Experimental
&
Clinical Article

Obstetrics; *Maternal Fetal Medicine and Perinatology*

Over-Expression of MicroRNA-210 and MicroRNA-185-5p in Normotensive Late-Fetal Growth Restriction: Preliminary Cohort Study

Esra ALTAN ERBILEN[1], Gulizar Fusun VAROL[2], Necdet SUT[3], Nebiye Pelin TURKER[4], Niyazi Cenk SAYIN[5]

Malatya, Türkiye

ABSTRACT

OBJECTIVE: Late Fetal Growth Restriction (LFGR), a condition associated with perinatal and long-term neurodevelopmental problems, is caused by inadequate placental transfer of oxygen and nutrients to the fetus. This preliminary study evaluates the expression profiles and clinical significance of hypoxia-sensitive microRNA-210 (miR-210) and microRNA-185-5p (miR-185-5p) in placental and maternal circulation. The potential clinical implications of this research could significantly enhance the management of LFGR.

STUDY DESIGN: In this prospective cohort study conducted at the Gynecology and Obstetrics Clinic of Trakya University Faculty of Medicine Hospital, miR-210 and miR-185-5p expression was evaluated in the placenta of healthy (n=30) and LFGR (n=30) cases. In healthy (n=15) and LFGR (n=15) cases, miRNA levels in maternal plasma were studied. We determined these two miRNAs using Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) and statistically analyzed them with clinical data to determine robustness. We provided a comprehensive evaluation.

RESULTS: Both the placenta and maternal plasma samples exhibited significant correlations with miR-210 and miR-185-5p expression ($r=0.615$; $r=0.771$, $p<0.01$). The expression levels in both placenta and plasma were significantly higher in the LFGR group. Placental miR-210 demonstrated an AUC (0.908), sensitivity (93.3%), and specificity (96.7%), while plasma miR-210 showed AUC (0.738), sensitivity (86.7%), and specificity (66.7%). In contrast, placental miRNA 185-5p displayed a diagnostic performance with AUC (0.926), sensitivity (76.1%), and specificity (63.8%); plasma miRNA 185-5p exhibited an AUC (0.778), sensitivity (86.7%), and specificity (86.7%). However, placental miR-210 and miR-185-5p did not fully correlate with birth weight, placental weight, and fetal cerebroplacental Doppler ratio.

CONCLUSION: Placental miRNA-210 showed superior diagnostic performance over placental miRNA 185-5p in LFGR pregnancies. However, it is crucial to emphasize that further studies are needed to validate these findings and correlate them with clinical data, underscoring the ongoing importance of research in this field.

Keywords: Late-fetal growth restriction; miR-210; miR-185-5p

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[1] *Malatya Training and Research Hospital, Gynecology and Obstetrics Perinatology, Malatya, Türkiye*
[2] *Kırklareli Training and Research Hospital, Department of Gynecology and Obstetrics, Perinatology Clinic, Kirklareli, Türkiye*
[3] *Trakya University Faculty of Medicine, Department of Biostatistics, Edirne, Türkiye*
[4] *Trakya University, TUTAGEM, Edirne, Türkiye*
[5] *Trakya University Faculty of Medicine, Department of Gynecology and Obstetrics, Perinatology Edirne, Türkiye*

Address of Correspondence: *Gulizar Fusun Varol*
Kirklareli Training and Research Hospital, Department of Gynecology and Obstetrics, Perinatology Clinic, 22030 Kirklareli, Türkiye
fgvarol@yahoo.com

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OCID IDs of the authors: EAE: 0000-0002-2859-1039
GFV: 0000-0003-1918-4746 NS: 0000-0001-6678-482X
NPT: 0000-0001-6060-3557 NCS: 0000-0001-7286-472X

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Introduction

Late fetal growth restriction (LFGR) is characterized by a deceleration in fetal growth during the later stages of gestation. While the pathophysiology of early- and late-onset FGR differs, early FGR is accompanied by significant Doppler changes, and deterioration in biophysical parameters is common. Still, in LFGR, there are mildly elevated cerebral Doppler findings with minimally increased umbilical artery Doppler parameters. There are no significant cardiovascular changes in LFGR. The frequency of preeclampsia in LFGR is not as high as in early FGR (1).

Fetuses experiencing LFGR are vulnerable to intrauterine hypoxia due to inadequate oxygen and nutrient supply at the fetal-maternal interface, which can lead to perinatal complica-

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tions and long-term neurodevelopmental issues. The existing tests for diagnosing fetal hypoxia often fail to identify many at-risk fetuses precisely. Therefore, new markers, in addition to Doppler ultrasound during antenatal monitoring of these fetuses, are becoming increasingly important (1-5).

MicroRNAs (miR-) are small, non-coding RNAs that play a role in the epigenetic regulation of various post-transcriptional biological processes. Trophoblasts are the primary source of placental miR-s and circulate in maternal blood during pregnancy (2). Placental miRNAs can be used to predict the degree of hypoxia. MicroRNA-210 affects mitochondrial metabolism and plays a role in angiogenesis, DNA damage response, cell proliferation, and apoptosis (6). In a hypoxic placenta, suppressing certain mitochondrial functions and increasing miR-210 expression may contribute to impaired fetal growth (7). FGR-associated upregulation of miR-210 and miR-424 was reported in a systematic review to examine FGR-associated miRNAs. Analysis of target genes of these miRNAs and pathway analysis indicated the involvement of angiogenesis and growth signaling pathways, such as the phosphatidylinositol 3-kinase-protein kinase B (PI3K-Akt) pathway (8). Some studies have reported that miR-25-3p and miR-132-3p, along with miR-185-5p, were significantly overexpressed in the cord blood of LFGR fetuses. miR-185-5p has been studied in LFGR pregnancies, particularly concerning fetal hypoxia (9).

The evaluation of hypoxia-sensitive miRs, such as miR-210 and miR-185-5p, in both placenta and plasma, can potentially serve as a non-invasive method to assess placental function and detect fetal hypoxia in LFGR pregnancies (2-4, 9-11)

To date, these two miRNAs, in conjunction with clinical parameters, have not been thoroughly investigated in LFGR pregnancies. By obtaining sufficient evidence from studies on paired placental and maternal blood samples, FGR-related placental miRNAs may be used for clinical application for early diagnosis of FGR.

Continuing our previous research on LFGR pregnancies, this preliminary study aims to compare the expression of miR-210 and miR-185-5p in maternal plasma and placental tissues with healthy pregnancies. Additionally, we aim to contribute scientifically by evaluating clinical findings such as Doppler fetal cerebroplacental ratio (CPR), birth weight (BW), and placental weight in conjunction with the expression of these miR-s.

Material and method

Definition, Inclusion Criteria, and Sample Collection: In this prospective cohort study if the gestational age (GA) is ≥32 weeks and there are no congenital anomalies, a diagnosis of LFGR was established based on the following criteria: fetal abdominal circumference (AC)/estimated fetal weight (EFW) <3% or AC/EFW <10%, CPR <5%, or the umbilical artery pulsatility index (UA-PI) >95% (12,13).

Fetuses with normal Doppler parameters and AC/EFW >10% were included as controls. Fetal ultrasound examinations evaluated the UA-PI, middle cerebral artery (MCA), and ductus venosus (DV). Fetal CPR represents the ratio of MCA-PI to UA-PI. Birth weight (BW) in grams and placental weight (g) were measured immediately after birth. Gestational age or week (GA or GW) was confirmed with first-trimester crown-rump length. Our study is a preliminary cohort study. Patients with singleton pregnancies diagnosed with LFGR during their follow-up in our center, those who met the LFGR criteria at their first admission, were included in the study group. Multiple pregnancy, fetal congenital, or chromosomal abnormality was excluded. Mothers with cardiovascular disease, pregestational or gestational diabetes, gestational hypertensive disorder, chronic hypertension, preeclampsia, or HELLP developed during follow-up were excluded. The study was conducted using the patient's medical records. The patient's demographic information, medical history, clinical follow-up, and laboratory results were obtained from the hospital's medical records. Trakya University Faculty of Medicine Ethics Committee approved the study on April 4, 2022 (TUTF-GOBAEK-2022/137 07/29). This study was carried out by the ethical principles stated in the Declaration of Helsinki, and informed consent was obtained from all patients.

Placental samples were collected during cesarean section from 30 LFGR cases and 30 healthy pregnancies at Trakya University Medical School Hospital. The samples were excised from the placenta’s middle portion using a sterile lancet, showing no necrosis or inflammation. Placental tissue was washed with sterile isotonic fluid to remove blood and debris, immediately placed in sterile tubes, and stored at –80 °C until processing. Maternal plasma (n=15) from each group was collected and stored at –80 °C. None of the women were in active labor at the time of sample collection.

MicroRNA Isolation and Real-Time Quantitative Polymerase Chain Reaction: The accuracy and efficiency of nucleic acid quantification have grown with better reagents and assay design approaches, making quantitative PCR (qPCR) an even more potent tool for gene expression research. A reliable comparison between two or more samples must be made under consistent sampling conditions for most gene expression experiments. The study preserved placenta and maternal plasma samples from healthy and LFGR pregnancies at -80°C until RNA extraction. The total RNA (including miRNAs) was extracted from 500 µL plasma in accordance with the manufacturer's instructions. The concentration of total RNA (including miRNAs) free of cells was then determined using Thermo Scientific's NanoDrop One® UV spectrophotometer (Wilmington, DE, USA). The High-

Capacity cDNA reverse transcription kit (Applied Biosystems™-4368814) contains all the components necessary for converting up to 2 µg of total RNA to single-stranded cDNA in a single 20 µL reaction. For each sample, 10.0 µL of RNA, 2.0 µL of 10X RT Buffer, 0.8 µL of 25X dNTP Mix (100 mM), 2.0 µL of 10X RT Random Primers, 1.0 µL of MultiScribe™ Reverse Transcriptase, 4.2 µL of Nuclease-free H2O, and a total of 20 µL of mixed thermal cDNA synthesis reaction were used.

The primer sequences of target and reference genes used in qPCR Analysis were as follows;

GAPDH: F: AAGACCTTGGGCTGGGACTG, R: ACCAAATCCGTTGACTCCGA;

miR-210: F:CTGTGCGTGTGACAGCGGCTGA, R:GCGAGCACAGAATTAATACGAC;

miR-185-5p: F:CAGTAATTCTAGGCGATCGCTGGACGCACAGAACAGTC,

Reference gene: GATATTTTATTGCGGCCAGCTGTCGGCATCAGGACAGG.

Primers were analyzed by qRT-PCR using SYBR® Select Master Mix (Life Technologies, USA) on the QuantStudio™ 6 Flex Real-Time PCR System. All PCRs were performed in triplicate. The sample was considered positive if the amplification signal occurred before the 40th threshold cycle. The qPCR mix for each sample consisted of 6 µL of Sybr Green, 0.4 µL of forward and reverse primers, 2.2 µL of dH2O, and 2 µL of cDNA, making a final volume of 11 µL. The PCR profile included an initial denaturation at 95°C for 10 minutes, followed by 52 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Gene expressions were normalized using GAPDH mRNA expression levels. They were determined as relative fold change compared to the control group using the comparative cycle threshold (2-ΔΔCt) method (Applied Biosystems, CA). The experiment was repeated three times to ensure reproducibility.

Statistical analysis

Statistical analysis was conducted using SPSS (Statistical Package for Social Science, Chicago, IL, USA) 22.0 Windows. Descriptive statistics included mean, standard deviation, frequency, and percentage values. A normality test was performed for the distribution of parameters. When working with data that did not meet the normal distribution assumption, the Mann-Whitney U test, Kruskal-Wallis test, and similar non-parametric tests were used. The median values of the groups were compared with the Mann-Whitney U test. The Spearman correlation test was employed to assess correlations between variables. Since the population of the research is not fully known, the power and sample level of the research were calculated with reference to the study of Gemma Correras-Badosa et al.(14). It was predicted that n=30 experimental and 30 control group cases planned to be included in the study could represent the study with 90% power. Additionally, the effect size level of the study was determined as 0.38. A p-value of <0.05 was considered significant.

Results

The expression of miR-210 and 185-5p was evaluated in the placental samples (n=30) and maternal plasma samples (n=15) collected from LFGR and healthy pregnancies at 32-39 GW.

Population characteristics: Age (30 years vs. 27.5 years), BMI (27 kg/m^2 vs. 27 kg/m2), GA (252 days vs. 245 days), nulliparity (70% vs. 53.3%), and fetal sex (male) (40%) were similar between LFGR and controls (Table I). However, BW (2075 g vs. 2475 g), placental weight (345 g vs. 610 g), and APGAR at the 5th min in LFGR were lower in the LFGRs compared to the healthy group.

Correlation between Placental and Maternal Plasma miRs: Both miR-210 and miR-185-5p in the placenta were also expressed in maternal plasma, resulting in a notable positive correlation (r=0.615; r=0.771, p<0.01) between placental and plasma miRs (Figure 1 a, b).

Significance of miR-210 and miR- 185-5p in LFGR: Both placental and plasma miR-210 and miR-185-5p were significantly higher in the LFGR group (Table II and Figure 2). There were significant mean fold changes in both placental and maternal plasma miR-210 and miR-185-5p between the healthy group and LFGR (Table III a, b).

Diagnostic Performances of miR-210 and miR-185-5p: The diagnostic performance of miR-210 and miR-185-5p in both placental and maternal plasma samples is presented in Figures 3 a, b, c, and d. Placental miRNA-210 exhibited an AUC of 0.908, with a sensitivity of 93.3% and specificity of 96.7% (p<0.0001). For plasma miRNA-210, the AUC was 0.738, sensitivity was 86.7%, and specificity 66.7% (p=0.0123). On the other hand, placental miRNA-185-5p demonstrated a diagnostic performance with an AUC of 0.926, a sensitivity of 76.1%, and a specificity of 63.8% (p<0.0001). Plasma miRNA-185-5p was associated with an AUC of 0.778, sensitivity of 86.7%, and specificity of 86.7% (p=0.0067).

Relationship between Placental miR-210 and 185-5p, Birth Weight, and Placental Weight: No significant relationship was observed between miR levels and placental or birth weights in either the control group or the LFGR group (Figure 4). The values of the controls were more tightly clustered and displayed a linear distribution, whereas this distribution was more dispersed in the LFGR group. Notably, miR-210 was lower in fetuses with a birth weight of 1500 grams and below,

in LFGR: Due to their high diagnostic performance, placental miRs were further evaluated with fetal CPR. When CPR >1 was considered indicative of abnormal Doppler, no statistically significant difference was found between placental miRs and CPR (Table V).

where perinatal complications were common, compared to fetuses weighing above 1500 grams. However, such a relationship was not observed for miRNA 185-5p (Table IV).

Placental miR-210 and miR-185-5p vs. Fetal Doppler CPR

Table I: *The characteristics of the population studied*

	FGR	Control	p
Cases (n)	30	30	
Maternal age (year)			
Median (Min-Max)	30 (19-45)	27.5 (19-39)	
Mean±SD	30.8±7.7	28.0±6.0	0.23
Maternal BMI (kg/m^2)			
Median (Min-Max)	27 (21-43)	27 (22-33)	
Mean± SD	27.2±4.1	27.4±2.9	0.80
GA at delivery (days)			
Median(Min-Max)	252 (224-273)	245 (238-265)	
Mean± SD	250.8±11.9	247.4±7.0	0.18
Birth weight (g)			
Median (Min-Max)	2075 (950-2560)	2475 (2100-2750)	0.000
Mean± SD	1933.6±499.1	2453±186.4	<0.001
Placenta weight (g)			
Median (Min-Max)	345 (225-502)	610 (400-800)	
Mean± SD	349.96±64.86	598.16±109.63	0.001
Cesarean Section (%)	100	100	
Nulliparity (%)	70	53.3	
Gender (male) (%)	40	40	
1. minute APGAR score			
≤7 (%)	%23.3	%6.6	
5. minute APGAR score			
≤7 (%)	%6.6	%0	

p<0.05 at significance

Figure 1A

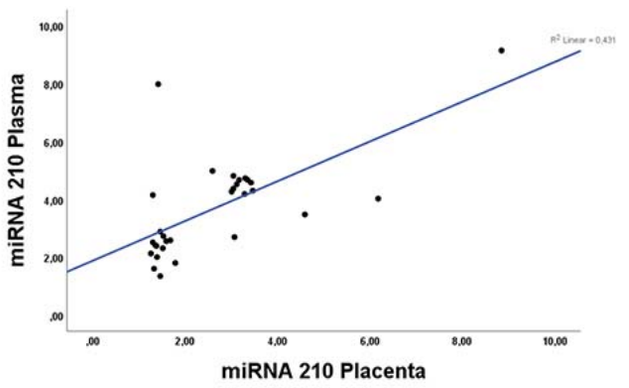

Figure 1A: *Correlation between placental and maternal plasma microRNA 210 (miRNA 210)*

Figure 1B

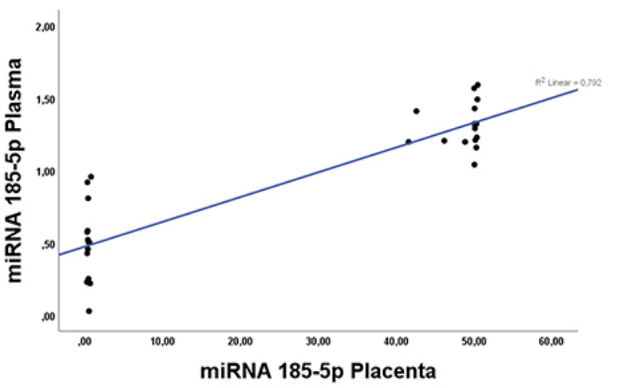

Figure 1B: *Correlation between placental and maternal plasma microRNA 185-5p (miRNA 185-5p)*

Table II: *Expression of microRNA 210 and microRNA 185-5p in the plasma and placenta of LFGR and controls*

	FGR	CONTROL	p
Cases (n)	30	30	
miR-210 Placenta			
Median (Min-Max)	3.21 (2.59-8.84)	1.44 (1.12-1.79)	0.000
Mean± SD	3.96±1.65	1.42±0.14	<0.001
Cases (n)	15	15	
miR-210 Plasma			
Median (Min-Max)	4.52 (2.70-9.15)	2.4 (1.34-7.98)	0.000
Mean± SD	4.62±1.37	2.75±1.58	<0.001
Cases (n)	30	30	
miR-185-5p Placenta			
Median (Min-Max)	50.03 (41.59-54.88)	0.44 (0.19-0.79)	0.000
Mean± SD	49.26±2.65	0.43±0.14	<0.001
Cases (n)	15	15	
miR-185-5p Plasma			
Median (Min-Max)	1.29 (1.04-1.59)	0.5 (0.03-0.96)	0.000
Mean± SD	1.31±0.15	0.47±0.26	<0.001

p<0.05 at significance; miR 210: microRNA 210; miR-185-5p:microRNA 185-5p

Figure 2A

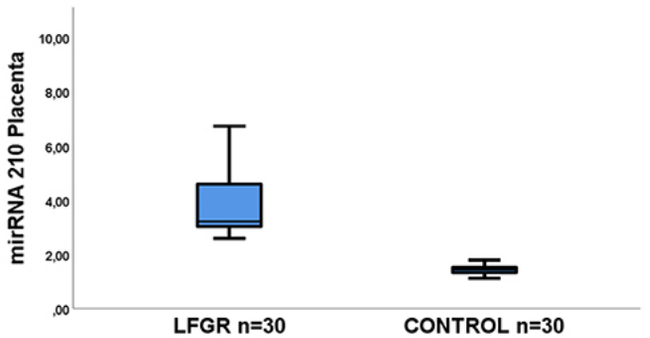

Figure 2B

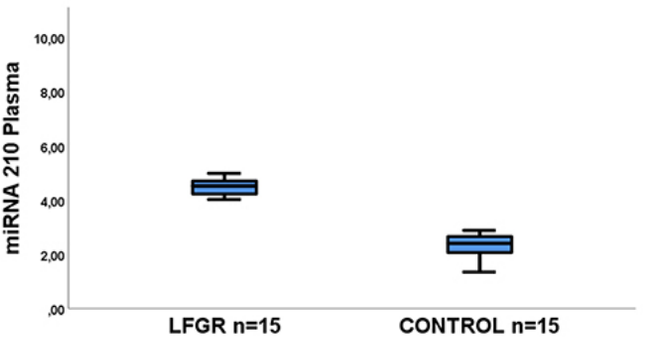

Figure 2C

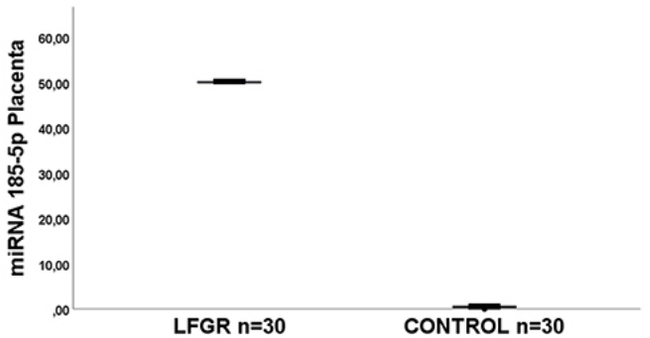

Figure 2D

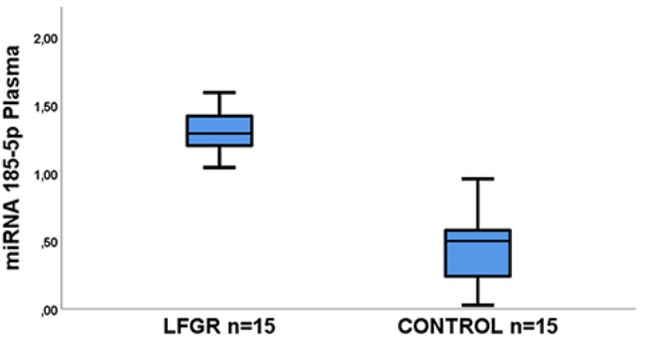

Figure 2AB: *microRNA 210 (miRNA 210) in the placenta and maternal plasma from Late Fetal Growth Restriction (LFGR) and controls.*
CD: *microRNA 185-5p (miRNA 185-5p) in placenta and plasma from Late Fetal Growth Restriction (LFGR) and controls*

Table III: Mean fold Changes of microRNA 210 (a) and microRNA 185-5p (b) in placenta and plasma

a:

miR-210	Mean Fold Change	SD(±)	p
Placenta	3.96	1.65	0.000, <0.001
Plasma	4.62	1.37	0.000, <0.001

b:

miR-185-5p	Mean Fold Change	SD(±)	p
Placenta	49.26	2.65	0.000, <0.001
Plasma	1.31	0.15	0.000, <0.001

p<0.05 at significance; miR 210: microRNA 210; miR-185-5p:microRNA 185-5p

Figure 3A

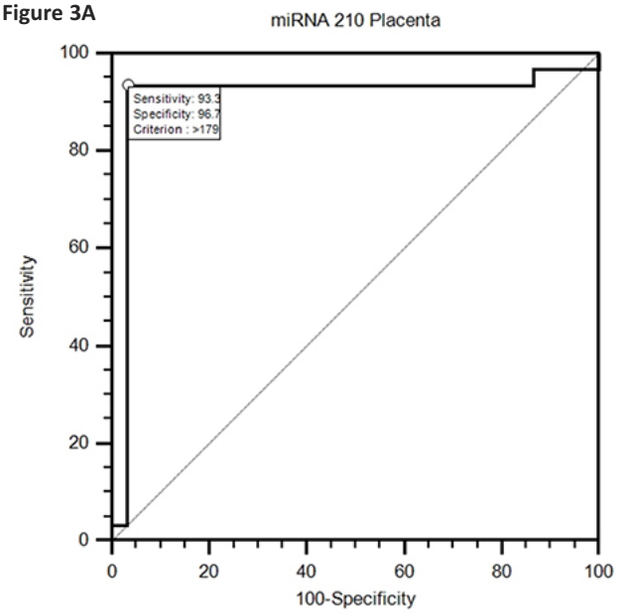

Figure 3B

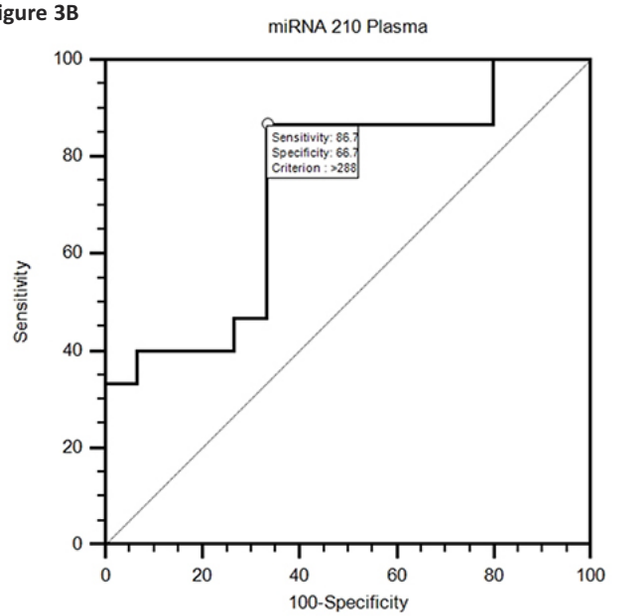

Figure 3C

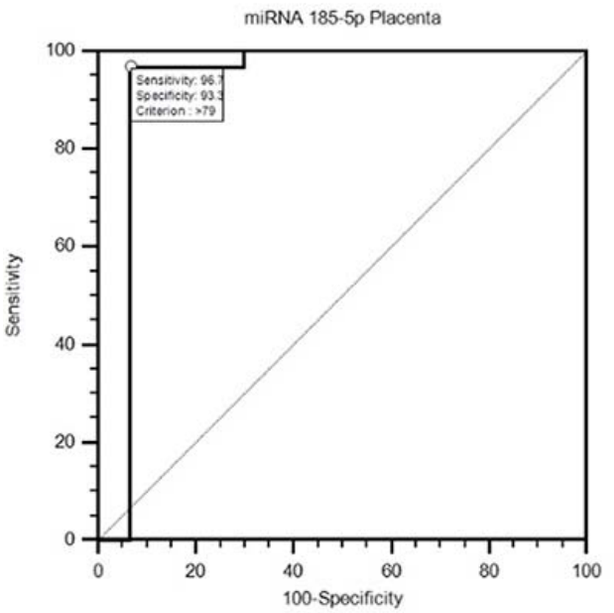

Figure 3D

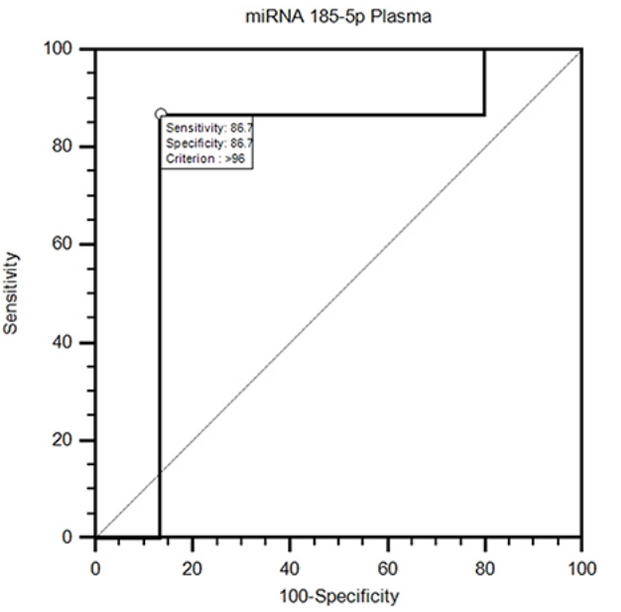

Figure 3:ABCD: *Diagnostic performances of microRNA 210 (miRNA 210) and microRNA 185-5p (miRNA 185-5p) in plasma and placenta*

Figure 4A

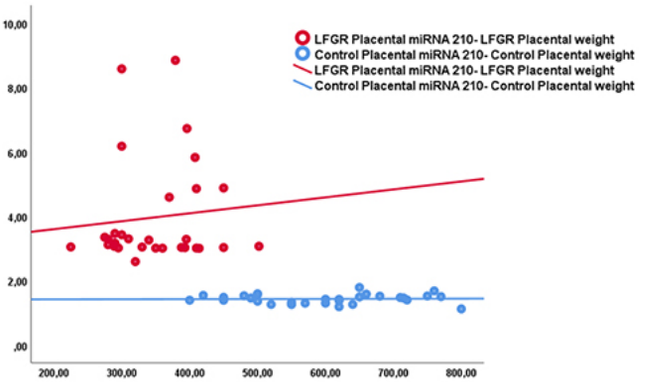

Figure 4B

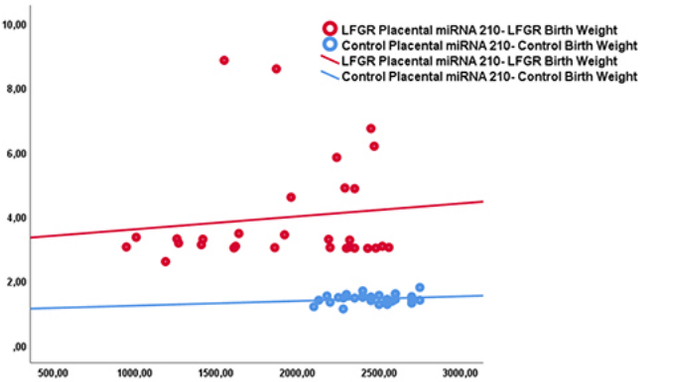

Figure 4C

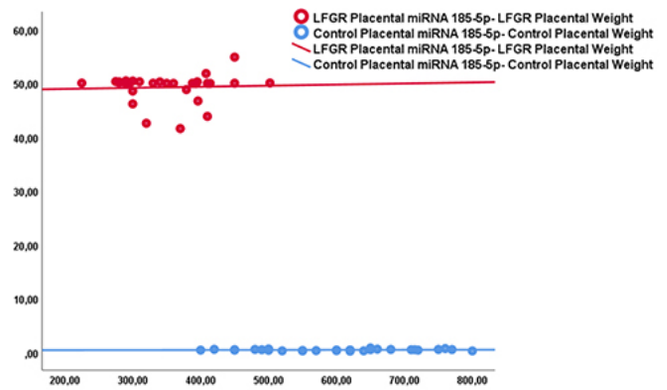

Figure 4D

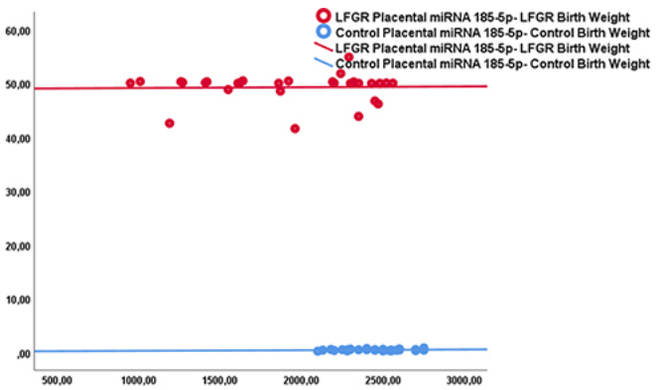

*Figure **4A:** Correlation between placental microRNA 210 (miRNA 210) and placenta weight. **B:** Correlation between placental microRNA 210 (miRNA 210) and birth weight. **C:** Correlation between placental microRNA 185-5p (miRNA 185-5p) and placenta weight. **D:** Correlation between placental microRNA 185-5p (miRNA 185-5p) and birth weight*

***Table IV:** microRNA expression and birth weight (BW) (≤ 1500 g)*

	>1500 g (n=23)	>1500 g (n=23)	p
Birth Weight(g)			
Median (Min-Max)	1260 (950-1419)	2290 (1550-2560)	
Mean± SD	1215.57±167.99	2152.17±317.65	
miR-210 Placenta			
Median (Min-Max)	2.59-3.35 (3.16)	3-8.84 (3.26)	
Mean± SD	3.11±0.25	4.22±1.81	0.009
miR-185-5p Placenta			
Median (Min-Max)	42.59-50.35 (50.16)	41.59-54.88 (50.02)	
Mean± SD	49.11±2.88	49.31±2.64	0.87

p<0.05 at significance; miR: microRNA

***Table V:** microRNA expression and fetal Doppler Cerebroplacental Ratio (CPR) in Late Fetal Growth Restriction (LFGR)*

	CPR <1 (n = 13)	CPR >1 (n = 17)	p
miR-210 Placenta			
Median (Min-Max)	3.28 (2.59-8.84)	3.07 (3.01-8.58)	
Mean± SD	3.92±1.74	3.99±1.64	0.908
miR-185-5p Placenta			
Median (Min-Max)	50.04 (41.59-50.46)	50.03 (43.86-54.88)	
Mean ± SD	48.54±3.08	49.82±2.19	0.195

p>0.05 not significant; miR 210: microRNA 210; miR-185-5p: microRNA 185-5p

Previous Studies on miRs and Fetal Hypoxia in FGR: Placenta-derived miRNAs found in the maternal circulation may serve as biomarkers, offering insight into placental function (2). In the context of a hypoxic placenta, alterations in mitochondrial energy metabolism and miRNA expression can impact fetal growth (7). However, the complex response of both placenta and fetus to intrauterine hypoxia results in varying fetal outcomes, which can potentially be elucidated through miRNA profiling (7,15). For instance, stillbirth oc-

Discussion

Principal findings: The expression of miR-210 and miR-185-5p exhibited a significant correlation in both the placenta and maternal plasma. LFGR cases demonstrated significantly higher expression levels of these miRNAs than controls. Notably, placental miRNA-210 displayed superior diagnostic performance over other miRNAs studied in identifying LFGR. However, these miRNAs did not fully correlate with BW, placental weight, and fetal CPR in LFGR cases.

plasma compared to controls (19). These diverse findings underscore the existence of various LFGR phenotypes and the differential expression patterns of different miRNAs.

In our study, miR-210 and miR-185-5p emerge as reliable biomarkers for placental hypoxia, given their strong diagnostic performance. Considering that fetuses with a birth weight of 1500 g and below are prone to significant complications, we used 1500 g as the cutoff value and examined its relationship with miRNAs. Interestingly, we found that miR-210 expression was significantly lower in fetuses with a BW of less than 1500 g suggesting a potential compensatory mechanism. However, further studies are needed to clarify the role of miR-210 in BW and fetal Doppler blood flow.

In our study, where no significant relationship was observed between miR levels and placenta or birth weight in the control group and LFGR group, it was noteworthy that miR-210 was lower in fetuses with a birth weight of 1500 grams and below, where perinatal complications are common, compared to fetuses over 1500 grams. In a study evaluating the level of miR-191-3p in low birth weight newborns, it was found to be reliably different compared to others, raising the idea that there may be a new player in the epigenetic mechanisms linking this condition and future endocrine-metabolic adverse outcomes (20).

In infants born with LFGR, certain pathways related to cardiac and neuronal cell death are overrepresented. Prioritizing synergistic miRNAs highlights the role of cholesterol efflux and starvation response in the overexpression of miR-185-5p in LFGR phenotyping (3). Our previous study, which analyzed the responses of three molecules (Tyrosine-kinase-2, Angiopoetin-2, and Thrombomodulin, unpublished data), emphasized the heterogeneity in LFGR pathophysiology. This further supports the idea that placental and circulating miRNAs may exhibit differential expression patterns in the context of LFGR with such diverse pathophysiology. Additionally, adaptive angiogenesis in the FGR placenta results from an imbalance in vascular and placental growth factors during placental development (21).

Conclusion

High levels of miRNA-210, similar to miRNA-185-5p in maternal plasma, may predict placental levels. This may help us in the management of LFGR cases as non-invasive tests that can be used to optimize the timing of birth without the development of fetal hypoxia. The fact that high placental miR-185-5p and miR-210 levels in LFGR cases were also found to be correspondingly high in maternal plasma may be helpful in the diagnosis and management of LFGR. Our study makes an additional contribution to the few studies in the literature comparing placental and plasma miRNA levels.

Strengths and Limitations: All cases were meticulously curs in approximately 1 in 200 pregnancies, and prenatal hypoxia predisposes offspring to congenital anomalies and future chronic diseases (16). Therefore, quantifying hypoxia-regulated miRs like miR-210, which exhibits differential expression in the maternal blood and placenta, may help identify pregnancies at risk of fetal hypoxia (2,3,5-7,9-11). It is important to note that miR-210 not only affects mitochondrial metabolism but also plays roles in angiogenesis, DNA damage response, cell proliferation, and apoptosis (6).

However, the differential expression of placental miRs may not always be reflected in maternal circulation (5). Among the miRNAs implicated in FGR pathophysiology, only three miRs, 141, 424, and 7d, have shown increased expression in both placental and maternal circulation. Interestingly, miR-210, frequently associated with the pathogenesis of FGR linked to preeclampsia, has been reported to be upregulated solely in placentas (5). This differs from our results, as our study on LFGR indicates that miR-210 is expressed in both the placenta and maternal plasma, suggesting a different pathophysiological mechanism (9). Some studies have reported that miR-25-3p and miR-132-3p, along with miR-185-5p (which we also investigated), were significantly overexpressed in the cord blood of LFGR fetuses (3). Additionally, miR-185-5p expression in cord blood was significantly increased in 22 LFGR pregnancies compared with 18 healthy pregnancies, consistent with fetal hypoxia indicated by fetal CPR (9). In our study, miR-185-5p was also significantly upregulated in both placenta and maternal plasma. However, a clear correlation with fetal CPR values could not be established due to a limited number of cases showing altered fetal cerebral flow. Furthermore, it is worth noting that fetal Doppler alone may not always suffice to predict perinatal outcomes (17). Some studies even suggest that impaired CPR may increase the risk of neurological problems in early childhood (18). In cases of occult placental hypoxia, fetal cerebral flow changes may occur despite normal flow in the umbilical artery. In these situations, differentially expressed placental miRNAs in the maternal circulation, combined with Doppler ultrasound, could aid in diagnosing fetal hypoxia (2,3,5).

The significance of miR-185-5p and miR-25-3p in cholesterol efflux and their relationship with various LFGR phenotypes have been highlighted (3). Our study, however, underscores that miR-185-5p, like miR-210, is overexpressed in maternal plasma and placenta, displaying high diagnostic performance in LFGR between 32 and 39 weeks of gestation. Conversely, another study indicated that miR-16-5p, miR-103-3p, miR-107-3p, and miR-27b-3p expressed in maternal plasma could help diagnose FGR before 32 weeks of gestation, whereas miRNA103-3p and miRNA107-3p exhibited lower expression in FGR cases at 32 to 37 weeks of gestation compared to healthy controls (4). Some placenta-specific miRNAs related to FGR, such as 518b, 1323, 520h, and 519d, were reported not significantly to differ in circulating maternal

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chosen and prospectively monitored. However, this study employed two miRNAs. Since we could not provide sufficient funds to cover the costs of the kits used, plasma was studied by randomly selecting 15 cases. To establish the significance of BW and CPR in clinical modeling, a larger sample size is imperative. Furthermore, the question of whether cases at 32 weeks of gestation may exhibit early-onset FGR remains open to debate. Conversely, the substantial reduction in placental miR-210 in fetuses weighing less than 1500 g compared to those weighing over 1500g warrants further investigation.

Future Research: Exploring the biological significance of various miRNAs that target common transcripts responsible for key pathways disrupted in the placenta presents an avenue for future research. By investigating other hypoxia-sensitive miRNAs in larger cohort studies and examining the use of miRNAs in combination with other clinical markers, changes in gene expressions affecting placental angiogenesis and oxygen and nutrient transfer from mother to fetus can be better understood. Additionally, there is a pressing need for more comprehensive studies delving into placental angiogenesis and the alteration of gene expressions that impact oxygen and nutrient transfer from the mother to the fetus.

Declarations

Ethics approval and consent to participate: All participants signed informed written consent before being enrolled in the study. The study was reviewed and approved by the Trakya University Faculty of Medicine Ethics Committee. (Ethics approval reference number: TUTF-GOBAEK-2022/137 07/29 date 04.04.2022). All procedures were performed according to the Declaration of Helsinki.

Availability of data and materials: The data supporting this study is available through the corresponding author upon reasonable request. / The datasets and code used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests: The authors have no conflict of interest in any product mentioned in this article.

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