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Obstetrics; *Maternal Fetal Medicine and Perinatology*

Tyrosine Kinase-2, Angiopoietin-2, and Thrombomodulin Axes in the Late-Onset Fetal Growth Restriction: A Prospective Cohort Study

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ABSTRACT

OBJECTIVE: Investigating the interaction among three interconnected proteins, namely receptor Tyrosine Kinase-2 (Tie-2), the vascular remodeling cytokine Angiopoietin-2 (Ang-2), and the coagulation inhibitor Thrombomodulin (TM), may offer fresh insights into the multifactorial origins of late-onset fetal growth restriction (LFGR).

STUDY DESIGN: In this prospective cohort study, we assessed the maternal serum concentrations of Tie-2, Ang-2, and TM in pregnancies that developed LFGR (n=30) and a control group (n=59) within the gestational weeks of 32-39 gestational weeks at Trakya University Hospital (January 2021-December 2021). Concentrations were quantified using ELISA, and data analysis was conducted using the SPSS 22.0 Windows software package.

RESULTS: The 75th percentile concentrations of these proteins were significantly lower in cases of LFGR. Among heavy smokers, the risk of LFGR increased by 2.37-fold. A significant correlation was observed between these proteins in both LFGR and healthy pregnancies. However, the sensitivity and specificity of these proteins within the Tie-2, Ang-2, and TM axes were 51%, 56%, 51%, and 45%, 52%, and 50%, respectively. When we examined cases where all three proteins exhibited a consistent trend, LFGRs accounted for 23.33% with reduced levels and 30% with elevated levels, whereas this pattern was observed in 40.67% with reduced levels and 42.37% with elevated levels in healthy pregnancies.

CONCLUSION: Although our study underscores the significance of the intricate interactions between Tie-2, Ang-2, and TM proteins in LFGR pregnancies, it is evident that a more comprehensive investigation is required to make meaningful contributions to the clinical applicability of this subject.

Keywords: Angiopoietin-2; Late-onset fetal growth restriction; Thrombomodulin; Tyrosine kinase-2

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Introduction

The pathophysiology of fetal growth restriction (FGR) involves a complex interplay of mechanisms. Late-onset fetal growth restriction (LFGR), although considered a milder form of FGR, is of significant concern due to the risk it poses, including stillbirth, resulting from an imbalance in the exchange of oxygen and nutrients between the placenta and the fetus. Despite a lower degree of prematurity, LFGR is associated with various perinatal complications and long-term neurodevelopmental issues linked to hypoxia. Given all these facts, early prediction of LFGR and appropriate management of delivery timing to prevent fetal exposure to hypoxia presents substantial challenges (1-3).

Throughout fetal growth, the placenta undergoes extensive angiogenesis and vasculogenesis with remodeling of the spiral arteries continuing into the second trimester and potentially beyond (4). However, abnormal fetal growth is associated with impaired remodeling of uterine spiral arteries due to decreased vascularization (5). Nevertheless, the precise mechanisms underlying endothelial dysfunction and coagulation abnormalities in the context of abnormal fetal growth remain unclear.

Angiopoietin-2 (Ang-2), a vascular remodeling cytokine, primarily originates from the decidual endothelium and reaches its peak levels at the end of the first trimester (6,7). In vitro experiments have demonstrated that Ang-2, mediated through the receptor, Tyrosine Kinase-2 (Tie-2), inhibits

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Thrombomodulin (TM), suggesting an additional role of Ang-2 in regulating hemostasis (7,8). Ang-2 also induces proinflammatory responses by increasing endothelial permeability and vessel plasticity (9). Thrombomodulin, an endothelial cell surface protein, serves as a coagulation inhibitor (10). Furthermore, it stimulates angiogenesis and protects the endothelium via its G-protein coupled receptor and its fifth epidermal growth factor-like region (11). Both TM and Ang-2 act as markers for endothelial damage and may serve as predictors of adverse clinical outcomes (12-17). Interestingly, elevated plasma levels of Ang-2 have been observed in COVID-19 patients, correlating with disease severity, hypercoagulation, and mortality (14). Moreover, our prior research supports the notion of compensatory elevations of mid-trimester Ang-2 and TM in maternal serum as well as in amniotic fluid, as predictors of later FGR (15).

The impact of the interplay between the Tie-2 receptor-mediated angiogenic protein Ang-2 and a natural anticoagulant Thrombomodulin on the development of LFGR has been raised as a question. Therapeutic approaches based on understanding this relationship could be a subject for further studies. The quest to decipher early indicators of LFGR-related complications remains a complex and challenging endeavor. Given the wealth of studies on these proteins, examining the correlation patterns of maternal serum Tie-2, Ang-2, and TM holds promise for enhancing our understanding of the etiology of LFGR and its potential management strategies.

Material and Method

Subjects and Sample Collection: The pregnancies that developed LFGR (n=30) and a control group (n=59) who were at 32-39 gestational weeks (GW) and applied to Trakya University Faculty of Medicine Hospital Perinatology Clinic (January 2021-December 2021) were included in the study. The sample size for the study was determined using G power analysis, indicating a medium effect size. Informed consent was obtained from the participants. The study was approved by the Institutional Ethics Review Board of Trakya University Hospital (2021/311). Our study was carried out by the Principles of the Declaration of Helsinki.

In this prospective cohort study, we measured the concentrations of maternal serum Tie-2, Ang-2, and TM in both LFGR and healthy pregnancies. Gestational age (GA) (day or week) was calculated from the last menstrual period (LMP) and ultrasound measurements. Multiple pregnancies, early FGRs, a history of congenital fetal malformations, and maternal and fetal genetic anomalies were excluded. In the absence of any congenital abnormality, a diagnosis of LFGR was made in the presence of GA ≥32 GW, Abdominal Circumference/Estimated Fetal Weight (AC/EFW) <3rd centile (percentile), or AC/EFW <10th centile, Cerebroplacental Ratio (CPR) <5th centile, or Umbilical Artery Pulsatility Index (UA-PI) >95th centile, (AC/EFW) <3rd centile, or in the presence of AC/EFW <10th centile (1). Postnatally, FGR was confirmed as a birthweight below the 3rd centile for gestational age and with other diagnostic criteria.

Heavy smoking was defined as smoking more than 10 cigarettes per day.

The control group, which was a group of healthy pregnancies, was created during the sample collection for the LFGR group and delivered by cesarean section in similar weeks for other reasons. The indications for cesarean section in pregnancies with LFGRs and controls were previous cesarean section, fetal distress, and breech presentation.

Maternal serum samples were collected after the diagnosis of LFGR or at admission to the hospital. None of the pregnancies were in active labor at the time of blood collection. The blood samples were collected in dry tubes, followed by centrifugation, and then stored at −80°C until further analysis.

ELISA: The concentrations of Ang-2, Tie-2, and TM were measured using commercially available ELISA kits designed for human Ang-2 (NEPENTHE, NE010122101, Türkiye), human TEK Tyrosine Kinase, Endothelial, Tie-2 (NEPENTHE, NE010730801, Türkiye), and human Thrombomodulin (NEPENTHE, NE010118301, Türkiye). The assay demonstrated good precision, with inter-assay and intra-assay coefficients of variation being less than 10% and 8%, respectively, for the measurements of human Ang-2, Tie-2, and TM values. The detection range for human Ang-2 was 5-1000 ng/L, for Tie-2, it was 0.5-200 ng/ml, and for TM, it was 0.05-20 ng/ml. Each sample was assessed in triplicate for all three proteins to ensure the accuracy and reliability of the results.

Statistical Analysis

SPSS (Statistical Package for Social Science, Chicago, IL, USA) 22.0 Windows package program was used for statistical analysis. Descriptive statistics of the pregnancies were presented as mean, standard deviation, frequency, and percentage values. Comparisons according to study groups were performed using the Mann-Whitney U test and Chi-square tests. Correlations between variables were assessed using the Spearman correlation test.

Receiver operating characteristic (ROC) analysis was carried out to determine optimal cut-off points for the measurements, and ROC curves were generated. The areas under the receiver operating characteristic curves (AUROC) were calculated to compare the effectiveness of the ROC analyses.

Additionally, a logistic regression analysis was conducted to investigate the risk factors that may influence the development of LFGR. A p-value of less than 0.05 was considered statistically significant in all analyses.

Results

Characteristics of the Study Population: A total of 30 cases of LFGR and 59 healthy pregnancies were evaluated in the study. The population characteristics are summarized in Table I.

Maternal age, Body Mass Index (BMI), and GA at delivery were similar between the LFGRs and the controls. However, heavy smoking (>10 cigarettes/day), GA, and Estimated Fetal Weight (EFW) were identified as independent risk factors for LFGR through logistic regression analysis (Table II). Significantly, heavy smokers had a 2.37-fold increased risk of LFGR (95% CI: 1.05-3.79, p=0.03).

The 1st and 5th minute APGAR scores and birth weights

Table I: *The characteristics of the population studied*

	LFGR (n=30)	Control(n=59)	p
Maternal age (year)	31.5 (19-45)	29 (19-39)	
mean ± SD	30.97± 7.67	29.42±5.63	p=0.28
Maternal BMI			
median (min-max)	27 (21-43)	27 (20-33)	
mean± SD	27.69±4.23	28.02±2.95	p=0.67
GA at delivery (days)			
median (min-max)	252(224-273)	245(238-265)	
mean± SD	250.37±11.87	247.34±6.2	p=0.12
BW (gr)			
median (min-max)	2075 (950-2420)	2400 (1900-2800)	p=0.01*
mean± SD	1975.63± 505.65	2430.51±195.79	
Mode of delivery (CS, n)	100	100	
Nulliparity (n %)	70	55.9	p=0.01*
Gender (male) (%)	40	40.7	p=0.93
Smokers (%)	20	13.6	p=0.03*
>10 cigarettes (%)	50	37.5	
APGAR score 1min. <7 (%)	33.33	5.08	p<0.05*
APGAR score 5 min. ≤ 7 (%)	6.66	0/59	p<0.05*
Tie-2 (ng/mL)			
median (min-max)	9.8 (6.6-34.8)	10.1 (1.3-35.8)	
mean ± SD	12.35±7.33	15.77±10.42	p=0.11
25th percentile	8.12	7.80	
75th percentile	11.65	27.40	p=0.04*
Ang-2 (ng/L)			
median (min-max)	3.5 (0.5-34.4)	4.4 (1.1-37.8)	
mean ± SD	8.38±10.05	13.81±14.31	p=0.07
25th percentile	1.77	1.60	
75th percentile	9.15	30.90	p=0.03*
TM (ng/mL)			
median (min-max)	10.5 (2.0-35.3)	11.5 (1.4-37.1)	
mean ± SD	13.27±8.14	15.87±11.61	p=0.28
25th percentile	9.22	7.80	
75th percentile	16.17	26.60	p=0.006*

*BW: Birth weight; GA: Gestational Age; BMI: Body Mass Index; Tie-2: Tyrosine kinase-2; Ang-2: Angiopoetin-2; TM: Thrombomodulin; *p<0.05 at significance*

Table II: *Risk Factors in LFGR and Controls*

Characteristics	p	OR	95% CI	
			Lower	Upper
BMI >30	0.23	2.70	0.53	13.87
Smoking	0.03*	2.37	1.05	3.79
Newborn Gender (F/M)	0.25	2.53	0.52	12.22
Gravida	0.06	4.25	0.93	19.43

BMI: Body Mass Index, F/M: Female/Male, CI: Confidence Interval

(BW) of the LFGR group were significantly lower compared to those in the control group ($p<0.05$). The significant difference in APGAR scores and BW, even within our relatively small sample size, underscores the meticulous selection, and monitoring of our study group.

Evaluation of Maternal Serum Tie-2, Ang-2, and TM Concentrations: The notable finding was the significantly lower 75th percentile profile of these proteins in LFGR cases compared to controls ($p<0.05$) (Table I, Figure 1). Although the mean values of Tie-2, Ang-2, and TM tended to be lower in the LFGR group, the heterogeneity within the studied population prevented these differences from reaching statistical significance between LFGRs and controls.

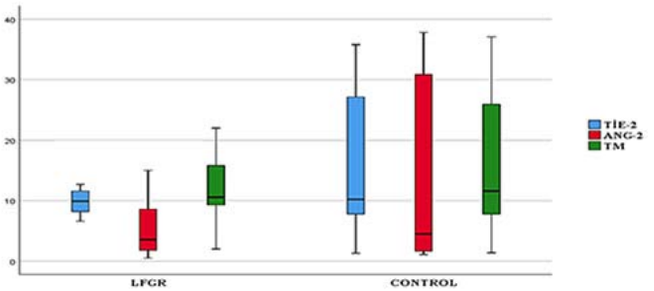


Figure 1: *Comparison of 75th percentiles of Tyrosine kinase (Tie-2), Angiopoietin-2 (Ang-2), and Thrombomodulin (TM) in LFGRs and controls.*

No significant distinct cut-off points were identified for Tie-2, Ang-2, or TM values in both LFGR and control groups ($p>0.05$) (Table III and Figure 2). The sensitivities for Tie-2 (51%), Ang-2 (56%), and TM (51%), as well as the specificities for Tie-2 (45%), Ang-2 (52%), and TM (50%) were determined. No significant distinct cut-off points were identified for Tie-2, Ang-2, or TM values in both LFGR and control groups ($p>0.05$) (Table III and Figure 2).

Smoking and the Tie-2/Ang-2/TM Axes: In the LFGR group, there were six smokers, with three of them being heavy smokers. In the control group, there were eight smokers, with three of them being heavy smokers (Table I). Interestingly, Tie-2/Ang-2/TM axes with values above the 75th percentile were associated with light smoking, and only one case with APGAR <8 and another with CPR<1 was observed. However, a significant pattern of high Ang-2 (>25th quartile) was observed in 50% of heavy smoker LFGR cases and 66.66% of LFGR cases with a Doppler CPR <1.

Table III: *Receiver Operating Characteristics for Tyrosine kinase-2 (Tie-2); Angiopoietin-2 (Ang-2); Thrombomodulin (TM)*

Variable	ROC Area	Cutoff	p	95% CI Lower	95% CI Upper
Tie-2	0.54	-	0.51	0.42	0.66
Ang-2	0.55	-	0.43	0.43	0.67
TM	0.54	-	0.53	0.42	0.66

*CI: Confidence Interval, *p<0.05*

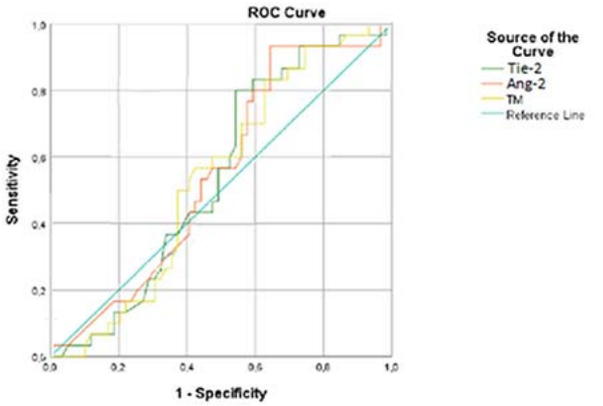


Figure 2: *Receiver operating characteristic curves and area under the Receiver operating caracteristics (AUROC) values for Tyrosine kinase-2 (Tie-2); Angiopoietin-2 (Ang-2); Thrombomodulin (TM).*

Correlation Between Tie-2/Ang-2/TM Proteins in Both LFGR and Control Groups: Significant positive correlations were found between these proteins in both LFGR and control groups (Table IV, Figures 3a, 3b, 3c). The correlation coefficients were as follows: Tie-2 and Ang-2 (rLFGR=0.653; rcontrol=0.867); Tie-2 and TM (rLFGR=0.611; rcontrol=0.819); Ang-2 and TM (rLFGR =0.485; rcontrol=0.745).

Table IV: *The correlation coefficients between Tyrosine kinase-2 (Tie-2), Angiopoietin-2 (Ang-2), and Thrombomodulin (TM) in LFGR and Control pregnancies*

	n	Mean	SD	Ang-2	TM
LFGR Tie-2	30	12.35	7.33	.653***	.611***
Control Tie-2	59	15.77	10.42	.867***	.819***
LFGR Ang-2	30	8.3	10.0		.485**
Control Ang-2	59	13.8	14.3		.745***

****p<.001 **p<.01*

Stratification of Median-Based Probable Correlations of Tie-2/Ang-2/TM in Both LFGR and Control Groups: The stratification of Tie-2/Ang-2/TM correlations in both LFGR and the controls is shown in Figure 4. The median values for Tie-2/Ang-2/TM of the LFGR vs. the controls were as follows: Tie-2: 9.8 vs. 10.1 ng/ml; Ang-2: 3.5 vs. 4.4 ng/L; TM: 10.5 vs. 11.5 ng/ml. Values below and above the median were categorized as "low and high", respectively.

Figure 3A

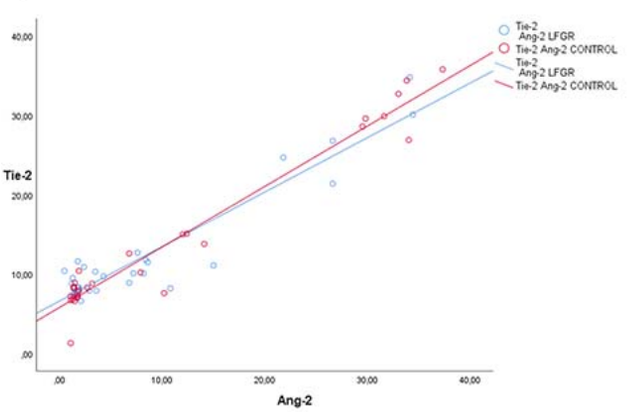


Figure 3B

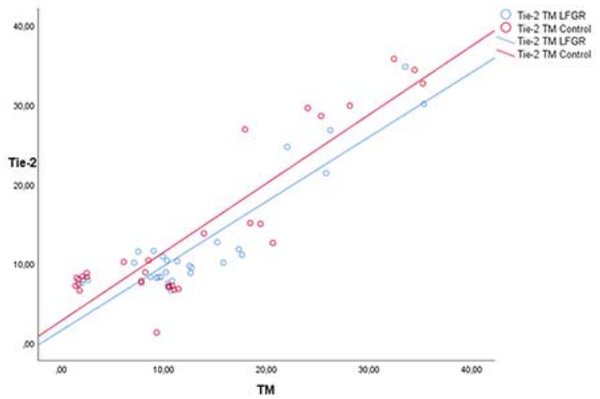


Figure 3B

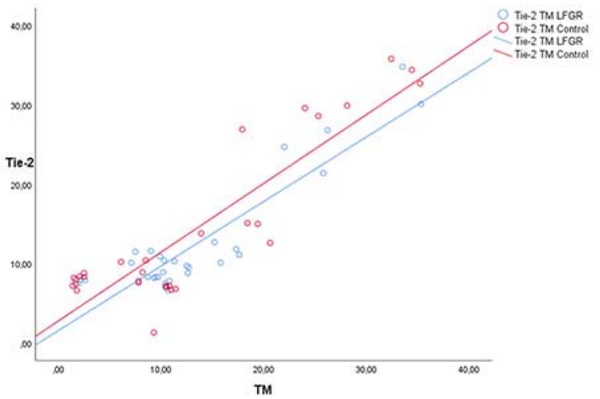
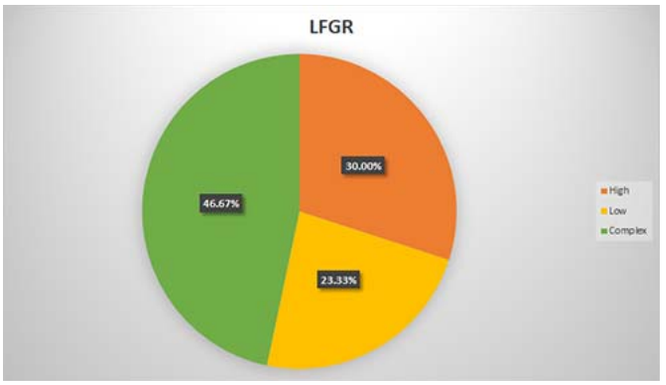


Figure 3a,b,c: *The correlations of Tyrosine Kinase-2 (Tie-2), Angiopoietin-2 (Ang-2), and Thrombomodulin (TM) in the LFGR and controls*



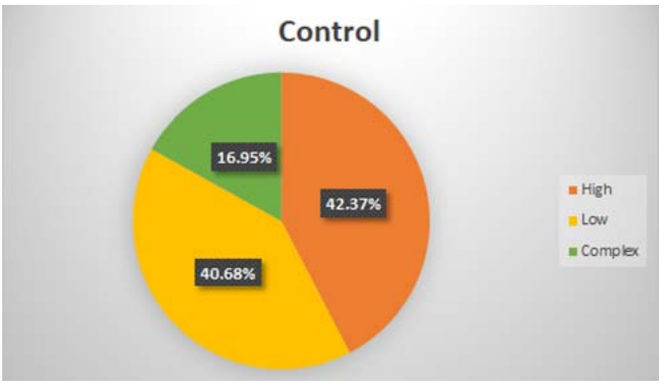


Figure 4: *Stratification of the median-based probable correlations: Tyrosine kinase-2 (Tie-2); Angiopoietin-2 (Ang-2); Thrombomodulin (TM) of the LFGRs (n=30) and the controls (n=59).*

The profile of the Tie-2-dependent Ang-2/TM axes (all three in a uniform directional pattern) was observed in 23.33% of LFGR cases as low and 30% as high. In contrast, this profile was more prominent among the controls: 40.67% were low and 42.37% were high. Additionally, the rest with more complex correlations among Tie-2/Ang-2/TM proteins were notably higher among the LFGR cases (46.66%) compared to the controls (16.94%).

The Low- and High-Profile Tie-2/Ang-2/TM Correlations and Their Perinatal Outcomes: Table V indicates that only 20% (6/30) of the LFGR cases had a CPR <1, while none of the controls did. Among LFGR cases with CPR<1, 44.4% (n=4/9) were in the high-profile group and 28.57% (n=2/7) in the low-profile group.

Regarding birth weights, no significant difference was ob-

Table V: *The featured correlations between Tyrosine kinase-2 (Tie-2), Angiopoietin-2 (Ang-2), Thrombomodulin (TM), and their perinatal outcomes. Gestational Age (GA), Birth Weight (BW), Doppler Cerebroplacental Ratio (CPR) <1(%), High (H): above the median; Low (L): below the median.*

	GA (week)	BW (gr)	CPR <1 (%)
LFGR (n=30)			
Tie-2/ Ang-2/ TM (H) 30% (n=9)	36.3±1.5	2101±423.3	44.4 (4/9)
Tie-2/Ang-2/TM (L) 23.33% (n=7)	36.0±1.4 p>0.05	1958±459.5 p>0.05	28.57 (2/7)
CONTROL (n=59)			
Tie-2/ Ang-2/ TM (H) 42.37% (n=25)	35.2±0.8	2413±160	-
Tie-2/ Ang-2/TM (L) 40.68% (n=24)	35.3±0.9 p>0.05	2475±189.5 p>0.05	-

served between the groups (p>0.05). However, it's worth noting that the birth weights of the two LFGRs with inverted A waves, presenting with high Ang-2s and low TMs, were 1010g and 950g, which may be of clinical significance.

Discussion

Our study indicates a close relationship between the proteins Tie-2, Ang-2, and TM, which appears to impact both the placenta in pregnancies with LFGR and healthy pregnancies. The detection of this relationship in both LFGR and control groups suggests that this phenomenon may not be specific to LFGR alone. However, the strength of this one-way relationship appears more complex in LFGR pregnancies.

Maintaining homeostasis is crucial for uncomplicated pregnancies; any disruption in the protein-to-protein relationship related to the maternal endothelium and placental function can lead to disturbances in fetal growth. A recent study has shown significant differences in placental proteome profiles between normal pregnancies and LFGR cases (18). Nevertheless, the factors contributing to placental dysfunction in LFGR remain complex and warrant further investigation. Therefore, studying Tie-2, Ang-2, and TM, which have demonstrated associations, in LFGR pregnancies may provide valuable insights (7,11,14,17). Stratified protein-to-protein correlations with a deeper mechanistic understanding could guide the development of more effective management strategies for LFGR pregnancies.

Notably, our study highlights the significantly lower profile of the 75th percentile of these proteins in the LFGR cases compared to the controls. Although these proteins exhibit significant correlations, they demonstrate low sensitivity and specificity for distinguishing LFGR cases.

It has been suggested that low maternal plasma soluble Tie-2 (sTie-2) in pregnancies with small for gestational age may create an antiangiogenic environment, affecting fetal growth (19). Consequently, the rising angiomodulatory imbalance observed in some LFGR pregnancies may disrupt communication between mother and fetus, leading to impairment of fetal growth (20). In cases where Tie-2 levels are low, one may anticipate low levels of Ang-2 and TM, as observed in some LFGR pregnancies, potentially influencing fetal growth.

Adverse obstetric outcomes associated with LFGR may be linked to primary maternal cardiovascular maladaptation (21). Abnormal placentation in the first trimester is believed to play a central role in the pathogenesis of early-onset preeclampsia (PE) and FGR. The hypovolemic state leads to placental hypoperfusion, potentially causing hypoxia-related obstetric complications in LFGR. Moreover, the placenta undergoes significant changes in angiomodulatory mediator signaling under hypoxia in the later trimesters (18,22).

The Tie-2/Ang-2 signaling system, especially in pathological vascular remodeling, angiogenesis, and pregnancy, plays a crucial role in regulating endothelial cells. However, data on this subject produced somewhat contradictory results (7,8,16). Thrombomodulin helps maintain endothelial quiescence by regulating procoagulant, proinflammatory, and angiogenic molecules in vascular endothelial cells (23). Its interaction with Tie-2 enhances endothelial function via the Ang/Tie-2 axis through the anticoagulant effect of activated protein C (24). Nevertheless, placental thromboinflammation may reduce placental TM, impair embryonic survival, and affect fetal growth (25). Whether low TM and high Ang-2 observed in 6.6% of LFGR cases in our study contribute to placental malperfusion and low birth weight remains to be clarified. Conversely, high maternal serum TM values have also been associated with very low birth weights in HELLP syndrome (26). These findings suggest that TM values may differ between preeclampsia-related FGRs and normotensive LFGR. Additionally, compensatory elevations of Ang-2 and/or TM in second-trimester serum and amniotic fluid may predict the development of FGR (15,27).

This underscores the complex relationship between an anticoagulant protein like TM and angiogenesis.

In our study, the results of the ROC analysis were not clinically significant, and the median-based stratification of unidirectional correlations appeared significantly different between LFGR and healthy pregnancies. This may suggest a multifactorial and mechanistically heterogeneous nature of the Tie-2, Ang-2, and TM axes, potentially contributing to LFGR pathogenesis (2,21,28).

Smoking may play a role in LFGR pathogenesis, particularly by increasing Ang-2 levels in maternal serum, and it is an acquired cause of placental dysfunction. Smoking in early gestation can lead to a higher umbilical artery resistance index, possibly contributing to abnormal fetal growth later on (29). Smoking has also been reported to stimulate Ang-2 in sputum in lung diseases (30). In our study, at least 50% of heavy-smoking LFGR cases exhibited elevated Ang-2 concentrations along with a Doppler CPR <1. Additionally, the occurrence of LFGR in nulliparous women supports its multifactorial and complex etiology.

We acknowledge certain limitations in our study. Our attempt to explain the role of Tie-2, Ang-2, and TM in LFGR pathogenesis was based on an analysis of protein-protein correlations with a limited number of LFGR pregnancies. This hypothesis deserves further validation in a larger cohort. Future research exploring the role of Tie-2, Ang-2, and TM during pregnancy and their potential to predict adverse pregnancy outcomes is eagerly anticipated.

Conclusion

LFGR is a complex obstetric complication with multiple

contributing factors, including variations in protein levels. A particularly noteworthy observation is the strong positive correlation between proteins Tie-2, Ang-2, and TM in both LFGR and healthy pregnancies. Especially, when we examined the stratification of these protein correlations, we observed a significant difference in the magnitude of these correlations in LFGRs compared to healthy pregnancies. Among LFGR cases, we observed more intricate correlations among Tie-2/Ang-2/TM proteins, accounting for 46.66% of cases, whereas in the control group, this phenomenon was present in only 16.94% of cases. These findings have the potential to provide valuable insights into the pathophysiology of LFGR and may have implications for clinical management.

Declarations

Ethics approval and consent to participate: All participants signed informed written consent before being enrolled in the study. The study was approved by the Institutional Ethics Review Board of Trakya University Hospital. (Ethics approval reference number: TUTF-BAEK 2021/311 14/23 date: 28.06.2021). 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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Author Contribution: Concept: G.F.V., Design: G.F.V., E.A.E., Data Collection or Processing: E.A.E., Analysis, and Interpretation: G.F.V., E.A.E., Literature Search: G.F.V., E.A.E., Writing: G.F.V., E.A.E., Critical Review: G.F.V., E.A.E. All authors read and approved the final manuscript.

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References

1. Lees CC, Stampalija T, Baschat A, da Silva Costa F, Ferrazzi E, Figueras F, et al. ISUOG Practice Guidelines: diagnosis and management of small-for-gestational-age fetus and fetal growth restriction. Ultrasound Obstet Gynecol. 2020;56(2):298-312. Doi: 10.1002/uog.22134. PMID: 32738107.
2. King VJ, Bennet L, Stone PR, Clark A, Gunn AJ, Dhillon SK. Fetal growth restriction and stillbirth: Biomarkers for identifying at risk fetuses. Front Physiol. 2022;13:959750. Doi: 10.3389/fphys.2022.959750. PMID: 36060697, PMCID: PMC9437293.
3. Sun C, Groom KM, Oyston C, Chamley LW, Clark AR, James JL. The placenta in fetal growth restriction: What is going wrong? Placenta. 2020;96:10-18. Doi: 10.1016/j.placenta.2020.05.003. PMID: 32421528.
4. Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. Hypertension. 2013;62(6):1046-54. Doi: 10.1161/HYPERTENSIONAHA.113.01892. PMID: 24060885.
5. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol. 2011; 204(3):193-201. Doi: 10.1016/j.ajog.2010.08.009. PMID: 21094932, PMCID: PMC3369813.
6. Woolnough C, Wang Y, Kan CY, Morris JM, Tasevski V, Ashton AW. Source of angiopoietin-2 in the sera of women during pregnancy. Microvasc Res. 2012 ; 84(3):367-74. Doi: 10.1016/j.mvr.2012.08.003. PMID: 22995870.
7. Daly C, Qian X, Castanaro C, Pasnikowski E, Jiang X, Thomson BR, et al. Angiopoietins bind thrombomodulin and inhibit its function as a thrombin cofactor. Sci Rep. 2018;8(1):505. Doi: 10.1038/s41598-017-18912-8. PMID: 29323190, PMCID: PMC5765006.
8. Thurston G, Daly C. The complex role of angiopoietin-2 in the angiopoietin-tie signaling pathway. Cold Spring Harb Perspect Med. 2012;2(9):a006550. Doi: 10.1101/cshperspect.a006650. PMID: 22951441, PMCID: PMC 3426817.
9. Lemieux C, Maliba R, Favier J, Théorêt JF, Merhi Y, Sirois MG. Angiopoietins can directly activate endothelial cells and neutrophils to promote proinflammatory responses. Blood. 2005;105(4):1523-30. Doi: 10.1182/blood-2004-09-3531. PMID: 15498854.
10. Takano S, Kimura S, Ohdama S, Aoki N. Plasma thrombomodulin in health and diseases. Blood. 1990;76(10): 2024-9. PMID: 2173634.
11. Pan B, Wang X, Nishioka C, Honda G, Yokoyama A, Zeng L, et al. G-protein coupled receptor 15 mediates angiogenesis and cytoprotective function of thrombomodulin. Sci Rep. 2017;7(1):692. Doi: 10.1038/s41598-017-00781-w. PMID: 28386128, PMCID: PMC5429650.
12. Liu KL, Lee KT, Chang CH, Chen YC, Lin SM, Chu PH. Elevated plasma thrombomodulin and angiopoietin-2 predict the development of acute kidney injury in patients with acute myocardial infarction. Crit Care. 2014; 18(3): R100. Doi: 10.1186/cc13876. PMID: 24886544, PMCID: PMC4075148.
13. Chan SH, Chen JH, Li YH, Lin LJ, Tsai LM. Increasing post-event plasma thrombomodulin level associates with

worse outcome in survival of acute coronary syndrome. Int J Cardiol. 2006;111(2):280-5. Doi: 10.1016/j.ijcard.2005.09.015. PMID: 16307806.

14. Hultström M, Fromell K, Larsson A, Persson B, Nilsson B, Quaggin SE, et al. Angiopoietin-2 inhibition of thrombomodulin-mediated anticoagulation-a novel mechanism that may contribute to hypercoagulation in critically ill COVID-19 patients. Biomedicines. 2022;10(6):1333. Doi: 10.3390/biomedicines10061333. PMID: 35740360, PMCID: PMC9220312.

15. Yener C, Varol F, Inan C, Sütcü H, Ateş S, Sayin C. Relationship between second-trimester amniotic fluid and plasma levels of angiopoietin-2 and thrombomodulin with adverse pregnancy outcome. J Obstet Gynaecol. 2022; 42(5):923-8. Doi: 10.1080/01443615.2021. 1960293. PMID: 34693872.

16. Kappou D, Sifakis S, Konstantinidou A, Papantoniou N, Spandidos DA. Role of the angiopoietin/Tie system in pregnancy (Review). Exp Ther Med. 2015;9(4):1091-6. Doi: 10.3892/etm.2015.2280. PMID: 25780392, PMCID: PMC4353758.

17. Tran V, Weckman AM, Crowley VM, Cahill LS, Zhong K, Cabrera A, et al. The Angiopoietin-Tie2 axis contributes to placental vascular disruption and adverse birth outcomes in malaria in pregnancy. EBioMedicine. 2021;73:103683. Doi: 10.1016/j.ebiom.2021.103683. PMID: 34758414, PMCID: PMC8590041.

18. Gęca T, Stupak A, Nawrot R, Goździcka-Józefiak A, Kwaśniewska A, Kwaśniewski W. Placental proteome in late-onset of fetal growth restriction. Mol Med Rep. 2022;26(6):356. doi: 10.3892/mmr.2022.12872. PMID: 36263610, PMCID: PMC9608316.

19. Gotsch F, Romero R, Kusanovic JP, Chaiworapongsa T, Dombrowski M, Erez O, et al. Preeclampsia and small-for-gestational age are associated with decreased concentrations of a factor involved in angiogenesis: soluble Tie-2. J Matern Fetal Neonatal Med. 2008;21(6):389-402. Doi: 10.1080/14767050802046069. PMID: 18570117, PMCID: PMC2780324.

20. Aplin JD, Myers JE, Timms K, Westwood M. Tracking placental development in health and disease. Nat Rev Endocrinol. 2020;16(9):479-94. Doi: 10.1038/s41574-020-0372-6. PMID: 32601352.

21. Mecacci F, Avagliano L, Lisi F, Clemenza S, Serena C, Vannuccini S, et al. Fetal Growth Restriction: Does an Integrated Maternal Hemodynamic-Placental Model Fit Better? Reprod Sci. 2021;28(9):2422-35. Doi: 10. 1007/s43032-020-00393-2. Epub 2020 Nov 19. PMID: 33211274; PMCID: PMC8346440.

22. Colson A, Sonveaux P, Debiève F, Sferruzzi-Perri AN. Adaptations of the human placenta to hypoxia: opportunities for interventions in fetal growth restriction. Hum Reprod Update. 2021 Apr 21;27(3):531-569. doi: 10.1093/humupd/dmaa053. PMID: 33377492.

23. Giri H, Panicker SR, Cai X, Biswas I, Weiler H, Rezaie AR. Thrombomodulin is essential for maintaining quiescence in vascular endothelial cells. Proc Natl Acad Sci USA. 2021 Mar 16;118(11):e2022248118. doi: 10.1073/pnas.2022248118. PMID: 33836597; PMCID: PMC7980409.

24. Minhas N, Xue M, Jackson CJ. Activated protein C binds directly to Tie2: possible beneficial effects on endothelial barrier function. Cell Mol Life Sci. 2017;74(10):1895-1906. doi: 10.1007/s00018-016-2440-6. Epub 2016 Dec 22. PMID: 28005151; PMCID: PMC11107519.

25. Kohli S, Singh KK, Gupta A, Markmeyer P, Lochmann F, Gupta D, Rana R, Elwakiel A, Huebner H, Ruebner M, Isermann B. Placental thromboinflammation impairs embryonic survival by reducing placental thrombomodulin expression. Blood. 2021 Feb 18;137(7):977-982. doi:10.1182/blood.2020005225. PMID: 32870264.

26. Varol FG, Ozgen L, Sayin NC, Demir M. Correlation between maternal plasma thrombomodulin and infant birth weight in hypertensive disorders of pregnancy. Clin Appl Thromb Hemost. 2009 Mar-Apr;15(2):166-70. doi:10.1177/1076029608325543. Epub 2008 Nov 19. PMID: 19022800.

27. Leinonen E, Wathén KA, Alfthan H, Ylikorkala O, Andersson S, Stenman UH, Vuorela P. Maternal serum angiopoietin-1 and -2 and tie-2 in early pregnancy ending in preeclampsia or intrauterine growth retardation. J Clin Endocrinol Metab. 2010 Jan;95(1):126-33. doi: 10.1210/jc.2009-0715. Epub 2009 Oct 29. PMID: 19875484.

28. Gris JC, Bouvier S, Cochery-Nouvellon É, Mercier É, Mousty È, Pérez-Martin A. The role of haemostasis in placenta-mediated complications. Thromb Res. 2019;181 Suppl 1:S10-S14. doi: 10.1016/S0049-3848(19)30359-7. PMID: 31477220.

29. Kho EM, North RA, Chan E, Stone PR, Dekker GA, McCowan LM; SCOPE consortium. Changes in Doppler flow velocity waveforms and fetal size at 20 weeks gestation among cigarette smokers. BJOG. 2009; 116(10): 1300-6. doi: 10.1111/j.1471-0528.2009.02266.x. Epub 2009 Jun 29. PMID: 19566582.

30. Kanazawa H, Asai K, Tochino Y, Kyoh S, Kodama T, Hirata K. Increased levels of angiopoietin-2 in induced sputum from smoking asthmatic patients. Clin Exp Allergy. 2009 Sep;39(9):1330-7. doi: 10.1111/j.1365-2222.2009.03262.x. Epub 2009 Apr 28. PMID: 19438587.