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Microplastics in the umbilical vein of fetal growth restricted and healthy fetuses: A preliminary Turkish selected case series

Fetal büyüme kısıtlılığı olan ve sağlıklı fetüslerin umbilikal veninde mikroplastikler: Ön seçilmiş Türk olgu serisi

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Abstract

Objective: Microplastics are ubiquitous environmental pollutants, yet their potential association with fetal growth restriction (FGR) remains unclear. This observational, analytical case-control study aimed to evaluate the presence and size characteristics of microplastics in umbilical venous blood samples from fetuses with FGR, compared with healthy controls.

Materials and Methods: Fourteen pregnant women with singleton pregnancies, aged 20-36 years, who delivered between 36+0 and 39+6 weeks' gestation were included. Pregnancies were classified as FGR (n=8) or healthy controls (n=6). Maternal and neonatal characteristics, including birth weight, Apgar scores, delivery mode, ultrasound and Doppler findings, and neonatal intensive care unit admission, were recorded. Umbilical vein blood samples collected at delivery were analyzed for the presence and particle size of microplastics using micro-Raman spectroscopy.

Results: Microplastics were detected in 83.3% of control cases and 50% of FGR cases, with no statistically significant difference between groups (p=0.198). Mean microplastic particle size was larger in the FGR group than in controls (54.41±20.15 µm vs. 36.28±16.33 µm), although the difference was not statistically significant (p=0.133). Birth weight was significantly lower in the FGR group (p=0.003).

Conclusion: Microplastics were detected in umbilical vein blood samples of both FGR and healthy fetuses. No significant association between the presence of microplastics and FGR was observed; however, further studies with larger sample sizes are required.

Keywords: Microplastics, fetal growth restriction, umbilical cord, IUGR

Öz

Amaç: Mikroplastikler çevrede yaygın olarak bulunan kirleticiler olup, fetal gelişim üzerindeki olası etkileri henüz net değildir. Bu analitik gözlemsel olgu-kontrol çalışmasının amacı, fetal büyüme kısıtlılığı (FBK) olan fetüsler ile sağlıklı kontrollerin umbilikal ven kan örneklerinde mikroplastik varlığını ve partikül boyut özelliklerini karşılaştırmalı olarak değerlendirmektir.

Gereç ve Yöntemler: Otuz altı+0 ile 39+6 gebelik haftaları arasında doğum yapan, 20-36 yaş aralığındaki 14 tekil gebelik çalışmaya dahil edildi. Olgular FBK tanısı alan gebelikler (n=8) ve sağlıklı kontrol grubu (n=6) olarak iki gruba ayrıldı. Maternal ve neonatal demografik özellikler, doğum ağırlığı, Apgar skorları, doğum şekli, ultrason ve Doppler bulguları ile yenidoğan yoğun bakım ihtiyacı kaydedildi. Doğum sırasında umbilikal venden elde edilen kan örneklerinde mikroplastik varlığı ve partikül boyutu mikro-Raman spektroskopisi ile analiz edildi.

PRECIS: Microplastics were detected in the umbilical vein of both fetal growth-restricted and healthy fetuses, with no statistically significant association observed between microplastic presence and fetal growth restriction in this preliminary cohort.

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Bulgular: Kontrol grubundaki olguların %83,3'ünde, FBK grubundaki olguların ise %50'sinde mikroplastik saptandı; gruplar arasında istatistiksel olarak anlamlı fark bulunmadı (p=0,198). Ortalama mikroplastik partikül boyutu FBK grubunda kontrol grubuna kıyasla daha büyük olmakla birlikte (54,41±20,15 µm'ye karşı 36,28±16,33 µm), bu fark istatistiksel olarak anlamlı değildi (p=0,133). Doğum ağırlığı FBK grubunda anlamlı derecede daha düşüktü (p=0,003).

Sonuç: Mikroplastikler hem FBK olan hem de sağlıklı fetüslerin umbilikal ven kan örneklerinde saptanmıştır. Mikroplastik varlığı ile FBK arasında istatistiksel olarak anlamlı bir ilişki gösterilememiştir. Bununla birlikte, bu ilişkinin daha net ortaya konabilmesi için daha geniş örneklemli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Mikroplastikler, fetal büyüme kısıtlılığı, umbilikal kord, FBK

Introduction

Fetal growth restriction (FGR) affects an estimated 5-10% of pregnancies and represents a major cause of adverse perinatal outcomes, ranking among the leading contributors to perinatal mortality[1]. Clinically, FGR is commonly defined by an estimated fetal weight or abdominal circumference below the 10th percentile for gestational age[2]. Its underlying causes are heterogeneous and include maternal medical conditions, multiple gestations, teratogenic exposures, infections, substance use, genetic abnormalities, fetal structural anomalies, and placental dysfunction[3].

Accumulating evidence suggests that environmental exposures play a meaningful role in the development of FGR[4]. Maternal smoking and alcohol intake have been consistently associated with an increased risk of impaired fetal growth[5]. Moreover, exposure to traffic-related air pollutants during the second and third trimesters has been linked to a higher likelihood of FGR[6]. In line with these findings, recent studies conducted in cold-climate settings have demonstrated that even low-level prenatal air pollution exposure may adversely affect birth weight outcomes[7].

Worldwide plastic production currently exceeds 360 million tons annually and continues to grow at an estimated rate of approximately 4% per year[8]. As plastic manufacturing expands, the environmental burden and potential health consequences associated with plastic-derived pollutants have likewise increased[8]. Plastic waste, which is translocated from its production environment to various other environments through multiple mechanisms, degrades over time due to various factors, resulting in the formation of microplastics (MP), defined as particles smaller than 5 mm, and nanoplastics, characterized as particles smaller than 1 micrometer[9]. The pervasive occurrence of MPs in environmental settings and consumables has raised significant concerns about human exposure. Contemporary research has identified the presence of MPs within human placental tissue, breast milk, and meconium[10,11]. Additionally, recent investigations involving animal models and in vitro cell cultures have indicated that exposure to MPs may have deleterious effects on placental tissue and fetal development[12-15]. In 2021, Amereh et al.[16] documented a notable correlation between the presence of MPs in human placental tissue and FGR. Utilizing an ex vivo placental perfusion system on rat placentas within a controlled laboratory environment, it has been established that nanopolystyrene particles can traverse from the maternal uterine circulation into the fetal circulation via the placenta[17,18]. Nevertheless, despite the established presence of MPs within human placental tissue, further elucidation is required regarding the translocation of MPs from the placenta into the umbilical vein[10,11]. In addition, recent human evidence from a systematic review and meta-analysis has reported a high prevalence of MPs in reproductive tissues and suggested a potential association between MP accumulation and adverse pregnancy outcomes, including FGR, although the available data remain limited and heterogeneous[19]. In the present investigation, we examined the presence of MPs in blood specimens collected from the umbilical veins of healthy fetuses and fetuses diagnosed with FGR.

Materials and Methods

Study Population

This prospective observational study was conducted at the obstetrics outpatient clinic of Kütahya Health Sciences University, Evliya Çelebi Training and Research Hospital between May 2021 and May 2022. Fourteen women with singleton pregnancies were enrolled. All participants were between 20 and 36 years of age and had a gestational age between 36+0 and 39+6 weeks at the time of delivery.

Participants were categorized into two groups. The FGR group included pregnancies with an estimated fetal weight below the 10th percentile accompanied by abnormal Doppler findings or with an estimated fetal weight below the 3rd percentile for gestational age. The control group consisted of healthy pregnancies without evidence of FGR[20]. Maternal and neonatal variables, including birth weight, Apgar scores, mode of delivery, ultrasonographic and Doppler findings, and neonatal intensive care unit admission, were prospectively recorded.

Ethical approval was obtained from the Kütahya Health Sciences University Non-Interventional Clinical Research Ethics Committee in Türkiye prior to study initiation (approval number: 2021/12-22, date: 08.07.2021). Written informed consent was obtained from all participants. The study was registered at ClinicalTrials.gov (identifier number: NCT05070715).

Exclusion criteria comprised recent use of medications affecting intestinal absorption, alcohol consumption, invasive dental procedures within the preceding two weeks, multiple

gestations, cardiovascular disease, chronic hypertension, autoimmune disorders, inflammatory bowel disease, gastrointestinal motility disturbances, gestational cholestasis, preeclampsia or eclampsia, sepsis, placental adhesion abnormalities, and known fetal chromosomal or congenital anomalies. Gestational age was determined based on first-trimester ultrasonography and the last menstrual period.

Sample Collection

Umbilical cord blood samples were obtained immediately after delivery, following cord clamping. Ten milliliters of blood were drawn from the umbilical vein using a PTFE syringe and transferred into sterile glass tubes. Samples were stored at -80 °C until further analysis. To minimize the risk of plastic contamination, latex-free neoprene sterile gloves were used during all sampling procedures.

Extraction of Samples

Upon arrival at the laboratory, samples were passed through a 45 µm mesh sieve. The retained material was transferred into sterile glass containers and rinsed with Milli-Q ultrapure water. Organic matter was removed using a highly alkaline digestion solution composed of potassium hydroxide and sodium hypochlorite, based on a modified protocol described previously[21].

The digestion mixture was prepared by combining ultrapure water with a saturated potassium hydroxide solution and sodium hypochlorite (containing active chlorine). Depending on the sample volume, approximately 250-500 mL of the solution was added to each container. Samples were then covered with aluminum foil and incubated in a closed environment at 50-60 °C until complete digestion of the organic material was achieved.

Following digestion, density separation was performed by transferring the solution into a separatory funnel and adding a potassium carbonate solution with a density of 1.6 g/cm^3. After a 24-hours settling period, the supernatant was carefully collected and vacuum-filtered through sterile GF/F filters with a pore size of 0.45 µm. Filter membranes were placed in sterile Petri dishes for subsequent microscopic evaluation.

MP Analysis with µ-Raman Spectroscopy

Filtered samples were examined using a confocal µ-Raman spectroscopy system (Renishaw InVia Qontor, Renishaw Plc., UK) equipped with 532 nm and 785 nm laser sources. Suspected particles were analyzed individually, and the obtained spectra were compared with reference polymer spectra in the instrument library to determine their chemical composition.

Quality Assurance (QA) and Contamination Control

All analytical procedures were conducted under strict contamination-control conditions. Sample preparation and analysis were performed in a laminar-flow cabinet, and all chemicals were prefiltered through GF/F filters. Laboratory equipment was rinsed with acetone prior to use and then stored wrapped in aluminum foil. Procedural blanks were processed alongside samples to assess potential contamination; no MP particles were detected in the procedural blanks.

Statistical Analysis

All statistical analyses were carried out using SPSS software (version 21). Continuous variables were summarized using both the mean (standard deviation) and the median (interquartile range, 25th-75th percentiles). Normality of the distribution was assessed using the Kolmogorov-Smirnov or Shapiro-Wilk test, as appropriate.

Group comparisons for continuous variables were performed using the independent samples t-test or the Mann-Whitney U test, depending on data distribution. Categorical variables were evaluated using the chi-square test. A two-sided p-value of less than 0.05 was considered statistically significant.

QA/Quality Control

This investigation strictly adhered to a protocol that minimized the risk of procedural contamination from the surrounding atmosphere, chemicals, surfaces, and equipment. In this setting, all analyses were conducted in a sealed laminar cabinet, and all chemicals utilized were filtered through a GF/F filter before examination. Furthermore, all equipment involved in the procedures was washed with acetone prior to use and stored wrapped in aluminum foil. Despite all precautions, a procedural blank was used to check for contamination, and all procedures applied to the samples were carried out with the blank. No MP particles were detected in the procedural blank. However, contamination likely resulted from the surgical environment and from the clothing worn by the medical staff who collected the samples during surgery. Plastics are pervasive in daily life and are integral to medical equipment. A substantial portion of medical equipment routinely employed in hospitals is manufactured from plastic or packaged in plastic[22]. Field et al.[22] documented a notable presence of MPs within the surgical environment, with polypropylene emerging as the second most prevalent polymer among these MPs. Therefore, it is essential to consider that the outcomes observed in this study could be attributed to the surgical setting and the sampled individuals.

Results

The mean age of the 14 pregnant women included in the study was 27.645.24 ±years. Eight cases were in the FGR group, and six were in the control group. Age, gravida, parity, number of living children, body mass index, gestation period, Apgar scores, education level, mode of delivery, newborn intensive care unit admission rates, and MPs in the umbilical vein were similar in the FGR and control groups. The FGR group had lower infant birth weights than the control group [2383 (2240-2680) g vs. 2983 (2745-3680) g; p=0.003].

The demographic, delivery, and MP data for the groups are presented in Table 1.

MPs were detected in the umbilical cord in 4/8 cases (50%) in the FGR group and in 5/6 cases (83.3%) in the control group (p=0.198). When the characteristics of MPs were analyzed, polypropylene was the most frequently detected MP, found in 4 cases in the FGR group and in 2 cases in the control group. Regarding color, blue was the most common in the FGR group, occurring in 2 cases, whereas all colors were detected at the same rate in the control group. MP size was 54.41±20.15 µm in the FGR group and 36.28±16.33 µm in the control group (p=0.133).

Two different MP types were detected in 2 samples: one in the FGR group and one in the control group. The characteristics of MPs detected in the umbilical vein are presented in Table 2.

Table 1. Demographic, delivery and microplastics values of groups

		FGR	Control	p
Age (years)		27 (26-34)	27 (21-32)	0.573[a]
Gravity		2 (1-3)	2 (1-2)	0.852[a]
Parity		2 (1-3)	2 (1-2)	0.662[a]
Live		2 (1-3)	2 (1-2)	0.662[a]
BMI (kg/m^2)		26.8 (23.5-29.4)	29 (24.6-30.86)	0.414[a]
GA (days)		265 (258-268)	270 (261-278)	0.228[a]
Birth weight (g)		2383 (2240-2680)	2983 (2745-3680)	**0.003**[a]
1′ Apgar score		9 (8-9)	9 (7-9)	0.950[a]
5′ Apgar score		10 (9-10)	10 (9-10)	0.950[a]
Education level	Less than graduate	5 (62.5%)	5 (83.3%)	0.393[b]
	Graduate	3 (37.5%)	1 (16.7%)	
Delivery mode	Vaginal delivery	3 (%37.5)	2 (33.3%)	0.872[b]
	Cesarean	5 (62.5%)	4 (66.7%)	
NICU admission	Yes	5 (62.5%)	4 (66.7%)	0.280[b]
	No	3 (37.5%)	2 (33.3%)	
Microplastics in umbilical vein	Yes	4 (50%)	5 (83.3%)	0.198[b]
	No	4 (50%)	1 (16.7%)	
Microplastic size (µm)		54.41±20.15	36.28±16.33	0.133[a]

[a]: Mann Whitney U test, [b]: Square test, FGR: Fetal growth restriction, NICU: Newborn ıntensive care unit, BMI: Body mass index, GA: Gestational age

Table 2. Microplastic characterics of patients

Patient number	Patient group	Particle type	Colour	Length (micron)	Polymer type
1	Control	Fragment	Blue	14.8	Polypropylene
2	FGR	Fragment	Brown	74.69	Polypropylene
3	Control	Fragment	Gray	61.59	Cellulose
4	FGR	Fragment	Blue	69.52	Polyethylene
		Fragment	Blue	23.16	Polypropylene
5	Control	Fragment	Yellow	29.59	Polyacrylamide
		Fragment	Black	47.84	Polypropylene
6	Control	Fragment	Yellow	29.13	Polystyrene
7	FGR	Fragment	Transparent	52.38	Polypropylene
8	Control	Fragment	Transparent	34.71	Polyamide
9	FGR	Fragment	Red	52.29	Polypropylene

FGR: Fetal growth restriction

μ-Raman spectroscopy images of MPs detected in FGR and control groups are shown in Figures 1 and 2, respectively.

Discussion

One of the main causes of neonatal mortality and morbidity is fetal growth retardation[23]. The impact of environmental pollutants on the etiology of FGR is becoming increasingly clear. Preterm birth and FGR are two negative pregnancy outcomes that have been linked in recent years to exposure to ambient air pollution during pregnancy[24,25]. MPs are widely utilized in a variety of industries that are closely tied to daily living, such as the synthesis of clothing fibers and regular chemical production[26]. According to recent research, MPs can be found in a variety of items, such as milk and honey[27] and tea bag drinks[28]. Additionally, MPs have been found in bodily fluids such as breast milk, newborn excrement, and meconium[29]. MPs have been found in healthy pregnant placenta tissue examined using Raman and laser direct infrared spectroscopy in recent research[10,11]. Similar to our investigation, Ragusa et al.[10] used Raman spectroscopy for their findings. The placentas from four women contained a total of twelve MP pieces (designated #1-#12). More precisely, three MPs were discovered in the chorioamniotic membranes: four on the maternal side and five on the fetal side. According to reports, home coatings, adhesives, plasters, polymers, and cosmetic and personal care goods are likely the source of MPs, with three of the MPs found being polypropylene[30]. Studies in cell cultures have demonstrated that nanoplastics negatively affect human placental cells. Human placental cells exposed to polystyrene nanoplastics (PS-NPs) had more intracellular reactive oxygen species, which caused deoxyribonucleic acid damage, cell cycle arrest in the G1 or G2 phase, inflammation, and apoptosis[31]. Research on animals produced similar results.

The study of Aghaei et al.[32] on mice showed that maternal MP exposure causes significant changes in placental metabolism. The study of Chen et al.[12] on mice revealed that PS-NPs caused fetal growth retardation and significantly impaired cholesterol metabolism in both the placenta and fetus. Zhang et al.[33] showed in a rat study that maternal exposure to PS-NPs damages the placental barrier and causes neurotoxicity. In the study published by Amereh et al.[16] in 2022, they reported that placental MPs may be associated with FGR. They examined placental tissue from 30 healthy pregnant women and 13 women diagnosed with FGR. Their study found MPs in the placentas of all 13 FGR cases but in only 3 of 30 control cases. When the properties of MPs were analyzed, polyethylene was the most common, accounting for 67.7%, followed by polystyrene at 33.3%. Raman spectroscopy was used in the investigations, as in our study. The study also reported negative correlations between MP load and birth weight, infant height, head circumference, and 1st-minute Apgar score. However, the relationship between MPs in the placenta and FGR could not be demonstrated at

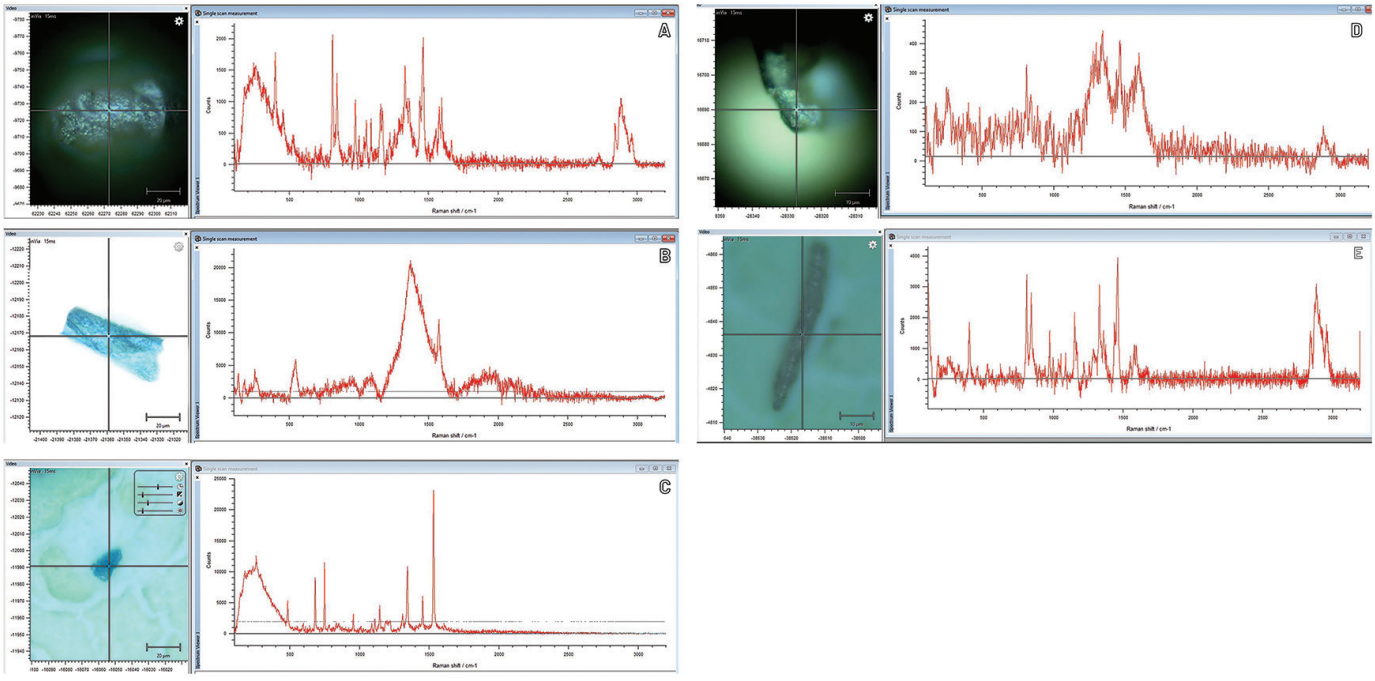

Figure 1. Representative μ-Raman spectroscopy images and spectra of microplastics detected in umbilical vein samples from the FGR group. Panels A-E demonstrate representative polypropylene and polyethylene microplastic fragments identified in the FGR group

FGR: Fetal growth restriction

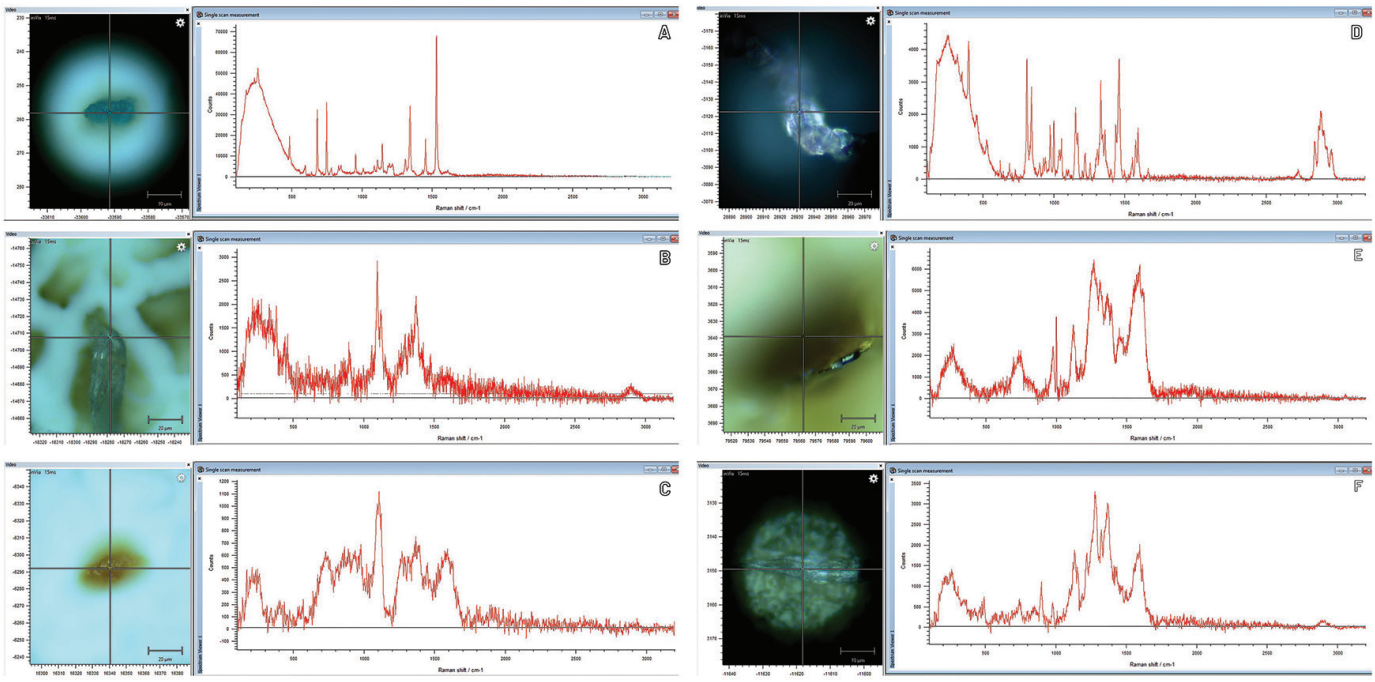

Figure 2. Representative µ-Raman spectroscopy images and spectra of microplastics detected in umbilical vein samples from the control group. Panels A-F demonstrate representative polypropylene, cellulose, polyacrylamide, polystyrene, and polyamide microplastic fragments identified in the control group

the molecular level in human studies. Recent evidence from a systematic review and meta-analysis including human studies has demonstrated a high prevalence of MPs in reproductive tissues and suggested a potential association with adverse pregnancy outcomes, including FGR[19]. However, these findings are based on a limited number of heterogeneous studies, each with relatively small sample sizes.

In contrast to these pooled findings, our study did not demonstrate a statistically significant association between MPs detected in the umbilical vein and FGR. This discrepancy may be explained by differences in biological compartments (placental tissue versus umbilical circulation), methodological variability, and the limited sample size of our study. This highlights the current gap between experimental and clinical evidence and underscores the need for translational studies that bridge experimental findings and real-world human data on the impact of MPs on fetal development.

Furthermore, there is currently limited evidence regarding the transfer of MPs from the placenta to the umbilical cord. In the current study, MP in the umbilical vein was detected in 5/6 patients (83.3%) in the control group and 4/8 patients (50%) in the FGR group (p=0.198). Therefore, our study did not observe a statistically significant association between FGR and MPs. When MP properties were examined, polypropylene was the most common MP, detected in four cases in the FGR group and two cases in the control group. Polypropylene was found to be the most common form of MP in the study by Ragusa et al.[10] that demonstrated the presence of MPs in the human placenta. Materials like food packaging and hospital intravenous cannulas contain polypropylene[34]. Identifying the source of MPs detected in human tissues and fluids is extremely challenging. Given that polypropylene was the most frequently found form of MP in the umbilical cord in our investigation and that hospitalized patients' intravenous cannulas were composed of the same material, intravenous cannulas may be a source of MPs.

Study Limitations

The small number of cases and the fact that patients' plastic consumption was not assessed may be considered limitations of our study. A major strength of our study is that, unlike most previous human studies focusing on placental tissue, we directly evaluated MPs in the umbilical vein, thereby providing novel and clinically relevant insight into potential fetal exposure. Taking all precautions to prevent plastic contamination during sample collection and subsequent processing is also a strength of our study.

Conclusion

Levels of MPs in the umbilical vein in the FGR and control groups were statistically comparable in this preliminary study of this Turkish cohort. Consequently, our results indicate that there was no statistically significant association between FGR and MPs in this small Turkish population. These findings should be interpreted in the context of emerging but still

limited human evidence regarding the clinical impact of MPs on pregnancy outcomes. Further large-scale, well-designed prospective studies are required to confirm these findings and better elucidate the clinical implications of MP exposure during pregnancy.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Kütahya Health Sciences University Non-Interventional Clinical Research Ethics Committee in Türkiye prior to study initiation (approval number: 2021/12-22, date: 08.07.2021).
Informed Consent: Written informed consent was obtained from all participants.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.B., Concept: İ.B., H.Ş., A.T., Design: İ.B., H.Ş., Data Collection or Processing: İ.B., Analysis or Interpretation: İ.B., H.Ş., Literature Search: İ.B., H.Ş., A.T., Writing: İ.B., H.Ş., A.T.
Conflict of Interest: No conflict of interest was declared by the authors.
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