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Assessment of biomarkers indicating activation of the complement system in pregnant women with fetal growth restriction

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ABSTRACT

Aims: To compare serum levels of sC5b-9, C3, C4, C1-INH, and CH50, which are indicators of complement system activation and regulatory processes, in pregnant women with and without fetal growth restriction (FGR).

Methods: This study enrolled eighty-six women with gestational age between 24 and 36 weeks. Maternal blood samples were obtained from 43 patients diagnosed with FGR and 43 from healthy pregnancies. Serum complement levels were measured using commercially available ELISA kits according to the manufacturer's instructions (SunRed, China).

Results: When the levels of complement activation biomarkers of pregnancies with FGR were compared with those of healthy pregnancies, the C1est level was significantly higher, C4 and CH50 levels were slightly lower, and Sc5b9 and C3 levels were similar.

Conclusion: While the exact role of complement activation in FGR remains fully elucidated, the elevated levels of C1-INH in women with FGR suggest a compensatory mechanism to mitigate thrombus formation and inflammation. This adaptive response may be a potential therapeutic target for improving placental function and pregnancy outcomes.

Keywords: Complement system, fetal growth restriction, pregnancy, placenta

INTRODUCTION

The complement system is an essential component of the innate immunity.[1] It acts as a bridge between innate and adaptive immunity and helps to clear immune complexes and apoptotic cells. Activation of the complement system is crucial for the immune system's ability to defend against pathogens; if it becomes excessive or targets the wrong areas, it can cause various disorders.[2,3] Studies have shown that the complement system is not only a defense mechanism against infection but is also involved in fundamental processes of pregnancy, such as placental angiogenesis and trophoblast invasion.[4-6] The complement system activity increases during pregnancy. However, complement inhibition is required at the implantation site during placental development and maintenance to maintain a normal placenta and ensure a healthy pregnancy. Abnormal or excessive activation of the complement system in the placenta is probably related to placental dysfunction, which can lead to pregnancy complications such as pre-eclampsia and fetal growth restriction (FGR).[7]

FGR is a significant obstetric condition affecting approximately 10% of pregnancies, in which the fetus does not achieve its full potential due to maternal, fetal, and placental factors. It is usually defined as a fetal abdominal circumference (AC) and estimated fetal weight (EFW) below the 10th percentile.[8] FGR increases the risk of perinatal morbidity and mortality and is associated with severe long-term health problems such as metabolic disorders and susceptibility to neurodevelopmental delays.[9,10] The placenta is vital during human pregnancy as it facilitates nutrient transfer, promotes immune tolerance, and adapts the mother's body to support the growing fetus.[11] Failure of deep placentation, underdevelopment of placental villi, reduced cytotrophoblast proliferation, and inadequate capillarization are common pathologies associated with FGR.[12,13]

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The complement system plays a crucial role in fetal growth restriction, as evidenced by various studies. Activation of the complement system, particularly through the C5a-C5aR interaction, has been identified as a key mediator of pregnancy loss and growth restriction.[14,15] In particular, soluble membrane attack complex (sC5b-9) complexes may directly affect placental functions by increasing cytokine synthesis and vascular permeability in endothelial cells.[16] Increased or decreased levels of C3 and C4 may indicate significant changes in the immune modulation capacity of trophoblast cells and the structural integrity of the placenta.[17] As C1-esterase inhibitor (C1-INH) is mainly involved in controlling the classical pathway of the complement system, abnormal changes in its levels may trigger abnormal complement activation at the maternal-fetal interface.[18,19] CH50, which reflects the total hemolytic complement activity in serum, is considered a general indicator of complement system activation.[20]

This study aimed to elucidate the role of the complement system in the pathogenesis of FGR and to provide new avenues for early diagnosis and management by comparing serum levels of sC5b-9, C3, C4, C1-INH, and CH50, which are indicators of complement system activation and regulatory processes, in pregnant women with and without FGR.

METHODS

In this prospective cross-sectional study conducted between 2021 and 2022, 86 singleton pregnant women participated, including 43 pregnant women with fetal growth restriction (FGR) and 43 healthy pregnant women, all between 24 and 36 weeks of gestation. All participants provided written informed consent after being informed of the study. This study was approved by the Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee (Date: 26.05.2021, Decision No: 99). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Fetuses with congenital malformations or diagnosed genetic syndromes, multiple pregnancies, and pregnant women with chronic diseases (pre-existing diabetes, autoimmune disorders, current cancer diagnosis, human immunodeficiency virus, and hepatitis) were excluded.

In all women included in the study, gestational age was confirmed in the first trimester using ultrasound biometry based on crown-rump length and menstrual history. All fetuses underwent a complete anatomical scan and Doppler imaging. Estimated fetal weight (EFW) was calculated using a formula ithat included ultrasound measurements of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL). The end-diastolic flow (EDF), resistive index (RI), pulsatility index (PI), systolic/diastolic velocity ratio (S/D), middle cerebral artery (MCA), ductus venous (DV), cerebroplacental ratio (MCA-PI/UA-PI), uterine artery pulsatility index were measured using Doppler velocimetry. All examinations were performed using an Arietta 850 ultrasound system (HITACHI, Tokyo, Japan).

The EFW was compared with the reference growth standard and evaluated as a percentile for gestational age. FGR was defined according to the following criteria: abdominal circumference (AC) and estimated fetal weight (FW) below the 3rd percentile, absence of umbilical artery (UA) end-diastolic flow (EDF), AC/estimated FW combination below the 10th percentile, and pulsatility index (PI) above the 95th percentile. In order to diagnose FGR, at least one of these parameters must be present before 32 weeks of gestation.[21] The control group comprised pregnant women without FGR.

Demographic and clinical data were obtained from the patients' medical files. Maternal factors analyzed included age, body mass index (BMI), gravidity/parity, miscarriage, and smoking. Perinatal outcomes such as mode of delivery, gestational age at delivery, indications for cesarean section, Apgar score, birth weight, neonatal sex, neonatal intensive care unit admission, neonatal morbidity, and mortality were analyzed.

Venous blood was collected from participants using heparinized tubes. Samples were centrifuged at 3000 RPM for 10 minutes to obtain serum. Serum samples were immediately frozen and stored at -80°C for subsequent analyses. Sc5b9, C3, C4, C1-INH, and CH50 levels were determined using commercially available ELISA kits according to the manufacturer's instructions (SunRed, China).

Statistical Analysis

G*Power version 3.1.9.7 was used for sample size estimation. According to Cohen's guidelines, it was calculated that 86 participants would be needed to detect significant differences between groups on the primary outcome measure with a medium effect size, an alpha value of 0.05, and a power value of 0.80. IBM SPSS v26 (USA) was used for the statistical analyses. Data are presented as medians and minimum and maximum values, numbers, and percentages as appropriate. The Kolmogorov-Smirnov test was used to determine the normal distribution of the numerical data. Where appropriate, The Mann-Whitney or chi-square test was used to compare numerical variables and proportions. The statistical significance level was set at $P<0.05$.

RESULTS

This study compared the serum concentrations of complement biomarkers between 43 women with healthy pregnancies in the control group and 43 women diagnosed with FGR. There were no significant differences in age, gravidity, parity, miscarriage, or BMI between the control and FGR groups (p>0.05) (Table 1). The FGR group exhibited lower gestational age, biparietal diameter, head circumference, abdominal circumference, femur length, estimated fetal weight, umbilical artery flow, and higher pulsatility index values in the uterine arteries than the control group (p<0.05). Fetal biometry and Doppler measurements are presented in Table 2.

Table 1. Maternal characteristics of the study groups

	Controls (n=43)	FGR (n=43)	Significance
Age (years)	29 (22-39)	29 (19-44)	0.726
Gravidity	2 (1-8)	2 (1-9)	0.704
Parity	1 (0-6)	1 (0-5)	0.332
Miscarriage	9 (20.9%)	10 (23.2%)	0.795
Smoking	3 (7%)	1 (2.3%)	0.306
BMI (kg/m²)	27.3 (21.1-39.7)	28.0 (18.4-40.0)	0.548

FGR: Fetal growth restriction, BMI: Body mass index
The Kolmogorov-Smirnov test was used to determine the normal distribution of the numerical data. Statistical significance was assessed using the Mann-Whitney U test for continuous variables and the Chi-square test for categorical variables. The statistical significance level was set at P<0.05.

The FGR group had lower Apgar scores, higher cesarean delivery and neonatal intensive care unit admission rates, and higher neonatal mortality and morbidity rates (P<0.05). Perinatal outcomes are presented in Table 3.

The median serum sC5b-9 concentration in the control group was 253.8 mg/L. In the FGR group, the median concentration was slightly lower at 251.6 mg/L. However, this difference was not statistically significant (p=0.431). Regarding C3 levels, both groups showed a median of 1.6 mg/L. The control group had a 1.2 to 2.5 mg/L range, while the FGR group was 0.3 to 2.0 mg/L, indicating no significant difference between the two groups (p=0.907).

Similarly, serum C4 levels were comparable between the two groups, with a median of 0.3 mg/L for controls (range 0.10 to 0.6 mg/L) and a slightly lower median of 0.2 mg/L for the FGR group (range 0.9 to 1.5 mg/L), which did not reach statistical significance (p=0.137).

Notably, the serum C1-INH concentration was significantly higher in the FGR group with a median of 83.4 mg/L (range 19.2 to 107.7 mg/L) compared to 68.4 mg/L (range 8.2 to 102.0 mg/L) in the control group (p=0.019). The CH50 levels were higher in the control group, with a median of 121 mg/L (range 13 to 179 mg/L), than in the FGR group, which had a median of 104 mg/L (range 65 to 133 mg/L). However, this difference was not statistically significant (p=0.083) (Table 4).

Table 2. Fetal biometry and Doppler parameters of study participants

	Controls (n=43)	Fetal growth restriction (n=43)	Significance
Gestational age by obstetric exam (w)	30 (24-35)	31 (25-37)	0.812
Gestational age by ultrasound exam (w)	31 (24-36)	27 (21-34)	0.001
Biparietal diameter (mm)	79 (59-90)	71 (51-85)	0.002
Head circumference (mm)	291 (217-332)	265 (199-311)	0.001
Abdominal circumference (AC) (mm)	269 (196-317)	225 (160-305)	0.000
AC percentile (%)	55 (6-90)	0 (0-8)	0.001
Femur length (mm)	58 (42-68)	50 (33-72)	0.003
Estimated fetal weight (EFW)	1700 (671-2762)	1016 (396-2570)	0.001
EFW percentile (%)	49 (9-98)	0 (0-9)	0.001
Absent end-diastolic flow	0	12 ()	
Umbilical artery pulsatility index (UA-PI)	0.9 (0.5-3.6)	1.1 (0.5-2.0)	0.001
Middle cerebral artery pulsatility index (MCA-PI)	2.0 (1.1-3.4)	1.5 (0.9-2.3)	0.001
Cerebroplacental ratio (MCA-PI/UA-PI)	2.1 (1.8-4.1)	1.4 (0.6-3.6)	0.001
Right uterine artery pulsatility index	0.8 (0.5-2.1)	1.5 (0.5-3.0)	0.001
Right uterine artery notch present	0 (0%)	17 (39.5%)	0.001
Left uterine artery pulsatility index	0.9 (0.4-1.7)	1.4 (0.6-4.0)	0.001
Left uterine artery notch present	0 (0%)	20 (46.5%)	0.001
Ductus venosus pulsatility index	0.6 (0.3-1.0)	0.6 (0.3-1.1)	0.318

Data are presented as number (%) or median (min-max). The Kolmogorov-Smirnov test was used to determine the normal distribution of the numerical data. Statistical significance was assessed using the Mann-Whitney U test for continuous variables and the Chi-square test for categorical variables. The statistical significance level was set at P<0.05.

Table 3. Perinatal outcomes of the study participants

	Controls (n=43)	Fetal growth restriction (n=43)	
Gestational age at delivery (weeks)	39 (33-41)	33 (25-37)	0.001
Mode of delivery			0.001
Vaginal	20 (46.5%)	6 (14%)	
Cesarean section	23 (53.5%)	37 (86%)	
Indications of cesarean section			
Fetal distress	6 (26.1%)	14 (38.9%)	
Previous cesarean section	11 (47.8%)	8 (22.2%)	
Abnormal labor	3 (13%)	1 (2.9%)	
Placental abruption	0 (0%)	4 (11.1%)	
The reverse flow of ductus venosus	0 (0%)	2 (5.6%)	
Preeclampsia	0 (0%)	4 (11.1%)	
Other	6 (12.9%)	3 (8.4%)	
Birth weight (g)	3190 (2390-4800)	1270 (440-2650)	0.001
Newborn gender			0.104
Female	18 (42.9)	26 (60.6)	
Male	24 (57.1%)	17 (39.5%)	
Apgar score at min 1	8 (1-9)	6 (0-8)	0.001
Apgar score at min 5	9 (2-10)	8 (0-9)	0.001
Admission to the neonatal intensive care unit	7 (16.3%)	35 (81.4%)	0.001
Neonatal mortality	1 (2.3%)	8 (18.6%)	
Neonatal morbidity	0 (0%)	3 (7%)	

Data are presented as number (%) or median (min-max). The Kolmogorov-Smirnov test was used to determine the normal distribution of the numerical data. Statistical significance was assessed using the Mann-Whitney U test for continuous variables and the Chi-square test for categorical variables. The statistical significance level was set at P<0.05.

Table 4. Studied complement biomarkers of the women with healthy pregnancy and FGR

	Controls (n=43)	FGR (n=43)	Significance
sC5b-9 (mg/L)	253.8 (171.9-2766.6)	251.6 (17.7-784.6)	0.431
C3 (mg/L)	1.6 (1.2-2.5)	1.6 (0.3-2.0)	0.907
C4 (mg/L)	0.3 (0.10-0.6)	0.2 (0.9-1.5)	0.137
C1-INH (mg/L)	68.4 (8.2-102.0)	83.4 (19.2-107.7)	0.019
CH50 (units/mL)	121 (13-179)	104 (65-133)	0.083

C1-INH: C1 esterase inhibitor, CH50: The total hemolytic complement, sC5b-9: Soluble membrane attack complex

DISCUSSION

Our study compared the serum levels of sC5b-9, C3, C4, C1-INH, and CH50 complement components between pregnant women with and without FGR. Our results showed that the serum C1-INH levels were significantly higher in women with FGR (p<0.05). C1-INH is mainly involved in controlling the classical pathway of the complement system, and abnormal changes in its levels may trigger abnormal complement activation at the maternal-fetal interface, leading to placental dysfunction and endothelial damage. C1-esterase inhibitor has been studied for its immunomodulatory effects, showing a reduction in proinflammatory cytokines and an increase in anti-inflammatory cytokines.[22] The literature shows that higher plasma levels of C1-inhibitor are associated with a lower risk of future venous thromboembolism.[23,24] In the case of FGR, higher levels of C1 esterase inhibitor may mean that the body is trying to stop thrombus formation and keep blood flow through the vessels open.C1-esterase inhibitor attenuates the inflammatory response during human endotoxemia.[25] In pregnant women with hereditary angioedema (HAE), a nanofiltered C1 esterase inhibitor was found to be safe and effective for managing HAE attacks during pregnancy, with a favorable risk-benefit profile and positive pregnancy outcomes.[26] Additionally, severe acute respiratory syndrome coronavirus-2 infection may lead to a deficiency in the C1 esterase inhibitor, potentially contributing to severe systemic abnormalities in patients with COVID-19.[27] These findings suggest that C1-esterase inhibitor modulates inflammatory responses and may affect fetal growth and development.

Complement system activity is increased during pregnancy. However, complement inhibition is required at the implantation site during placental development and maintenance to maintain a normal placenta and ensure a healthy pregnancy. Abnormal or excessive activation of the complement system in the placenta is

likely related to placental dysfunction, which can lead to pregnancy complications such as preeclampsia and FGR.[28]

Guillermina Girardi et al.[29] showed in their study of mice with spontaneous abortion and FGR that complement activation, especially C5a, is a crucial intermediate step in the development of antibody-independent placental and fetal damage. The study also suggested that complement activation may lead to an imbalance in the angiogenic factors necessary for proper placental development.

Lynch et al.[30] reported that increases in complement activation products in early pregnancy are associated with adverse pregnancy outcomes, including preeclampsia. The lack of a significant difference in serum C3 levels between our groups may suggest that alternative pathway activation may not be as critical in the etiology of FGR as other pregnancy complications. However, the consistency of C3 levels between both groups suggests that a basic level of complement activity is maintained during pregnancy and that it is essential to balance the protective and pathogenic roles of complement activation.

A mouse model was used in the study by Qu et al.[31] They demonstrated that deficiency of the C5 component provided a protective effect against fetal growth restriction and loss after unilateral uterine ischemia/reperfusion. These results suggest that C5 may be a potential vulnerability factor in these processes, and its deficiency may prevent adverse outcomes. In support of these findings, in a case described by Burwick and Feinberg[32], a patient with severe preeclampsia/HELLP syndrome at 26 weeks of gestation was treated with eculizumab targeting C5, resulting in a marked improvement in the clinical condition and complete normalization of laboratory parameters. Prolonged pregnancy treatment by 17 days. Inhibition of complement activation has shown promise in preventing angiogenesis failure and rescuing pregnancies affected by fetal loss and growth restriction.[33]

Derzsy et al.[2] emphasized that the C3a/C3 ratio and sC5b-9 levels are increased in preeclamptic pregnancies, indicating excessive complement activation. The fact that there was no significant difference in sC5b-9 levels between the control and FGR groups in our study suggests that the terminal pathway of complement activation indicated by sC5b-9 does not dominate FGR, unlike preeclampsia.

Limitations

The relatively small sample size, the use of a prospective cross-sectional design, and the examination of a limited number of complementary system components limit the generalizability of the findings and the establishment of causal relationships.

CONCLUSION

Although the exact role of complement activation in fetal growth restriction (FGR) remains fully elucidated, the elevated levels of C1-INH in women with FGR suggest a compensatory mechanism to mitigate thrombus formation and inflammation. This adaptive response may be a potential therapeutic target for improving placental function and improving pregnancy outcomes. Further research with a larger cohort and a comprehensive analysis of the complement system are necessary to confirm these findings and develop targeted interventions.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee. (Date: 26.05.2021, Decision No: 99).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Availability of Data and Material

The data analyzed during the study are available from the corresponding author upon request.

Authors' Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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