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Fetal Growth Restriction and Maternal Serum Phthalate Levels in Pregnancy: A Case-Control Study

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ABSTRACT

OBJECTIVE: The study aimed to investigate the association between maternal serum di-2-ethylhexyl phthalate (DEHP) levels-DEHP is a chemical widely used in plastics, as well as in the food and cosmetics industries-and fetal growth restriction (FGR, a condition where the fetus fails to achieve expected growth).

STUDY DESIGN: This study included 84 women between April and July 2019: 40 with fetal growth restriction and 44 gestational age-matched controls. Maternal plasma levels of DEHP were measured with sensitive, specific immunoassays and with Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS).

RESULTS: Patient ages ranged from 18 to 40 years (mean 27.16±5.70). Age, BMI, and smoking distributions did not differ significantly between groups (p>0.05). Serum DEHP levels were significantly higher in the FGR (+) group than in the FGR (−) group (p=0.001; p<0.01). Based on this, we calculated a serum DEHP cut-off using ROC analysis and diagnostic screening. The area under the ROC curve was 0.693 [95% CI: 0.580-0.807; p=0.002; p<0.01]. At a cut-off >107.93, sensitivity was 70.0%, specificity 68.2%, PPV 66.7%, NPV 71.4%, accuracy 69.1%, and Youden index 0.382.

CONCLUSION: This study demonstrates a significant association between higher maternal phthalate levels and fetal growth restriction. These findings contribute to emerging evidence linking phthalate exposure to impaired fetal development; however, maternal serum phthalate levels are not currently suitable for clinical prediction or early detection of FGR. To address these limitations, further studies with larger cohorts and longitudinal designs are needed to confirm these associations and explore underlying mechanisms.

Keywords: Fetal growth restriction; Fetus; Maternal; Phthalate; Pregnancy; Serum

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Introduction

Fetal growth restriction (FGR) is a condition in which the fetus fails to achieve its expected growth potential, usually defined as a fetal weight below the 10th percentile. FGR carries an increased risk of perinatal (around birth) complications. This condition is usually caused by uteroplacental insufficiency (reduced blood flow between the uterus and the placenta), genetic disorders, or maternal health problems. Maternal, fetal, and placental factors are implicated in its cause, and it is generally believed to develop following suboptimal utero-placental perfusion (impaired blood supply). Maternal causes include advanced age, chronic disease, and environmental factors (1). FGR, which affects 5 to 10 per 100 pregnancies, increases perinatal mortality and morbidity and contributes to adverse outcomes such as low APGAR (a newborn health score) scores and an increased need for neonatal (newborn) care. Long-term consequences include neurodevelopmental disorders (2).

Di-ethyl-Hexyl-Phthalate (DEHP) is a chemical found in

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plastics and some personal care products. These compounds disrupt hormone regulation when they enter the body (3). Exposure to phthalates during pregnancy can impact fetal health. Phthalates have been linked to embryotoxicity, teratogenicity, and placental factor disruption, potentially leading to fetal growth restriction (4). Studies suggest phthalates may affect fetal development during pregnancy and increase the risk of FGR. Animal studies suggest an association between phthalate exposure and FGR. However, research on maternal serum levels in diagnosed cases is limited (5). Among phthalates, DEHP is one of the most widely used high-molecular-weight compounds. It is found in food packaging, medical devices, household plastics, cosmetics, and personal care products. DEHP is a major source of human phthalate exposure in many populations. Both DEHP and its metabolites cross the placenta, raising concerns about fetal development. We specifically focused on DEHP, not a mixture of phthalates, to evaluate the most clinically relevant exposure in pregnancy.

In this study, we aimed to compare maternal serum phthalate levels in healthy pregnant women and those with FGR (fetal growth restriction). In this way, we aimed to evaluate whether maternal phthalate burden is associated with the development of FGR. Although prior experimental studies have demonstrated a potential association between phthalate exposure and impaired fetal development, evidence based on human maternal serum levels in pregnancies complicated by FGR remains extremely limited. To our knowledge, this study is among the very few to investigate circulating maternal DEHP (di(2-ethylhexyl) phthalate) levels in confirmed FGR cases using a validated UPLC-MS (ultra performance liquid chromatography-mass spectrometry) method, aiming to contribute clinical evidence to this emerging field.

Material and method

Approval for this study was granted by the Clinical Research Ethics Committee of the Tertiary Care Center on April 16, 2019 (16/04/2019#74/2019). After being invited to the study, all participants were informed and provided written and verbal consent. The study complied with the principles of the Declaration of Helsinki.

A case-control study was conducted to assess the association between maternal DEHP plasma levels in pregnant women with fetal growth restriction (n=40) and healthy pregnant volunteers (n=44). Case and control groups were chosen from the same clinical population in the same period. Participants were enrolled consecutively based on eligibility. All eligible women attending the antenatal clinic from April to July 2019 were screened. Pregnancies diagnosed with FGR by ultrasound formed the case group. Those with appropriate-for-gestational-age fetuses and no complications in the same period formed the control group. The FGR group included women with estimated fetal weights or abdominal circumference measurements below the 5th percentile for gestational week. The control group comprised women with an estimated fetal weight above the 10th percentile. Maternal age, weight, height, BMI, obstetric history, gestational age, and blood pressure were recorded.

Gestational age was determined from the last menstrual period or from biometric ultrasound in the 1st or 2nd trimester. Fetal measurements (biparietal diameter, femoral length, and abdominal circumference) were assessed using obstetric ultrasound. The estimated fetal weight was calculated with the Hadlock formula. Amniotic fluid index was measured by ultrasound; oligohydramnios was identified when the deepest pocket was less than 2 cm. Fetuses with anomalies and multiple pregnancies were excluded. Ultrasound examinations were performed with a Voluson 730 Pro machine with a 3.5 MHz convex probe. Doppler exams measured the pulsatility index, resistivity index, and systolic/diastolic ratio of the umbilical, middle cerebral, and uterine arteries. All Doppler measurements were performed by a single experienced operator to eliminate inter-observer variability; repeated measurements were averaged to minimize intra-observer error. APGAR scores and birth weights were recorded after delivery. Pregnant women diagnosed with FGR, aged 18-40 years, and at 28-40 weeks of gestation were included in the FGR group. FGR was defined as estimated fetal weight or abdominal circumference below the 5th percentile. Healthy pregnant women aged 18-40 years with no systemic or perinatal conditions, and whose fetal development matched gestational age, were included in the control group. Exclusion criteria included refusal to participate, age outside 18-40 years, systemic maternal diseases (such as diabetes, chronic renal insufficiency, hypertension, liver or hematologic diseases, chronic inflammatory diseases), maternal infections requiring FSE or causing FGR (e.g., CMV, Rubella, Herpes, VZV, Syphilis, Listeria, Toxoplasma), congenital fetal anomalies, cannabis or cocaine addiction, smoking or alcohol use, poor obstetric history, preeclampsia, and multiple pregnancies.

The antecubital venous blood samples from 84 subjects were collected using a BD® brand sterile disposable needle and filled into the yellow BD Vacutainer® SST tube. To minimize the potential influence of diurnal fluctuations in phthalate metabolism, samples were collected between 09:00 and 11:00 in the morning at the maternity clinic and brought to the laboratory within 20 minutes. The blood samples collected for phthalate measurement were centrifuged in Nüve (Nüve, Ankara, Turkey) NF800 centrifuge devices at 4000 rpm for 10 minutes. After separation of the serum portion, the samples were placed in Eppendorf tubes and stored at -80 ˚C until the study day. On the day of the first blood collection from the patients, a 0.9% NaCl solution, stored in a glass bottle, was collected using the same type of needles and tubes used for blood collection from the patients and placed in 5 Eppendorf tubes. These 5 tubes were stored together with the sera until the day

of analysis. The aim was to determine the extent of phthalate exposure from the intended materials using these samples, which were collected with the same materials and stored in the same conditions as the mothers' serum samples. These last 5 samples were referred to as "blanks".

The analysis of di-2-ethyl-hexyl phthalate (DEHP) in maternal serum was performed using the Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) method on the Acquity™ UPLC BEH C18 (2.1×50 mm, 1.7 µm) column of the Waters™ UPLC-MS instrument at Duzen Norwest Laboratory. Di-2-ethylhexyl phthalate (DEHP) standards (catalog number ULM 6241) and di-2-ethylhexyl phthalate-13C2 (DEHP-IS) internal standards (catalog number CLM 6238) were obtained from Cambridge Isotope Laboratories. The injection volume of the samples was set to 5 µL. 0.1% formic acid (in ultrapure water) was used in mobile phase A, and 0.1% formic acid (in methanol) was used in mobile phase B. The flow rate was set to 0.60 mL/min. The auxiliary chemicals used were Merck® brand methanol (catalog number 1.06007), hexane (catalog number 1.04371), and acetone (catalog number 1.00020). For the analysis, DEHP intermediate standard solutions were prepared in acetone at concentrations of 1000 µg/L and 100 µg/L. DEHP-IS was prepared in acetone at a concentration of 1000 µg/L. The standards were prepared in acetone in a concentration range of 10-1000 µg/L. During the analysis procedure, 200 µL of the serum samples and the blank samples were removed and placed in a glass tube. 40 µL of the internal standard was added to all samples. 2 mL of acetone was added to the mixture for protein precipitation. After shaking the mixture for 30 seconds, it was placed in an ultrasonic bath for 2 minutes. The mixture was centrifuged at 2000 rpm for 10 minutes to separate the precipitated proteins. The upper phase (liquid part) was transferred to another glass tube and evaporated to dryness under nitrogen at 40˚C. Then, 0.5 mL of distilled water containing 0.1% formic acid was added, and after shaking for 30 seconds, the mixture was placed in an ultrasonic bath for 2 minutes. Then, 1.5 mL of hexane was added for extraction. After shaking for 1 minute, it was centrifuged at 2000 rpm for 10 minutes. The supernatant (hexane) was transferred to another glass tube. The extraction was repeated with 1.5 mL of hexane. The resulting extract was evaporated to dryness under nitrogen at 40 ˚C. 200 µL of a 70:30 methanol:ultrapure water mixture (with 0.1% formic acid) was taken, shaken for 30 seconds, placed in an ultrasonic bath for 2 minutes, and the sample was dissolved. The sample was placed in a vial and analyzed in the device (6). After receiving the analytical results, 16 randomly selected samples were re-analyzed by adding 200 µg/L standards. The 200 value was subtracted from the results found and compared to the previously found values. During this validation process, the recovery rate for these 16 samples ranged from 98% to 102%. The results of 5 blank samples were 5.32, 5.69, 6.13, 4.60, and 4.82 µg/L.

Statistical Methods

The Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) program was used for the statistical analysis. During data evaluation, descriptive statistics (mean, standard deviation, median, first quartile, third quartile, frequency, percentage, minimum, maximum) were used. The normality of quantitative data was assessed using the Shapiro-Wilk test and graphical methods. Student's t-test was used for comparisons between two groups with normally distributed quantitative variables, and the Mann-Whitney U test was used for comparisons between two groups with non-normally distributed quantitative variables. Pearson chi-square test, Fisher's exact test, and the Fisher-Freeman-Halton test were used to compare qualitative data. Diagnostic screening tests and ROC analysis were used to determine the cut-off value for serum DEHP level. Sensitivity was defined as the test's ability to correctly identify patients, whereas specificity was the test's ability to correctly identify healthy individuals. The positive predictive value represented the probability that a subject truly had the disease when the test result was positive, while the negative predictive value represented the probability that a subject was truly healthy when the test result was negative. Statistical significance was accepted as $p<0.05$.

Results

The study was performed in a total of 84 pregnant women, 47.6% (n=40) with fetal growth restriction and 52.4% (n=44) with normal development. The subjects' ages ranged from 18 to 40 years, with a mean of 27.16±5.70 years. Demographic data of the study groups are given in Table I. There was no statistically significant difference in age, BMI, or smoking distribution between the groups ($p>0.05$). There was a statistically significant difference in systolic blood pressure between the groups, with the FGR (+) group showing higher values than the FGR (-) group ($p=0.003$; $p<0.01$). There was no statistically significant difference between the groups in diastolic blood pressure or body temperature ($p>0.05$). Pulse rate measurements were significantly lower in the FGR (+) group compared to the FGR (-) group ($p=0.032$; $p<0.05$). Weight gain during pregnancy and history of FGR in previous pregnancy did not show a statistically significant difference between the groups ($p>0.05$).

There was no statistically significant difference between the groups in terms of alanine aminotransferase (ALT), platelet count (PLT), blood urea nitrogen (BUN), and creatinine measurements, and proteinuria rates ($p>0.05$). Aspartate aminotransferase (AST) measurements of the FGR (+) positive group were significantly lower than the FGR (-) group ($p=0.043$; $p<0.05$). Oligohydramnios rate ($p=0.001$; $p<0.01$) and estimated fetal weight (EFW) percentiles <3 and 3-5 ($p=0.001$; $p<0.01$) were higher in the FGR (+) group compared to the FGR (-) group (Table II).

Table I: *Evaluation of Descriptive Characteristics by Groups*

		FGR (+) (n=40)	FGR (-) (n=44)	p
Age (years)	Mean±SD	27.36±5.78	26.99±5.69	0.774[a]
BMI (kg/m²)	Mean±SD	26.93±4.59	28.41±4.74	0.150[a]
Smoking; n(%)	No	36 (90.0)	40 (90.9)	0.588[b]
Smoking; n(%)	Yes	4 (10.0)	4 (9.1)	0.588[b]
Systolic blood pressure (mmHg)	Mean±SD	108.13±6.64	103.95±5.79	**0.003****[a]
Diastolic blood pressure (mmHg)	Mean±SD	69.13±5.72	68.80±4.64	0.772[a]
Heart rate (bpm)	Mean±SD	90.08±6.57	92.64±3.47	0.032*[a]
Body temparature (°C)	Mean±SD	36.61±0.23	36.65±0.21	0.439[a]

*a: Student's t-test, b: Pearson Chi-Square Test, *p<0.05, **p<0.01, FGR: Fetal growth restriction, SD: Standard deviation, Bpm: Beats per minute*

Table II*. Evaluation and comparison of the laboratory results and fetal ultrasound measurements between the groups*

		FGR (+) (n=40)	FGR (-) (n=44)	p
Proteinuria n(%)	No	38 (95.0)	43 (97.7)	0.603[d]
Proteinuria n(%)	Yes	2 (5.0)	1 (2.3)	0.603[d]
ALT		14 (12; 19)	15 (14; 18.5)	0.149[c]
AST		11;5 (10;16)	14;5 (11; 19)	**0.043***[c]
PLT		243325.00±84650.23	264272.73±88449.79	0.273[a]
BUN		18.40±7.27	18.32±6.35	0.956[a]
Creatinine		0.58±0.11	0.59±0.07	0.703[a]
EFW (gr)		1986.13±467.94	2313.32±813.40	**0.025***[a]
EFW Percentile n(%)	<3	30 (75.0)	0 (0)	**0.001****[e]
EFW Percentile n(%)	3-5	10 (25.0)	0 (0)	**0.001****[e]
EFW Percentile n(%)	10-25	0 (0)	1 (2.3)	**0.001****[e]
EFW Percentile n(%)	25-50	0 (0)	30 (68.2)	**0.001****[e]
EFW Percentile n(%)	50-75	0 (0)	12 (27.3)	**0.001****[e]
EFW Percentile n(%)	75-90	0 (0)	1 (2.3)	**0.001****[e]
Amniotic Fluid n(%)	Anhydramnios	1 (2.5)	0 (0)	**0.001****[e]
Amniotic Fluid n(%)	Oligohydramnios	16 (40.0)	2 (4.5)	**0.001****[e]
Amniotic Fluid n(%)	Normal	23 (57.5)	42 (95.5)	**0.001****[e]

*a: Student t Test, c: Mann Whitney U Test, d: Fisher's Exact Test, e: Fisher freeman Halton test, *p<0.05, **p<0.001, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, PLT: Platelet, BUN: Blood urea nitrogen, EFW: Estimated fetal weight*

Umbilical artery systolic/diastolic (S/D) (p=0.001), pulsatility index (PI) (p=0.001), and the resistance index (RI) (p=0.001) measurements were significantly higher in the FGR (+) group compared to the FGR (-) group (p<0.01). Umbilical Artery end-diastolic flow loss, middle cerebral artery (MCA) PI, and RI measurements were not significantly different between the groups (p>0.05). Uterine artery PI measurements (p=0.001; p<0.01) and uterine artery notch incidence rate (p=0.012; p<0.05) of the FGR (+) group were significantly higher than the FGR (-) group (Table III).

The FGR (+) group had significantly lower birth week (p=0.001; p<0.01), birth weight measurements (p=0.001 and p<0.01), APGAR 1 (p=0.001; p<0.01), and APGAR 5 (p=0.001; p<0.01) scores compared to the FGR (-) group. C/S delivery rate was higher in the FGR (+) group compared to the FGR (-) group (p=0.001; p<0.01) (Table IV).

Table III: *Evaluation of Doppler ultrasound results by group.*

		FGR (+) (n=40)	FGR (-) (n=44)	p
Umb. Artery S/D		2.6 (2.3;3.1)	2.1 (1.9;2.2)	**0.001****[c]
Umb. Artery PI		1 (0.9;1.2)	0.9 (0.9;1)	**0.001****[c]
Umb. Artery RI		0.9 (0.7;1)	0.6 (0.5;0.7)	**0.001****[c]
Absent or reversed end-diastolic velocity; n(%)	No	38 (95.0)	44 (100)	0.224[d]
Absent or reversed end-diastolic velocity; n(%)	Yes	2 (5.0)	0 (0)	0.224[d]
MCA PI		1.2 (1.1;1.3)	1.2 (1.1;1.4)	0.175[c]
MCA RI		0.8 (0.7; 1)	0.8 (0.7;0.9)	0.272[c]
Uterine Artery PI		1(0.8;1.3)	0.8(0.7;0.8)	**0.001****[c]
Uterine Artery Notch n(%)	No	32 (80.0)	43 (97.7)	**0.012***[d]
Uterine Artery Notch n(%)	Yes	8 (20.0)	1 (2.3)	**0.012***[d]

*c: Mann Whitney U test, d: Fisher's exact test, *p<0.05, **p<0.001 S/D: Systolic/diastolic, PI: Pulsatility index, RI: Resistance index, Umb: Umbilical, MCA: Middle cerebral artery*

Table IV: *Evaluation of birth outcomes and Di-ethyl-hexyl Phthalate results between groups*

		FGR (+) (n=40)	FGR (-) (n=44)	p
Week of birth		36.80±1.92	38.77±1.47	**0.001****a
Birthweight (gr)		2079.75±450.85	3281.32±369.05	**0.001****a
Delivery Method n(%)	Vaginal	12 (30.0)	31 (70.5)	**0.001****b
Delivery Method n(%)	Cesarean section	28 (70.0)	13 (29.5)	**0.001****b
APGAR 1	Median(Q1,Q3)	6 (5.6)	7 (7.7)	**0.001****c
APGAR 5	Median(Q1,Q3)	8 (7.8)	9 (9.9)	**0.001****c
Serum DEHP (µg/l)		11.7-312.2 (132.4) 138.17±71.74	11-221,6 (91.6) 91.45±56.64	**0.001****a

*a: Student t test, b: Pearson chi-square test, c: Mann Whitney U test, *p<0.05, **p<0.001, Q1: First quarter, Q3: Third quarter, DEHP: Di-ethyl-Hexyl phthalate*

There was a statistically significant difference in serum DEHP measurements between groups (p=0.001; p<0.01), with the FGR (+) group showing higher levels than the FGR (-) group (Table IV). Based on this significance, we considered calculating the cut-off point for serum DEHP using ROC analysis and diagnostic screening tests. The area under the curve obtained by ROC analysis was 0.693 [AuROC (95% CI): 0.693 (0.580-0.807); (p=0.002; p<0.01)] (Figure 1). A serum DEHP level > 107.93 had a sensitivity of 70.0%, specificity of 68.2%, PPV of 66.7%, NPV of 71.4%, accuracy of 69.1% and the Youden index value of 0.382.

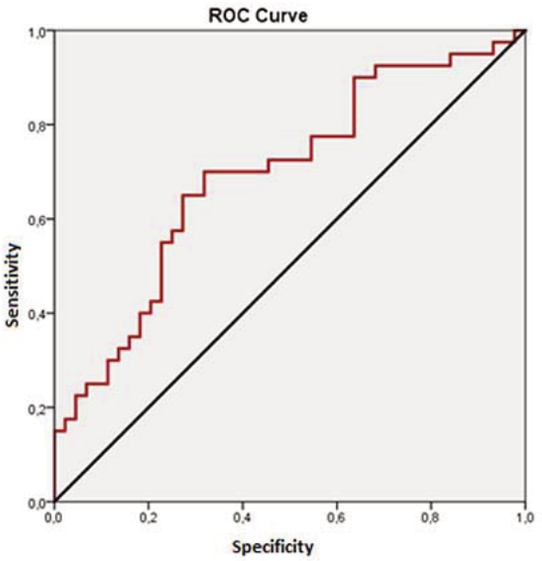

Figure 1: *ROC curve for serum DEHP levels by groups.*

There was no statistically significant correlation between serum DEHP and weight gained during pregnancy, Umbilical Artery S/D, PI, RI, MCA PI, RI, and Uterine Artery PI measurements (p>0.05). A statistically significant negative correlation was found between EFW percentile and serum DEHP (r:–0.216; p=0.048; p<0.05) (Table 5). The distribution of serum DEHP measurements across gestational weeks in the groups is shown in Figure 2.

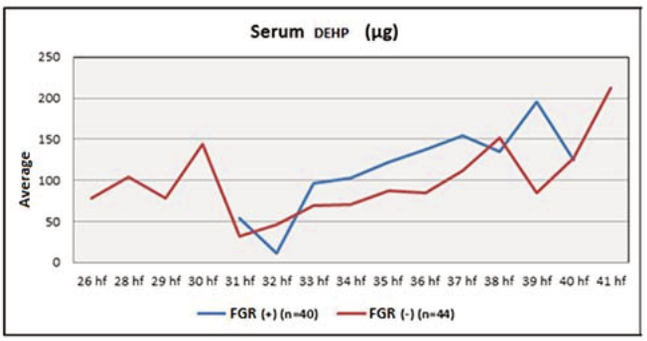

Figure 2: *Distribution of serum DEHP measurements by gestational week in the groups.*

There was a statistically significant positive correlation between gestational week and serum DEHP levels in the FGR (+) patient group (r = 0.438; p = 0.005; p < 0.01). In the control group, there was no statistically significant correlation between gestational week and serum DEHP levels (r = 0.255; p = 0.095; p > 0.05).

Table V: *Association of Serum DEHP Measurements with Doppler USG Results, Weight Measurements During Pregnancy, and EFW*

	Serum DEHP (µg) r	Serum DEHP (µg) p
Umb Artery S/D	0.182	0.097
Umb Artery PI	-0.056	0.612
Umb Artery RI	0.062	0.575
MCA PI	-0.191	0.081
Middle Cerebral Artery RI	0.062	0.577
Uterine Artery PI	0.016	0.883
Weight gained during pregnancy	-0.136	0.217
Estimated Fetal Weight percentile	-0.216	**0.048***

*r: Spearman's correlation coefficient, *p<0.05 S/D: Systolic/diastolic, PI: Pulsatility index, RI: Resistance index, Umb: Umbilical, MCA:Middle cerebral artery.*

Discussion

FGR is a disease that can have a variety of negative effects, including fetal death. It can have long or short-term effects and complications in the fetal, perinatal, neonatal, or infantile period. Due to the serious complications, awareness, recognition, and management of this disease are very important.

Although FGR was removed from the criteria for preeclampsia in the 2013 update of the ACOG Task Force on Hypertensive Disorders of Pregnancy, the overlapping physiopathologic processes leading to uteroplacental insufficiency and the knowledge that hypertensive disorders can often accompany FGR remain current (7). In our study, maternal systolic blood pressure was higher in the FGR group than in the control group, but no significant differences were observed in diastolic blood pressure or proteinuria. When the relationship between maternal heart rate and fetal development is examined, the literature provides limited data. Low maternal heart rate is associated with FGR and low birth weight, as shown in the studies by Everett et al. and Odendaal et al (8,9). In our study, maternal heart rate was significantly lower in the FGR (+) group than in the control group.

In our study, AST levels were significantly lower in the FGR group within the reference intervals considered normal than in the control group. It is known that systemic diseases that also affect the liver can accompany or cause FGR, but we found no studies in the literature reporting that low AST levels are associated with FGR, in contrast to elevated liver function test values indicating liver damage or dysfunction.

In the PORTO study, it was shown that the amniotic index begins to decline progressively when the estimated fetal weight falls below 3p (10). A review by Figueras et al. showed a strong association between FGR and oligohydramnios (11). In our study, the amniotic fluid index was significantly lower in the FGR group.

The causes of FGR include placental, maternal, fetal, and genetic factors. Although the causes and primary physiopathology vary, it is now known that almost all causes lead to inadequate uteroplacental perfusion and fetal malnutrition, which in turn results in FGR. Further data are also lacking for many of the management algorithms, interventions, and treatments considered preventative. When considering systemic maternal conditions such as insulin-dependent and vasculopathy-related diabetes, heart disease, renal disease, cyanotic lung disease, acquired thrombophilia, and hypertension, these conditions are known to cause FGR, but their treatment improves maternal quality of life, reduces maternal mortality and morbidity; however, outcomes in terms of achieving the desired level of fetal development are sometimes not very encouraging. The literature discusses which treatments or interventions can prevent FGR or its recurrence or improve fetal outcomes in mothers or expectant mothers who have been diagnosed with FGR or who belong to the at-risk group. The fact that the etiopathogenesis is still unclear is considered an important factor in this context (12). The results of a database analysis of 49 studies by Abalos et al. have shown that antihypertensive treatment of pregnant women with hypertension does not improve fetal development (13). Prevention of FGR remains a topic that requires further research. In our study, pregnant women with additional systemic diseases were excluded according to the inclusion and exclusion criteria.

The most common routes of exposure to phthalates are through diet, direct contact, and inhalation. In preclinical experimental animal studies conducted separately by Yu et al., Shen et al., Zhao et al., and Ahbab et al., phthalate exposure has been shown to be associated with FGR (4,5,14,15). In our study, DEHP serum levels were significantly higher in the FGR patient group than in the control group. In addition, a negative correlation was observed between DEHP serum levels and the EFW percentile. The suspicion that phthalates, also known as endocrine disruptors, are associated with FGR is supported by our finding that DEHP levels in maternal serum were significantly higher in the FGR group than in the control group. In our study, DEHP, which had higher blood serum concentrations in the FGR patient group compared with the control group, was found to be commonly used in the manufacture of vinyl plastic, which is widely used in the packaging of plastic solid or liquid food, and in the manufacture of vinyl plastic, which is widely used as a material for storage containers, and we think that the consumption of food preserved with plastic in daily life may be associated with an increased risk of FGR although the mechanism is not yet fully understood. One limitation of our study is the lack of adjustment for potential confounders such as diet, lifestyle, socioeconomic background, or environmental exposure, which are known to influence phthalate burden. Although groups were similar in basic maternal characteristics, residual confounding cannot be ruled out. Future studies with multivariable models and comprehensive exposure assessments are warranted. Gestational age may influence serum DEHP concentrations due to changes in metabolism and placental transfer throughout pregnancy. Although the groups were matched for gestational age, we cannot exclude residual confounding related to the timing of sampling. Additionally, blank samples were prepared using saline instead of serum matrix, which may not entirely reflect matrix-dependent extraction efficiency. Future studies should incorporate matrix-matched calibration to better evaluate phthalate recovery. Another limitation of our study was the inability to rule out inflated Type I errors due to multiple statistical tests. These findings should be considered hypothesis-generating and confirmed in larger studies with adjusted analyses. Also, it should be noted that the most mechanistic data are derived from experimental models, and their direct applicability to human pregnancy remains uncertain.

In our study, no statistically significant correlation was found between the Doppler parameters and DEHP concentrations. In animal studies, phthalates have been shown to affect

fetal development through possible mechanisms such as decreased LINE-1 gene expression, which provides global DNA methylation closely related to fetal development, and disruption of thyroid hormone receptor signaling in the placenta, but further human clinical studies are needed to fully elucidate this mechanism (4,14). In addition to the interventions and treatments mentioned in the previous paragraph, we believe that dietary habits that minimize phthalate exposure in pregnant women may be one of the measures that can prevent FGD, although the mechanism remains to be elucidated by human studies, and the association needs to be clearly established.

At a DEHP serum level > 107.93, the sensitivity was 70.0%, the specificity was 68.2%, the PPV was 66.7%, the NPV was 71.4%, the accuracy was 69.1%, and the Youden index was 0.382, which is a cut-off value consistent with the results of our study. Based on this result, we believe it is statistically very unlikely that DEHP levels are a useful marker for predicting FGR.

A review by Berkley et al., published by the Publications Committee of the International Society of Maternal-Fetal Medicine, recommends using umbilical artery Doppler flow evaluation as a primary monitoring tool for suspected FGR (16). Baschat et al. demonstrated that a 30% loss of function of the villous vascular bed leads to increased resistance in the umbilical artery and thus reduced end-diastolic flow (17). Processes leading to impaired uteroplacental perfusion, including abnormal Doppler findings of the umbilical artery, such as decreased diastolic flow, increased systolic-to-diastolic (S/D) flow ratio, and increased pulsatility index (PI), have been closely associated with perinatal mortality and morbidity in the literature (16). In our study, the PI, RI, and S/D ratio of the umbilical artery were found to be statistically significantly higher in pregnant women with FGR compared with the control group; however, end-diastolic flow loss was not statistically significantly different between the groups. PI, RI, and S/D ratio of the umbilical artery were not statistically significantly associated with serum levels of DEHP.

As described in the literature, Doppler parameters of MCA, PI, RI, and S/D values decrease with increasing severity of restriction and hypoxia in fetuses diagnosed with FGR (17-20). The return of MCA Doppler findings to their previous normal state in the ongoing process may indicate that the autoregulatory mechanisms have deteriorated. In our study, no significant difference was observed between the groups for MCA PI and RI. There was no statistically significant correlation between DEHP serum levels and MCA PI and RI values.

A review of the literature shows that uterine artery Doppler is used to determine the risk of developing FGR. Conditions such as an end-systolic notch in the uterine artery and a high PI value are a risk for abnormal placentation and ultimately the development of FGR (21). In our study, uterine artery PI and notch rate were higher in the FGR group than in the control group. This point may be considered a limitation and a potential confounder.

FGR is known to increase perinatal and neonatal mortality and morbidity, as shown in a study of 63,400 pregnant women by Chauhan et al. The same study also showed that obstetric interventions and cesarean section rates were higher in pregnancies diagnosed with FGR than in pregnancies without FGR (22). In our study, APGAR scores at 1 and 5 minutes were significantly lower in the FGR group than in the control group. In our study, the cesarean delivery rate was higher in pregnancies with FGR than in the control group.

FGR is a condition that increases perinatal mortality and morbidity. Although the causes vary, uteroplacental insufficiency and fetal malnutrition are considered a common pathway linking all causes. In our study, a statistically significant association was found between pregnancies with FGR and DEHP serum levels. Compared with the control group, patients with FGR had significantly higher mean DEHP levels. Umbilical artery PI, RI, and S/D values, i.e., umbilical artery Doppler parameters, were found to be higher in pregnancies diagnosed with FGR than in the control group. When the data collected from the patients and the results of the laboratory analyses were analyzed together, it was found that although an association between FGR and abnormal umbilical artery Doppler findings was shown, the DEHP serum level, which was higher on average in patients with FGR compared with the control group, was not directly related to abnormal umbilical artery Doppler findings.

Based on our study, which showed a significant association between phthalates, which increase the hardness and flexibility of plastics and to which pregnant women are exposed due to their dietary, cosmetic, and other daily habits, and the development of FGR, we believe that strategies to reduce phthalate exposure may contribute to improved pregnancy outcomes, but further research is needed to clarify causality.

Declarations

Author's Contribution: All of the authors, UK, MCI, and YEU, have contributed to project development, data collection, data analysis, and writing of the manuscript.

Competing Interests: The authors declare no conflicts of interest.

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Ethics approval and consent to participate: All participants provided written informed consent before enrollment in the study. The study was reviewed and approved by the Ethics Committee of Ankara Dr. Zekai Tahir Burak Education and Research Hospital (Ethics approval reference number: 74/2019, date: 16.04.2019). All procedures were performed in accordance with the Declaration of Helsinki.

Availability of data and materials: The data supporting this study are available through the corresponding author upon reasonable request.

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