screening of fetal aneuploidy during the 1st and 2nd trimesters may be helpful in the detection of women under increased risk of pregnancy complications. However because of their low sensitivity and positive predictive value, the combination of maternal serum analytes with maternal features and Doppler measurements appears to be the most effective method in predicting adverse pregnancy outcomes. The earlier detection of high risk populations will enable better monitorization and management of these patients with a subsequent reduction in fetal-maternal morbidity and mortality rates.

References

1. Walker JJ. Pre-eclampsia. Lancet. 2000; 356: 1260-1265.
2. Gaccioli F, Aye ILMH, Sovio U, Charnock-Jones DS, Smith GCS. Screening for fetal growth restriction using fetal biometry combined with maternal biomarkers. Am J Obstet Gynecol 2018; 218: S725-S737.
3. Krause TG, Christens P, Wohlfahrt J, Lei U, Westergaard T, Norgaard-Pedersen B, et al. Second-trimester maternal serum alpha-fetoprotein and risk of adverse pregnancy outcome. Obstet Gynecol 2001; 97: 277-282.
4. Gagnon A, Wilson RD, Audibert F, et al Society of Obstetricians and Gynaecologists of Canada Genetics Committee. Obstetrical complications associated with abnormal maternal serum markers analytes. J Obstet Gynaecol Can 2008; 30: 918-949.
5. Dugoff L. Society for Maternal-Fetal Medicine. First- and second-trimester maternal serum markers for aneuploidy and adverse obstetric outcomes. Obstet Gynecol 2010; 115: 1052-1061.
6. Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (The FASTER Trial). Am J Obstet Gynecol 2004; 191: 1446-1451.
7. Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin Summary, Number 222. Obstet Gynecol 2020; 135: 1492-1495.
8. Yaron Y, Cherry M, Kramer RL, et al. Second-trimester maternal serum marker screening: maternal serum alpha-fetoprotein, beta-human chorionic gonadotropin, estriol, and their various combinations as predictors of pregnancy outcome. Am J Obstet Gynecol 1999; 181: 968-967.
9. Dugoff L, Cuckle HS, Hobbins JC, et al. FaSTER Trial Research Consortium. Prediction of patient-specific risk for fetal loss

using maternal characteristics and first- and second-trimester maternal serum Down syndrome markers. Am J Obstet Gynecol. 2008; 199: 290.

10. Spencer K, Cowans NJ, Nicolaides KH. Low levels of maternal serum PAPP-A in the first trimester and the risk of pre-eclampsia. Prenat Diagn 2008; 28: 7-10.
11. Krantz D, Goetzl L, Simpson JL, et al. First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. Association of extreme first-trimester free human chorionic gonadotropin-beta, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. Am J Obstet Gynecol 2004; 191: 1452-1458.
12. 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: 471-477.
13. Spencer K. Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome. Prenat Diagn 2000; 20: 652-656.
14. Morris RK, Cnossen JS, Langejans M, 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.
15. Chandra S, Scott H, Dodds L, Watts C, Blight C, Van Den Hof M. Unexplained elevated maternal serum alpha-fetoprotein and/or human chorionic gonadotropin and the risk of adverse outcomes. Am J Obstet Gynecol 2003; 189: 775-781.
16. Dugoff L, Hobbins JC, Malone FD, et al. Quad screen as a predictor of adverse pregnancy outcome. Obstet Gynecol. 2005; 106: 260-267.
17. Towner D, Gandhi S, El Kady D. Obstetric outcomes in women with elevated maternal serum human chorionic gonadotropin. Am J Obstet Gynecol 2006; 194: 1676-1682.
18. Onderoğlu LS, Kabukçu A. Elevated second trimester human chorionic gonadotropin level associated with adverse pregnancy outcome. Int J Gynaecol Obstet 1997; 56: 245-249.
19. Kowalczyk TD, Cabaniss ML, Cusmano L. Association of low unconjugated estriol in the second trimester and adverse pregnancy outcome. Obstet Gynecol 1998; 91: 396-400.
20. Sayin NC, Canda MT, Ahmet N, Arda S, Süt 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: 47-53.
21. Schleifer RA, Bradley LA, Richards DS, Ponting NR. Pregnancy outcome for women with very low levels of maternal serum unconjugated estriol on second-trimester screening. Am J Obstet Gynecol 1995; 173: 1152-1156.
22. Spencer K, Yu CK, Cowans NJ, Otigbah C, Nicolaides KH. Prediction of pregnancycomplications by first-trimester maternal serum PAPP-A and free beta-hCG and withsecond-trimester uterine artery Doppler. Prenat Diagn 2005; 25: 949-953.
23. Elsandabesee D, Srinivas M, Kodakkattil S. The clinical value of combining maternal serum screening and uterine artery Doppler in prediction of adverse pregnancy outcome. J Obstet Gynaecol 2006; 26: 115-117.
24. Onwudiwe N, Yu CK, Poon LC, Spiliopoulos I, Nicolaides KH. Prediction of pre-eclampsia by a combination of maternal history, uterine artery Doppler and mean arterial pressure. Ultrasound Obstet Gynecol 2008; 32: 877-883.
25. Poon LC, Kametas NA, Maiz N, et al. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension 2009; 53: 812-818.
26. Poon LC, Shennan A, Hyett JA, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. Int J Gynaecol Obstet 2019; 145: 1-33.
27. Poon LC, Syngelaki A, Akolekar R, Lai J, Nicolaides KH. Combined screening for preeclampsia and small for gestational age at 11-13 weeks. Fetal Diagn Ther 2013; 33: 16-27.