Gokhan Tosun*, Ayca Nazli Akar, Derya Burkankulu, Venhar Ceyhan and Emine Aydin

Effect of combination of uterine artery doppler and vitamin D level on perinatal outcomes in second trimester pregnant women

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Abstract

Objectives: To investigate the effect of the combination of uterine artery (UtA) Doppler and vitamin D levels on perinatal outcomes in second trimester pregnants was aimed.
Methods: UtA Dopplers and vitamin D levels of 226 pregnant women in the second trimester were measured. Patients were followed for adverse pregnancy outcomes. The relationship of this combination with these pregnancy outcomes was evaluated.
Results: Sensitivity and specificity values are respectively; Left UtA-S/D ratio for preeclampsia was 85.29 and 81.77 %, left UtA-RI was 90.62 and 43.30 % for preterm birth, and 76.19 and 66.34 % for the development of fetal distress had the best predictive effect. Vitamin D values had no predictive value for any parameter (p>0.005).
Conclusions: UtA Doppler has a good predictive value for many adverse pregnancy outcomes. However, in order to correlate these results with second trimester vitamin D levels, more homogeneous and specific groups are needed.

Keywords: uterine artery Doppler; vitamin D; preeclampsia; premature birth; fetal growth restriction

***Corresponding author: Gokhan Tosun**, Associate Professor, Izmir Faculty of Medicine, Department of Obstetrics and Gynecology, University of Health Sciences, Izmir, Türkiye, E-mail: drgokhan77@gmail.com. https://orcid.org/0000-0001-6229-2270
Ayca Nazli Akar, Izmir Faculty of Medicine, Department of Obstetrics and Gynecology, University of Health Sciences, Turkey, Izmir, Türkiye. https://orcid.org/0000-0002-7495-5470
Derya Burkankulu, Department of Obstetrics and Gynecology, Izmir Democracy University, Buca Seyfi Demirsoy Education and Research Hospital, Izmir, Türkiye
Venhar Ceyhan, Department of Obstetrics and Gynecology, Kayseri City Education and Research Hospital, Kayseri, Türkiye
Emine Aydin, Department of Obstetrics and Gynecology, Division of Perinatology, Istanbul Medipol University, Istanbul, Türkiye

Introduction

Uterine artery (UtA) Doppler (UAD) continues to be used increasingly as the best indicator of uteroplacental blood flow, even in the early weeks, to predict adverse pregnancy outcomes that may occur in advanced pregnancy [1]. The leading adverse pregnancy outcome is preeclampsia (PE), which is also an important cause of maternal death [2]. While the basic mechanism in PE is impaired placentation with abnormal trophoblast invasion, increased vascular resistance and impaired uteroplacental flow can present many conditions, including result in olgohydramniosis, spontaneous preterm birth (SPB), small for gestational age (SGA) newborn, fetal growth retardation (FGR) and fetal death [3, 4]. With this, various studies continue to be published regarding the gestational age at which UAD should be evaluated, and it takes its place in the literature day by day, both alone and with additional marker research to strengthen this prediction of UAD [5, 6]. In the literature, many markers, from histidine to high-temperature requirement protease A4 (HtrA4), from high mobility group box-1 (HMGB-1) to serum micro RNA-210, are being studied together with UAD in predicting negative future pregnancy outcomes [2, 7–9]. Studies on vitamin D, one of these markers, have produced different results in terms of the prediction of this marker alone on adverse pregnancy outcomes, especially PE [10–14]. While some studies suggest this predictive effect of vitamin D level [10–12], others reveal that it has no relationship [13, 14]. However, there is only one study in the literature that includes the relationship between UAD and vitamin D levels regarding this prediction [10].

In this context, the aim of this study is to investigate the effect of the combination of UAD and vitamin D level in predicting adverse pregnancy outcomes between 20^{+0}–24^{+0} weeks of gestation.

Materials and methods

This study was carried out at Istanbul Medpiol University Faculty of Medicine Hospital between June 2021 and March 2024. UAD and vitamin D levels were measured in pregnant women between 20^{+0}-24^{+0} weeks. In this context, all

pregnant women between 20 + 0–24 + 0 weeks included in the study were evaluated for age, height, body weight, gravida, parity, abortion, previous obstetric history, additional internal diseases, previous surgery history, smoking status, aspirin and low molecular weight heparin. (LMWH) usage was asked and the information was recorded. Pregnant women were placed in a supine position on a flat stretcher with a full bladder, and the ultrasound (Voluson e6™) abdominal probe (c2-9 Mhz) was moved laterally from the midline in the longitudinal axis, from the mons pubis level to the spina iliac anterior superior (SIAS), and the iliac arteries were visualized. Then, it was colored and the right and left uterine arteries were visualized by angling the probe. Then, Doppler was turned on and uterine artery pulsatility index (PI), resistance index (RI) and systole/diastole ratio (S/D ratio) were obtained. Additionally, the presence of notch was evaluated. During the same weeks of pregnancy, the patients' vitamin D levels were measured after a 12-h fast. For this, patients gave approximately 5 cc of blood. In the routine obstetric examinations of these patients in the following weeks of pregnancy, PE, SGA, FGR, fetal distress, oligohydramnios, decrease in baby movements, whether SPB developed or not, as well as birth weeks, delivery method, birth weight of the babies and APGAR scores were determined. The relationship of these results with the combination of UAD and vitamin D levels was determined.

The study received approval from the Istanbul Medipol University Ethics Committee (date: 25.05.2021, number: E–10840098-772.02-2385), informed consent was obtained from all participating volunteers, and the study was carried out in accordance with the Declaration of Helsinki.

Definitions

AGA: (babies with normal weight according to birth week) babies with birth weights between the 10th and 90th percentile, SGA; babies with birth weight below the 10th percentile and FGR. It was defined as being<10th percentile according to the baby's birth week and accompanied by findings such as loss of end daystolic flow in umbilical artery Doppler. Preterm birth: it was defined as births occurring before 37 + 0 weeks of gestation. Accordingly, while early preterm birth was defined as births at 33 + 6 weeks and below, late preterm birth was defined as births between 34 + 0–36 + 6 weeks. Gestational hypertension: after the 20th week of gestation, blood pressure of 140/90 mmHg and above was defined as arterial presence, while the presence of at least 300 mg/day protein in urine was defined as PE. Severe PE: it was defined as arterial blood pressure≥160/100 mmHg, accompanied by proteinuria, as well as maternal organ damage or HELLP syndrome, which is accompanied by hemolysis, eclamptic convulsions, elevated liver enzymes and low platelets. Fetal distress was defined as late decelerations occurring after contraction in the non-stress test (NST) and/or the presence of an inverted A wave in ductus venosus Doppler.

Laboratory evaluation

Venous blood samples were collected after 8–12 h of fasting. After centrifugation, vitamin D level was measured by the chemiluminescence immunoassay method using the Advia Centaur XPT device (Siemens Healthcare Diagnostics Inc, Tarrytown, NY, USA). Fresh blood samples were used. The same devices and kits were used throughout the entire study.

Statistical analysis

Mean, standard deviation, median, minimum and maximum values in descriptive statistics of continuous variables; In defining categorical variables, frequency (n) and percentage (%) values are given. Normality assumptions of the variables were examined with the Kolmogorov Smirnov test. Mann–Whitney U test was used to compare continuous variables that did not show normal distribution between two groups. Relationships between categorical variables were examined with Chi-square/Fisher exact analysis. Univariate and multivariate logistic regression analysis was performed to determine the variables that predict adverse pregnancy outcomes. Receiver operating characteristics (ROC) analysis was performed to calculate sensitivity, specificity, positive and negative predictability values. IBM SPSS.25 program and MedCalc were used in the analyses, and $p<0.05$ was accepted as the significance level.

Results

According to power analysis, 226 patients were included in the study [7, 15]. The demographic characteristics and Doppler findings of the patients are given in Table 1. Comparative data of UAD parameters and vitamin D levels in pregnant women with and without PE, preterm birth and fetal distress are given in Table 2, and comparative data in pregnant women with and without SGA and FGR are given in Table 3. Univariate and multivariate logistic regression analyzes related to PE, preterm birth, fetal distress, SGA and

Table 1: Medical and demographic characteristics of the patients.

Parameters	Number	%	Mean ± SD	Median (Min.-Max.)
Age, year	226		27.75 ± 5.33	27 (18–41)
BMI, kg/m^2	226		26.80 ± 4.65	26.3 (17.74–47.65)
Gravida	226		2.50 ± 1.38	2 [1–9]
Parity	226		1.17 ± 1.09	1 (0–5)
Systolic blood pressure arterial, mmHg	226		109.52 ± 13.25	110 (80–147)
Diastolic blood pressure arterial, mmHg	226		66.39 ± 8.71	67.5 (50–96)
Pregnancy week on USG, week	226		22.21 ± 1.48	22 (20–24)
Pregnancy week in blood sample, week	226		22.21 ± 1.48	22 (20–25)
Vitamin D, µg/L	226		15.77 ± 9.39	13.39 (3.55–87)
Right uterine artery pulsatility index (PI)	226		1.09 ± 0.74	0.99 (0.13–7.59)
Right uterine artery resistance index (RI)	226		0.86 ± 3.82	0.57 (0.19–58)
Right uterine artery systole/diastole (S/D) ratio	226		3.42 ± 1.87	2.93 (0.13–9.87)
Left uterine artery PI	226		1.46 ± 4.26	0.99 (0.06–64)
Left uterine artery RI	226		0.60 ± 0.17	0.57 (0.26–1)
Left uterine artery S/D ratio	226		3.53 ± 2.22	2.79 (0.45–18.5)
APGAR 1st minute	226		7.96 ± 1.00	8 [4–9]
APGAR 5th minute	226		9.07 ± 0.89	9 [2,5–9]
Pregnancy week at birth, week	226		38.29 ± 1.91	38.5 (28–42)
Baby birth weight, gram	226		3,118.77 ± 515.40	3,200 (1,100–4,200)
Presence of additional disease	33	14.6		
Epilepsy	1	0.44		
Goiter	6	2.65		
Chronic renal failure	1	0.44		
Hypthyroidism	10	4.4		
Obsessive compulsive disorder	1	0.44		
Rheumatic disease	1	0.44		
Cholelithiasis	2	0.88		
Pituitary adenoma	1	0.44		
Migraine	1	0.44		
Behçet	1	0.44		
Tuberculosis	1	0.44		
Varicosis	1	0.44		
Bipolar affective disorder	1	0.44		
Asthma	3	1.31		
FMF	2	0.88		
Cigarette	20	8.8		
Aspirin	7	3.1		
LMWH	5	2.2		
Notch in the right uterine artery	12	5.3		
Notch in left uterine artery	14	6.2		
Preterm birth	32	14.2		
Early preterm birth	7	21.9		
Late preterm birth	25	78.1		
SGA	22	9.7		
FGR	17	7.5		
Preeclampsia	34	15		
Fetal distress	21	9.3		
Oligohydramnios	4	1.8		
Decrease in baby movements	4	1.8		
Type of birth				
Vaginal birth	148	65.5		
Cesarean birth	78	34.5		
Cause of birth				
Travail	188	83.2		
Fetal distress	23	10.2		
Preeclampsia	1	0.44		
FGR	1	0.44		
Oligohydramnios	4	1.8		
Decrease in baby movements	5	2.2		
Premature membrane rupture	3	1.31		
Postmaturity	1	0.44		

BMI, body mass index; FMF, familial mediterranean fever; LMWH, low molecular weight heparin; SGA, small for gestational age; FGR, fetal growth retardation.

FGR are given in Table 4, and the sensitivity and specificity values of the uterine artery in predicting PE, preterm birth, fetal distress, SGA and FGR are given in Table 5.

There was no significant difference in UAD parameters and vitamin D levels between patients with and without oligohydramniosis (p>0.05). However, as a result of univariate logistic regression analysis, it was found that left UtA-PI (p=0.013) and left UtA-RI (p=0.009) values significantly predicted oligohydramniosis status. In multivariate regression analysis, it was found that neither parameter significantly predicted oligohydramniosis (p>0.05). The optimal cut-off value of the left UtA-PI value in predicting oligohydramniosis was found to be≤0.70, with a sensitivity of 75.00 and a specificity of 81.98. The optimal cut-off value of the left UtA-RI value was found to be≤0.47, sensitivity 75.00 and specificity 78.83. There was no significant difference in any parameter between patients with and without decreased baby movements, smoking, low molecular weight heparin (LMWH) use and aspirin use (p>0.05).

Table 2: Comparison of uterine artery and vitamin D parameters according to preeclampsia, preterm birth and fetal distress status.

	No preeclampsia		Preeclampsia		
	Mean ± SD	**Median (min-max)**	**Mean ± SD**	**Median (min-max)**	**p-Value**
Vitamin D	16.20 ± 9.70	13.70 (5.01–87)	13.32 ± 7.03	11.39 (3.55–31.49)	0.078
Right uterine artery PI	1.09 ± 0.73	1 (0.13–7.59)	1.11 ± 0.81	0.95 (0.50–5.09)	0.703
Right uterine artery RI	0.90 ± 4.15	0.57 (0.19–58)	0.59 ± 0.15	0.57 (0.33–0.99)	0.495
Right uterine artery S/D ratio	3.16 ± 1.72	2.70 (0.58–9.87)	4.83 ± 2.08	4.97 (0.13–8.5)	**<0.001**
Left uterine artery PI	1.12 ± 0.69	0.97 (0.06–4.79)	3.42 ± 10.79	1.08 (0.54–64)	**0.014**
Left uterine artery RI	0.59 ± 0.17	0.57 (0.26–1)	0.66 ± 0.18	0.62 (0.37–0.99)	**0.028**
Left uterine artery S/D ratio	3.06 ± 1.69	2.59 (0.45–8.76)	6.19 ± 2.90	6.12 (1.20–18.5)	**<0.001**
	n	**%**	**n**	**%**	
Right uterine artery notch (–)	182	94.8	32	94.1	0.698
Right uterine artery notch (+)	10	5.2	32	94.1	
Left uterine artery notch (–)	182	94.8	30	88.2	0.236
Left uterine artery notch (+)	10	5.2	4	11.8	
	No preterm birth		**Preterm birth**		
	Mean ± SD	**Median (min-max)**	**Mean ± SD**	**Median (min-max)**	**p-Value**
Vitamin D	15.81 ± 9.65	13.10 (3.55–87)	15.54 ± 7.77	14.83 (4.32–32.43)	0.931
Right uterine artery PI	1.10 ± 0.77	0.98 (0.13–7.59)	1.05 ± 0.45	1.02 (0.42–2.68)	0.769
Right uterine artery RI	0.89 ± 4.12	0.57 (0.19–58.00)	0.64 ± 0.18	0.57 (0.29–0.99)	0.327
Right uterine artery S/D ratio	3.38 ± 1.80	2.89 (0.58–9.87)	3.62 ± 2.29	2.99 (0.13–8.50)	0.980
Left uterine artery PI	1.43 ± 4.57	0.97 (0.06–64)	1.70 ± 1.27	1.31 (0.58–6.22)	**<0.001**
Left uterine artery RI	0.58 ± 0.17	0.56 (0.26–1)	0.69 ± 0.17	0.65 (0.46–0.99)	**<0.001**
Left uterine artery S/D ratio	3.53 ± 2.27	2.80 (0.45–18.5)	3.55 ± 1.91	2.76 (0.57–8.19)	0.696
	n	**%**	**n**	**%**	
Right uterine artery notch (–)	183	94.3	31	96.9	1.00
Right uterine artery notch (+)	11	5.7	1	3.1	
Left uterine artery notch (–)	182	93.8	30	93.8	1.00
Left uterine artery notch (+)	12	6.2	2	6.3	
	No fetal distress		**Fetal distress**		
	Mean ± SD	**Median (min-max)**	**Mean ± SD**	**Median (min-max)**	**p-Value**
Vitamin D	15.86 ± 9.64	13.30 (3.55–87.00)	14.86 ± 6.58	14.20 (5.84–28.82)	0.992
Right uterine artery PI	1.08 ± 0.76	0.95 (0.13–7.59)	1.20 ± 0.45	1.07 (0.52–2.68)	**0.025**
Right uterine artery RI	0.88 ± 4.01	0.56 (0.19–58)	0.64 ± 0.12	0.64 (0.40–0.99)	0.057
Right uterine artery S/D ratio	3.36 ± 1.85	2.79 (0.58–9.87)	3.93 ± 2.04	3.95 (0.13–7.98)	0.104
Left uterine artery PI	1.45 ± 4.46	0.97 (0.06–64)	1.61 ± 1.23	1.25 (0.29–6.22)	**0.005**
Left uterine artery RI	0.59 ± 0.18	0.56 (0.26–1)	0.68 ± 0.13	0.66 (0.46–0.99)	**0.002**
	n	**%**	**n**	**%**	
Right uterine artery notch (–)	196	95.6	18	85.7	0.88
Right uterine artery notch (+)	9	4.4	3	14.3	
Left uterine artery notch (–)	194	94.6	18	85.7	0.106
Left uterine artery notch (+)	11	5.4	3	14.3	

PI, pulsatility index; RI, resistance index; S/D, systole/diastole ratio; (–), absent; (+), present; bold text is statistical significant.

Discussion

In this study, the effect of UAD and vitamin D combination on adverse pregnancy outcomes, especially FGR and PE, in pregnant women between 20^{+0}-24^{+0} weeks of gestation was investigated. According to our results, while UAD parameters have a predictive effect on PE, FGR, SGA, premature birth and cesarean delivery due to fetal distress, they do not

Table 3: Comparative data of uterine artery Doppler parameters and vitamin D levels in pregnant women with and without SGA and FGR.

	No SGA		SGA		
	Mean ± SD	**Median (min-max)**	**Mean ± SD**	**Median (min-max)**	**p-Value**
Vitamin D	15.88 ± 9.48	13.50 (3.55–87)	14.75 ± 8.64	11.54 (5.01–40)	0.532
Right uterine artery PI	1.09 ± 0.76	0.97 (0.13–7.59)	1.12 ± 0.46	1.02 (0.45–2.11)	0.412
Right uterine artery RI	0.88 ± 4.02	0.57 (0.19–58)	0.62 ± 0.15	0.58 (0.38–0.99)	0.327
Right uterine artery S/D ratio	3.30 ± 1.83	2.78 (0.13–9.87)	4.48 ± 1.93	3.97 (1.60–7.98)	0.003
Left uterine artery PI	1.49 ± 4.48	0.97 (0.06–64)	1.25 ± 0.48	1.11 (0.53–2.34)	0.072
Left uterine artery RI	0.59 ± 0.17	0.57 (0.26–1)	0.66 ± 0.17	0.65 (0.34–0.99)	0.045
Left uterine artery S/D ratio	3.38 ± 2.15	2.76 (0.45–18.5)	4.91 ± 2.35	4.78 (1.24–8.5)	0.003
	n	**%**	**n**	**%**	
Right uterine artery notch (–)	194	95.1	20	90.9	0.329
Right uterine artery notch (+)	10	4.9	2	9.1	
Left uterine artery notch (–)	192	94.1	20	90.9	0.633
Left uterine artery notch (+)	12	5.9	2	9.1	
	No FGR		**FGR**		
	Mean ± SD	**Median (min-max)**	**Mean ± SD**	**Median (min-max)**	**p-Value**
Vitamin D	15.89 ± 9.65	13.21 (3.55–87)	14.29 ± 5.34	15.05 (6.50–27.46)	0.805
Right uterine artery PI	1.06 ± 0.68	0.99 (0.13–7.59)	1.46 ± 1.23	0.95 (0.13–5.09)	0.300
Right uterine artery RI	0.74 ± 0.19	0.71 (0.45–1.02)	0.86 ± 3.17	0.57 (0.19–0.58)	**0.002**
Right uterine artery S/D ratio	3.29 ± 1.76	2.79 (0.13–8.5)	4.93 ± 2.52	4.16 (1.40–9.87)	0.005
Left uterine artery PI	1.46 ± 4.42	0.97 (0.29–64)	1.51 ± 1.46	1.25 (0.06–6.77)	0.133
Left uterine artery RI	0.59 ± 0.17	0.57 (0.26–1)	0.68 ± 0.19	0.66 (0.30–0.99)	**0.029**
Left uterine artery S/D ratio	3.38 ± 1.92	2.78 (0.45–8.76)	5.36 ± 4.18	4.32 (0.58–18.5)	**0.034**
	n	**%**	**n**	**%**	
Right uterine artery notch (–)	200	95.7	14	82.4	0.051
Right uterine artery notch (+)	9	4.3	3	17.6	
Left uterine artery notch (–)	198	94.7	14	82.4	0.077
Left uterine artery notch (+)	11	5.3	3	17.6	

PI, pulsatility index; RI, resistance index; S/D, systole/diastole ratio; (–), absent; (+), present; bold text is statistical significant, SGA, small for gestational age; FGR, fetal growth retardation.

have any prediction for decrease in baby movements, smoking, aspirin and LMWH use. In oligohydramnios, left UtA-PI (p=0.013) and left UtA-RI (p=0.009) values were significantly predictive only in the univariate logistic regression analysis, but this was not observed in the multivariate logistic regression analysis. However, it was concluded that vitamin D levels were not predictive of all parameters.

UAD studies continue to increase day by day in order to obtain early predictions for many adverse pregnancy outcomes, especially PE. In fact, the underlying pathology stems from the vital importance of the invasion of trophoblasts into the uterine decidua and decidual vessels in the formation of the placenta. Placentation defects PE, FGR and SGA are closely related to neonatal etiologies, and it is possible to make predictions about these important vascular events during pregnancy with UAD. In 2022, a very large review examining UAD and its impact on pregnancy complications, including 55,974 women and revealing a 38-year study, found that UAD was used to predict pregnancy complications such as recurrent pregnancy loss, PE, FGR, stillbirth, and SPB. It shows that the combination of UAD with other biochemical markers provides a predictive result of over 90 % for early-onset PE/FGR, where poor placental implantation is a factor. It also emphasized that a UtA-PI greater than 1.45 in low-risk women in the second trimester is a critical indicator in predicting PE [1]. In our study, in the second trimester, left UtA-PI was statistically significantly higher in pregnant women with PE (p=0.014), but especially the left UtA-S/D ratio was 2 times higher in pregnant women with PE compared to the control group (p<0.001). While the sensitivity of the same value was determined as 85.29 % and the specificity as 81.77 %, PPV; was 45.3 and NPV was 96.9. Although UAD offers a strong exclusion rate in cases where PE will not occur, it

Table 4: Univariate and multivariate logistic regression analyzes associated with preeclampsia, preterm birth, fetal distress, SGA and FGR.

	Univariate logistic regression analyzes				Multivariate logistic regression analyzes			
	B	Odds ratio	95 % CI	p	B	Odds ratio	95 % CI	p-Value
Preeclampsia								
Right uterine artery S/D ratio	0.414	1.513	1.259–1.818	<0.001	−0.187	0.829	0.611–1.125	0.229
Left uterine artery PI	0.464	1.590	1.116–2.265	0.01	0.364	1.439	0.692–2.993	0.329
Left uterine artery RI	2.182	8.865	1.215–64.661	0.031	−2.188	0.112	0.002–6.415	0.289
Left uterine artery S/D ratio	0.669	1.952	1.577–2.416	<0.001	0.814	2.258	1.628–3.130	<0.001
Preterm birth								
Left uterine artery PI	0.012	1.012	0.943–1.086	0.738				
Left uterine artery RI	3.352	28.572	3.708–220.178	0.001				
Fetal distress								
Right uterine artery S/D ratio	0.175	1.192	0.736–1.930	0.476				
Left uterine artery PI	0.008	1.008	0.922–1.101	0.866				
Left uterine artery RI	2.798	16.416	1.512–178.287	0.021				
Left uterine artery S/D ratio	0.163	1.177	0.997–1.389	0.055				
SGA								
Right uterine artery S/D ratio	0.284	1.328	1.081–1.632	0.007	0.154	1.167	0.873–1.559	0.296
Left uterine artery S/D ratio	0.236	1.266	1.065–1.504	0.007	0.149	1.160	0.920–1.463	0.209
Left uterine artery RI	1.957	7.081	0.668–75.093	0.104	–	–	–	–
FGR								
Right uterine artery S/D ratio	0.377	1.458	1.162–1.830	0.001	0.240	1.271	0.944–1.713	0.115
Left uterine artery RI	2.704	14.946	1.103–202.443	0.042	1.640	5.153	0.244–1.728	0.292
Left uterine artery S/D ratio	0.285	1.330	1.096–1.613	0.004	0.115	1.122	0.877–1.434	0.360
Right uterine artery RI	−0.011	0.989	0.832–1.175	0.898	–	–	–	–

SGA, small for gestational age; FGR, fetal growth retardation.

Table 5: Sensitivity and specificity values of uterine artery in predicting preeclampsia, preterm birth, fetal distress, SGA and FGR.

	Cut off value	AUC	Sensitivity, %	Specificity, %	PLR	NLR	PPV	NPV
Preeclampsia								
Left uterine artery S/D ratio	>4.25	0.849	85.29	81.77	4.68	0.18	45.3	96.9
Preterm birth								
Left uterine artery RI	>0.53	0.693	90.62	43.30	1.60	0.22	20.9	96.6
Fetal distress								
Left uterine artery RI	>0.61	0.709	76.19	66.34	2.26	0.36	18.8	96.5
SGA								
Right uterine artery S/D ratio	>3.11	0.694	81.82	55.88	1.85	0.33	16.7	96.6
Left uterine artery S/D ratio	>4.3	0.695	63.64	75.98	2.65	0.48	22.2	95.1
FGR								
Right uterine artery S/D ratio	>3.42	0.706	76.47	63.64	2.10	0.37	14.6	97.1
Left uterine artery RI	>0.64	0.659	64.71	72.25	2.33	0.49	15.9	96.2
Left uterine artery S/D ratio	>4.17	0.655	64.71	73.21	2.41	0.48	16.4	96.2

PLR, positive likelihood ratio; NLR, negative likelihood ratio; PPV, positive predictive value (positive predictive values); NPV, negative predictive value (negative predictive values); S/D ratio, systol/diastole ratio; SGA, small for gestational age; FGR, fetal growth retardation.

still seems to need additional markers to predict PE. In fact, when we look at the literature, we see that new studies are being conducted every day to strengthen this prediction and increase the possibility of predicting real PE cases. Some of these markers presented in combination with UAD are histidine-rich glycoprotein (the combination of HRG and

UtA-PI revealed 91 % sensitivity and 62 % specificity for preterm PE) [7], protease A4 (HtrA4) (Serum HtrA4 levels and UtA-PI The combination of > 95th percentile provided sensitivity, specificity, PPV and NPV of 79.4 , 86.1, 37 and 97.6 %, respectively, for PE prediction) [8], serum high mobility group box-1 (HMGB1) (Using mean UtA-PI above the 95th percentile with abnormal serum HMGB1 levels, sensitivity, specificity, PPV, and NPV for predicting PE were 88.0 , 50.8, 10.8, and 98.4 %, respectively. This study showed that serum HMGB1 at 16^{+0}–20^{+6} weeks of gestation was effective in predicting PE, but the addition of UtA-PI did not improve the prediction performance) [9] and even serum micro RNA-210 (for predicting PE when abnormal serum micro RNA-210 level, abnormal mean PI or uterine artery diastolic notches were used, sensitivity, specificity, PPV and NPV were found to be 95.5 , 54.9, 10.0 and 99.6 %, respectively. And in this study, sensitivity, specificity, PPV, and NPV for predicting early-onset PE were 100.0 , 53.2, 3.3, and 100.0 %, respectively) [2]. A study was added to these studies in 2021, showing that the combination of vitamin D level and UADs predicts the risk of PE. In this study, it was revealed that first trimester UtA-PI and calciferol levels showed 83.87 % sensitivity and 90.9 % specificity for the development of PE, and the overall accuracy of the test was 86.79 % [10]. Theoretically, the relationship between Vit D and PE development has been tried to be explained by the effect of Vitamin D on implantation, angiogenesis and endothelial status, regulation of immune response, Renin-Angiotensin and Aldosterone system (RAAS) and calcium metabolism [11, 12]. However, the contribution of Vitamin D on placentation has been suggested [13], but the exact role of vitamin D in this process has still not been elucidated. It is also stated that the beneficial effect of Vitamin D on pregnancy development can only be observed when supplementation is initiated during placental implantation [16]. And of course, the role of vitamin D on steroid hormone synthesis has also been held responsible for this effect [17, 18].

In our study, vitamin D levels measured in the second trimester were found to be an average of 16.20 ng/mL in patients who did not develop PE, and an average of 13.32 ng/mL in patients who developed PE. Similarly, the average was 15.86 ng/mL in pregnant women who did not develop oligohydramnios, while the average was 10.72 ng/mL in those who did not. However, in both cases, this difference was not statistically significant. However, vitamin D level gave similar results between both groups in all other pregnancy outcomes, including SPB, SGA newborn, and FGR. FGR, which often occurs as a result of PE, actually consists of two phenotypic conditions that manifest themselves with different developmental stages and consequences. While early-onset FGR (usually detected earlier than the 32nd week of gestation) presents a typical deterioration pattern progressing from increased abnormalities in Doppler parameters to abnormal biophysical parameters [3], in late-onset FGR, mildly abnormal cerebral Doppler along with normal or It has minimally elevated umbilical artery Doppler indices but does not exhibit any significant cardiovascular or biophysical changes [4]. Studies show that, unlike early-onset FGR, the relationship between PE and late-onset FGR is weaker [4]. In early-onset FGR, the main Doppler changes are at the level of the umbilical artery, with a progressive increase in PI until the absence of end diastolic flow (EDF) or reverse flow. Changes in cerebral, cardiac, and ductus venosus circulation are often present, but with different sequences. Late-onset FGR is determined by third-trimester placental insufficiency leading to fetal hypoxia. Cerebro-placental ratio (CPR) and middle cerebral artery pulsatility index (MCA-PI) appear to be the main markers for both diagnosis and obstetric treatment, while umbilical artery Doppler PI is often normal. In our study, a significant increase in the right UtA-RI, left UtA-RI and left UtA-S/D ratio was observed in cases with FGR. However, the FGR rate of the patients in our study was 7.5 % (17/220) and only 3 of them (1.36 %) had early-onset FGR. In our study, univariate logistic regression analysis found that right UtA-S/D ratio (p=0.001), left UtA-RI value (p=0.042) and left UtA-S/D ratio (p=0.004) significantly predicted FGR status. In multivariate logistic regression analysis, it was determined that all three parameters did not significantly predict FGR status (p>0.05). We think that the fact that all UAD parameters were not affected is due to the late onset of the majority of the FGR population.

Another condition in PE is excessive thromboxane production, which is already known. For this reason, aspirin, which inhibits thromboxane production, is used prophylactically in PE [19]. Studies on this subject have revealed that a statistically significant decrease in the occurrence of PE is observed with the use of aspirin, however, it is also stated that the reduction in risk is small to moderate and many women will need to be treated to prevent a PE case [19]. In fact, this is because the high-risk subgroups that may benefit most clinically from aspirin have not been identified [19]. In our study, the rate of patients using aspirin was 3.1 % (n=7), and all of them had experienced PE in their previous pregnancies. However, none of these patients developed PE in the index pregnancies. Additionally, no difference was observed in terms of UAD parameters between patients using and not using aspirin.

Another issue is that UAD evaluated prenatally carries some clues for SGA, which can often be only constitutional and has a limited relationship with other negative

pregnancy outcomes. This situation mostly involves a search for foresight regarding the risks that the suspected SGA newborn will face in the postpartum period. Considering the clinical high-risk factors, UAD has a better predictive effect on PE and SGA newborns at 22–24 weeks of gestation in high-risk women [5]. However, unlike the high-risk group, low-risk pregnancies intended to result in term birth already show increased diastolic blood flow velocity and early diastolic notch loss by the 22nd week of pregnancy. However, pregnancies with early diastolic notches and persistent high resistance waveforms are at risk of premature birth due to PE, placental abruption and FGR [6]. In a meta-analysis published in 2019 that included 17 observational studies, the best predictor of third trimester UAD for suspected SGA fetuses was for perinatal mortality and it was emphasized that the worst prediction was for the composite adverse perinatal outcome consisting of admission to the neonatal intensive care unit, cesarean delivery due to intrapartum fetal risk, 5th minute APGAR score below 7, and neonatal acidosis [20]. In our study, the best predictive parameter for the development of fetal distress was left UtA-RI with 76.19 % sensitivity and 66.34 % specificity. In pregnancies that developed SGA, both right and left UtA-S/D ratios were significantly higher than in pregnancies that did not develop SGA. However, no association was found with decreased baby movements. In addition to all this, no correlation was detected between vitamin D level and these parameters.

Studies have also focused on the relationship between UAD and spontaneous preterm birth (SPB) and investigated whether UAD is predictive of this extremely important adverse pregnancy outcome, which is a precursor of neonatal morbidity and mortality. In a cohort study from England conducted between 1999 and 2002, 234 SPB and 5472 term births were compared and no significant correlation was found between UAD and week of birth [21]. In their study published in 2022, Camen et al. found no statistical relationship between UAD and preterm birth before the 32nd week of gestation, but UtA-S/D ratio and UtA-PI were correlated with preterm birth in pregnant women between 32 and 35.5 weeks ($p<0.005$) and emphasized that women with second trimester abnormal UAD should be examined more closely [22]. In fact, the underlying reason for the predictive value of the uterine artery on SPB is the 'major obstetric syndrome' associated with the failure of the physiological transformation of the spiral arteries [23]. Previous studies have suggested that women with signs of placental dysfunction have higher UtA blood flow impedance, resulting in failure of physiological transformation of the spiral arteries [23, 24]. In fact, studies have reached contradictory results regarding the relationship between SPB and UtA resistance [25, 26]. While one study stated that UAD evaluated in the first trimester is not a predictor of preterm birth, but when combined with smoking and previous preterm birth history, it can predict births below 34 weeks with a false positive rate of 8 % [25], in another one, it was highlighted that UtA-PI measured between 22 and 24 weeks was higher in SPBs before the 33rd week than in women giving birth at 33 weeks or later, however, UtA-PI did not provide a significant improvement in the SPB prediction provided by the mother's demographic characteristics and previous obstetric history [26]. In addition, a study stated that fetal gender has an effect on Doppler parameters, suggesting that second trimester UtA resistance and the frequency of notching in UtA are higher in women carrying male fetuses [27]. In the light of all these studies, we think that the importance of the gestational age at which UAD is evaluated should be considered. In a study in which UAD was evaluated in three different periods of pregnancy: 18^{+0}-22^{+6} weeks, 28^{+0}-31^{+6} weeks and 32^{+0}-35^{+6} weeks, a relationship was detected between UtA-S/D ratio and UtA-PI and preterm birth only in the measurement between 32^{+0}-35^{+5} weeks, and this relationship was a low-grade relationship with late preterm birth [22]. In our study, only the left UtA-RI value gave significant results in univariate logistic regression analysis between 20^{+0}-24^{+0} weeks of gestation. Left UtA-RI, sensitivity was 90.62 % and specificity was 43.30 %. PPV was 20.9 and NPV was 96.6. Only 1 of these cases was iatrogenic preterm birth and the other 31 patients were SPB cases. This clearly demonstrates the success of ruling out SPB with a normal UtA-RI value. However, in case of positive values, it is important to evaluate and follow the patient together with other risk factors. Vitamin D studies on preterm birth did not yield the expected results, and despite a small number of studies suggesting that vit D levels and preterm birth are correlated [28], no significant relationship could be established between SPB and vitamin D levels [14]. However, there are also studies suggesting that vitamin D deficiency is associated with births below the 37th week, even after confounding factors such as ethnic factors, race, and geographical conditions are eliminated and this result is mostly attributed to the relationship between vitamin D deficiency and bacterial vaginal infections and SPBs that develop secondary to these infections [29, 30]. A study from Poland could not find a full correlation between vitamin D deficiency and preterm birth and stated that only severe vitamin D deficiency could be a risk factor for preterm birth [31]. In our study, no relationship could be established between vitamin D levels and SPB, as with all other negative pregnancy outcomes. The average vitamin D level of the patients included in our study was 15.77 ± 9.39 ng/mL, and severe vitamin D deficiency (<10 ng/mL) was not observed, but according to our results, no difference was observed

between vitamin D levels in the patient group who developed SPB and those who did not. Of course, there are many factors that cause SPB, especially infectious, inflammatory and connective tissue diseases. However, when the literature is examined, it is seen that in order to associate SPB with vitamin D deficiency, there is a need to conduct randomized controlled studies in groups with more specific and larger sample sizes, in which all other causes and risk factors are excluded.

Limitations

The limitation of the study is that it does not go into etiological detail for each adverse pregnancy outcome and it works with a heterogeneous group.

Strengths

The strengths of the study are that it is the second study conducted in this combination and the study with the largest sample size.

Conclusions

In the light of all these data, we conclude that the combination of UAD and vitamin D is not predictive of adverse pregnancy outcomes. Vitamin D, either alone or on its own, does not have any predictive effect on the effects of UAD. As known so far, UAD continues to be a sensitive antenatal test in the prediction of both PE, early-onset FGR, and SPB and SGA newborns. However, in our study, it was concluded that the S/D ratio, instead of PI, which is more emphasized in the literature, and the left UtA parameters are more predictive than the right UtA. In this context, there is a need for randomized controlled, double-blind studies with specific and homogeneous groups and larger sample sizes, free from all other confounding factors, both for the isolated effect of vitamin D and its combined effect with UAD.

Research ethics: The study received approval from the Istanbul Medipol University Ethics Committee (date: 25.05.2021, number: E–10840098-772.02-2385), and the study was carried out in accordance with the Declaration of Helsinki.
Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

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References

1. Tian Y, Yang X. A review of roles of uterine artery doppler in pregnancy complications. Front Med 2022;9:813–43.
2. Trongpisutsak A, Phupong V. Prediction of preeclampsia using a combination of serum mic ro RNA-210 and uterine artery doppler ultrasound. Sci Prog 2021;104:1–5.
3. Turan OM, Turan S, Gungor S, Berg C, Moyano D, Gembruch K, et al. Progression of doppler abnormalities in intrauterine growth restriction. Ultrasound Obstet Gynecol 2008;32:160–7.
4. Muresan D, Rotar IC, Stamatian F. The usefulness of fetal Doppler evaluation in early versus late onset intrauterine growth restriction. Review of the literature. Med Ultrasonography 2016;18:103.
5. Coleman MA, McCowan LM, North RA. Mid-trimester uterine artery Doppler screening as a predictor of adverse pregnancy outcome in high-risk women. Ultrasound Obstet Gynecol 2000;15:7–12.
6. Parry S, Sciscione A, Haas DM, Grobman WA, Iams JD, Mercer BM, et al. Role of early second-trimester uterine artery doppler screening to predict small-for-gestational-age babies in nulliparous women. AJOG 2017;59:1–10.
7. Bolin M, Wikström AK, Wiberg-Itzel E, Olsson AK, Ringvall M, Poroma-Sundström I, et al. Prediction of preeclampsia by combining serum histidine-rich glycoprotein and uterine artery doppler. Am J Hypertens 2012;25:1305–10.
8. Siricharoenthai P, Phupong V. The first-trimester serum high-temperature requirement protease A4 and uterine artery doppler for the prediction of preeclampsia. Sci Rep 2023;13:8295.
9. Wairachpanich V, Phupong V. Second-trimester serum high mobility group box-1 and uterine artery doppler to predict preeclampsia. Sci Rep 2022;12:6886.
10. Manasova GS, Kuzmin NV, Drozd AV, Arbuzova EA, Badiuk NS. On the relationship of vitamin D status and indicators of the uterine arteries pulsation index in pregnant women at risk of preeclampsia developing: cross-sectional study. Fharmacol Online 2021;3:1206–17.
11. Xu L, Lee M, Jeyabalan A, Roberts JM. The relationship of hypovitaminosis D and IL-6 in preeclampsia. Am J Obstet Gynecol 2013;210:1–7.
12. Gathiram P, Moodley J. The role of the renin-angiotensin-aldosterone system in preeclampsia: a review. Curr Hypertens Rep 2020;22:1–9.
13. Rezavand N, Tabarok S, Rahimi Z, Vaisi-Raygani A, Mohammadi E, Rahimi Z. The effect of VDR gene polymorphisms and vitamin D level on blood pressure, risk of preeclampsia, gestational age, and body mass index. J Cell Biochem 2018;120:6441–8.
14. Wetta LA, Biggio JR, Cliver S, Abramovici A, Barnes S, Tita AT. Is midtrimester vitamin D status associated with spontaneous preterm birth and preeclampsia? Am J Perinatol 2014;31:541–6.
15. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143:29–36.

16. Hollis BW. Vitamin D status during pregnancy: the importance of getting it right. EBioMedicine 2018;39:23–4.
17. Barrera D, Avila E, Hernández G, Méndez I, González L, Halhali A, et al. Calcitriol affects hCG gene transcription in cultured human syncytiotrophoblasts. Reprod Biol Endocrinol 2008;6:1–8.
18. Zabul P, Wozniak M, Slominski AT, Preis K, Gorska M, Korozan M, et al. A proposed molecular mechanism of high-dose vitamin D3 supplementation in prevention and treatment of preeclampsia. Int J Mol Sci 2015;16:13043–64.
19. Knight M, Duley L, Henderson-Smart DJ, King JF. Antiplatelet agents for preventing and treating pre-eclampsia. Cochrane Database Syst Rev 2000;CD000492. https://doi.org/10.1002/14651858.CD000492.
20. Martinez-Portilla RJ, Caradeux J, Meler E, Lip-Sosa DL, Sotiriadis A, Figueras F. Third-trimester uterine artery doppler for prediction of adverse outcome in late small-for-gestational-age fetuses: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2020;55:575–85.
21. van Zijl MD, Koullali B, Mol B, Snijders RJ, Kazemier BM, Pajkrt E. The predictive capacity of uterine artery doppler for preterm birth – a cohort study. Acta Obstet Gynecol Scand 2020;99:494–502.
22. Camen IV, Manolea MM, Vrabie SC, Sandulescu M-S, Serbanescu M-S, Boldeanu MV, et al. The ability of doppler uterine artery ultrasound to predict premature birth. Curr Heal Sci J 2022;48:277.
23. Familiari A, Bhide A, Morlando M, Scala C, Khalil A, Thilaganathan B. Mid-pregnancy fetal biometry, uterine artery Doppler indices and maternal demographic characteristics: role in prediction of small-for-gestational-age birth. Acta Obstet Gynecol Scand 2016;95: 238–44.
24. Cnossen JS, Morris RK, ter Riet G, Mol BWJ, Joris AM, Post VD, et al. Use of uterine artery doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. CMAJ 2008;178:701–11.
25. Parra-Cordero M, Sepulveda-Martinez A, Rencoret G, Valdes E, Pedraza D, Munoz H. Is there a role for cervical assessment and uterine artery Doppler in the first trimester of pregnancy as a screening test for spontaneous preterm delivery? Ultrasound Obstet Gynecol 2014;43:291–6.
26. Fonseca E, Yu CK, Singh M, Papageorghiou AT, Nicolaides KH. Relationship between second-trimester uterine artery doppler and spontaneous early preterm delivery. Ultrasound Obstet Gynecol 2006; 27:301–5.
27. Broere-Brown ZA, Schalekamp-Timmermans S, Hofman A, Jaddoe V, Steegers E. Fetal sex dependency of maternal vascular adaptation to pregnancy: a prospective population-based cohort study. BJOG 2016; 123:1087–95.
28. Kalok A. Maternal serum vitamin D and spontaneous preterm birth. Clin Exp Obstet Gynecol 2020;47:16–20.
29. Bodnar LM, Platt RW, Simhan HN. Early-pregnancy vitamin D deficiency and risk of preterm birth subtypes. Obstet Gynecol 2015;125:439–47.
30. Bodnar LM, Klebanoff MA, Gernand AD, Platt RW, Parks WT, Catov JM, et al. Maternal vitamin D status and spontaneous preterm birth by placental histology in the US Collaborative Perinatal Project. Am J Epidemiol 2014;179:168–76.
31. Baczyńska-Strzecha M, Kalinka J. Assessment of correlation between vitamin D level and prevalence of preterm births in the population of pregnant women in Poland. Int J Occup Med Environ Health 2017;30: 933–41.