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Fetal vascular malperfusion score is linked with developing preeclampsia in women with gestational diabetes mellitus: a retrospective cohort study

Selim Afsar[1*], Gulay Turan[2], Ayse Yigit Sonmez[3], Ceyda Sancakli Usta[1], Akın Usta[1]

SUMMARY

OBJECTIVE: Fetal vascular malperfusion is associated with poor perinatal outcomes in women with preeclampsia and gestational diabetes mellitus. The aim of this study was to determine the association between fetal vascular malperfusion score and syncytiotrophoblast basement membrane thickness and clinicopathological variables, such as developing preeclampsia in women with gestational diabetes mellitus.

METHODS: This retrospective cohort study included 65 pregnant participants (34 with gestational diabetes mellitus and 31 controls) between January 2019 and January 2022. Gestational diabetes mellitus was diagnosed as ≥2 of 4 elevated values on a 3-h, 100-g oral glucose tolerance test. The fetal vascular malperfusion score was evaluated by endothelial CD34 positivity in the villous stroma of the placenta. The association between fetal vascular malperfusion score and syncytiotrophoblast basement membrane thickness with clinicopathological variables in women with gestational diabetes mellitus was evaluated.

RESULTS: It was revealed that the gestational diabetes mellitus group had greater fetal vascular malperfusion scores than the control group (gestational diabetes mellitus group fetal vascular malperfusion score: 34.2±9.1 and control group fetal vascular malperfusion score: 26.5±8.7, respectively, p=0.0009). Syncytiotrophoblast basement membrane thickness was correlated with the development of preeclampsia, trophoblast proliferation, and fetal vascular malperfusions (0.3952, p=0.0129; 0.3487, p=0.0211; and 0.4331, p=0.0082, respectively). On the contrary, fetal vascular malperfusions were correlated with the development of preeclampsia, villous edema, and trophoblast proliferation (0.3154, p=0.0343; 0.2922, p=0.4123; and 0.3142, p=0.0355, respectively).

CONCLUSION: The gestational diabetes mellitus group displayed significantly higher fetal vascular malperfusion scores and thickening of the syncytiotrophoblast basement membrane than the control group. There is a correlation between developing preeclampsia and the fetal vascular malperfusion scores and the syncytiotrophoblast basement membrane thickness.

KEYWORDS: Diabetes, gestational. Chorionic villi. Antigens, CD34. Pre-eclampsia.

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized as impaired glucose tolerance or overt diabetes occurring during pregnancy[1,2]. The prevalence of GDM varies worldwide (2–38%) among racial and ethnic groups, and recently, it has been increasing gradually owing to advanced maternal age and obesity outbreaks[3,4].

The human placenta serves as a temporal organ that might be considered a two-way mirror reflecting the metabolic status of both mother and fetus; therefore, it might be used to denote metabolic dysregulation during pregnancy, such as GDM[5,6]. Hyperglycemia is an essential factor in the formation of histopathological alterations[5]. Maternal hyperglycemia might lead to alterations in the placental structure and function that compromise fetal development, with an increased risk of perinatal morbidity and mortality. The degree to which the maternal plasma level of glucose promotes placental alterations has yet to be unveiled[6].

Recent studies have revealed that GDM is associated with histopathological alterations, including increased placental thickness and weight, perivillous fibrin deposits, villous immaturity and edema, cytotrophoblastic hyperplasia, and thickening of the syncytiotrophoblast basement membrane[7,8]. The villous immaturity leads to an excessive gap between the intervillous space and fetal vasculature that endangers maternal–fetal oxygen transport[9]. The transporting unit in the human placenta is the syncytiotrophoblast membrane, which facilitates glucose transport across the placenta. It is hypothesized that the basement membrane of the syncytiotrophoblast is the rate-limiting step in glucose transport[10].

[1]Balikesir University, School of Medicine, Department of Obstetrics and Gynecology – Balikesir, Turkey.
[2]Balikesir University, School of Medicine, Department of Pathology – Balikesir, Turkey.
[3]Adana Yuregir State Hospital, Department of Obstetrics and Gynecology – Adana, Turkey.
*Corresponding author: drselim@istanbul.edu.tr
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The most prominent histopathological characteristic of fetal vascular malperfusion (FVM) is the loss of vasculature in chorionic villi, which can be detected readily with hematoxylin and eosin (H&E) staining at the later stages of FVM. The utilization of CD34 immunostaining for detecting the early stages of FVM has just come into the pathological practice to overcome the inefficiencies of H&E staining[11]. The overidentification of FVM based on CD34 immunostaining in the lobular villous vasculature and endothelium empowers the correlation of FVM with umbilical cord compromise and stillbirth, as well as poor perinatal outcomes in maternal preeclampsia, maternal diabetes mellitus, and intrauterine growth restriction (IUGR)[11-13]. However, it can be appreciated in the normal population in the short term[14]. In light of all the facts mentioned above, identifying FVM has the utmost importance in the histopathogenesis of GDM and its correlation with perinatal outcomes.

This study aimed to determine the association between the FVM score and syncytiotrophoblast basement membrane thickness with clinicopathological variables, such as developing preeclampsia in women with GDM.

METHODS

Ethical statement

This study was held in parallel with the Helsinki Committee's essentials. Ethical approval was obtained from the Ethics Committee of Balikesir University with the approval number 2021-195, and this retrospective cohort study included 65 participants between January 2019 and January 2022.

Study design

Women with singleton pregnancies underwent a two-step approach to detecting GDM, and they were followed until delivery. Women with singleton pregnancies were screened with a 1-h 50-g glucose challenge test (GCT) from the 24th to 28th weeks of pregnancy. Women with positive GCT results (glucose ≥140 mg/dL) proceeded to a diagnostic 3-h, 100-g oral glucose tolerance test (OGTT). Women with negative GCT results were included in the control group. Women with two or more elevated values on a 3-h, 100-g OGTT based on Carpenter and Coustan criteria[1] were included in the GDM group.

Based on these results, 65 age-matched women participated in the study in either the GDM (n=34) or the control group (n=31), and their placental specimens were retrieved after delivery. Women with a history of hypertension, pregestational diabetes, multiple pregnancies, intrauterine infections, and fetal anomalies were excluded from the study.

Immunohistochemistry and fetal vascular malperfusion score evaluation

Standardized tissue preparation protocols were followed during the histopathological examination of the placentas, as in the literature[15]. Afterward, anti-human monoclonal CD34 antibody (anti-CD34 ab, Abcam, Cambridge, MA) was applied to the slides, and the tissue extracts were rinsed again with phosphate-buffered saline (PBS), followed by staining with H&E and periodic acid–Schiff (PAS) to describe the placental alterations.

Villous immaturity is defined as the combination of reduced terminal villous surface area, irregular villous contour, syncytial knots, villous edema, fibrin deposition, trophoblast proliferation, and increased layer thickness. It was evaluated under light microscopy with H&E and PAS staining. The addition of an anti-CD34 antibody, which is primarily used to empower the diagnosis of FVM, is a valuable marker for highlighting the villous vasculature and endothelium (Figure 1)[10]. The pictures of three randomly selected areas of the terminal villi (40×) were analyzed by an image processing system (ImageJ open access program from the National Institute of Health). The pictures were uploaded to the program and then converted into 8-bit images. Afterward, the CD34 staining intensity was evaluated by adding area fractions, which correspond to the FVM scores.

Statistical analysis

Statistical and power analyses of this study were performed with the open-source Jamovi statistical software (version 2.3.21) and G*Power software (version 3.1.9.7). According to the literature, the minimum sample size was calculated as 36 per group based on α error: 0.001, power: 0.95, and effect size d: 1. The distribution and homogeneity of groups were evaluated by skewness, kurtosis, Levene's test, and Kolmogorov-Smirnov test. The variables between the study groups were compared using

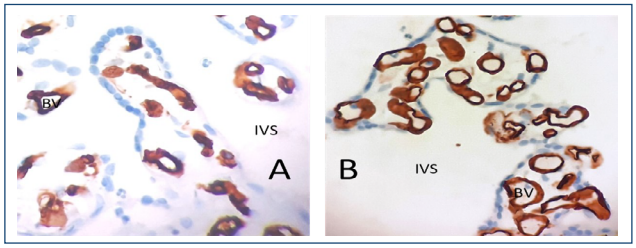

Figure 1. CD34 immunostaining of terminal villi in the control and the gestational diabetes mellitus groups. (A) Weak CD34 (+) immunostaining in the control group (low fetal vascular malperfusion score) (40×). (B) Strong CD34 (+) immunostaining in the gestational diabetes mellitus group (high fetal vascular malperfusion score) (40×). FVM: fetal vascular malperfusion; BV: blood vessel; IVS: intervillous space.

the Mann-Whitney U and chi-squared tests. Spearman's correlation analysis was performed on the variables. Statistical significance was determined as $p<0.05$.

RESULTS

Placental tissues were retrieved from 65 women (n=34 for the GDM group and n=31 for the control group=), and there were no significant differences in the context of age, parity, gestational age, developing preeclampsia, fasting blood glucose level, fetal macrosomia, neonatal hypoglycemia, or fetal birth weight between the study groups. The clinical features of the study groups are summarized in Table 1.

The morphological assessment concluded that there were no differences between the study groups regarding placental weight. The placental tissue of the GDM group displayed significantly higher villous immaturity, trophoblastic cell proliferation, and thickening of the syncytiotrophoblast basement membrane compared with the control group (p=0.0002, p=0.0126, and p=0.0002, respectively) (Table 2).

However, there were no statistical differences between the study groups in the context of villous edema or fibrin thrombus in placental tissue (p= 0.6430 and p= 0.7685, respectively). It was revealed that the GDM group had higher FVM scores than the control group (34.2±9.1 versus 26.5±8.7, respectively, p=0.0009) (Table 2).

Regarding the association between pathological alterations of the placenta and GDM group variables, the syncytiotrophoblast basement membrane thicknesses were correlated with developing preeclampsia, trophoblast proliferation, and FVM scores (ρ=0.395, p=0.01; ρ=0.348, p=0.02; and ρ=0.433, p=0.008, respectively). On the contrary, FVM scores were correlated with developing preeclampsia, villous edema, and trophoblast proliferation (ρ=0.315, p=0.03; ρ=0.292, p=0.41; and ρ=0.314, p=0.03, respectively) (Table 3).

Table 1. The clinical features of the study groups.

Participants' characteristics	GDM (n=34)	Control (n=31)	p-value
Age (years), median	30.5 (24–40)	29 (22–42)	0.47
Parity (n)	1 (0–3)	1 (0–4)	0.91
Fasting blood glucose (mg/dl)	88.5 (68–121)	76 (67–94)	0.09
Gestational age (weeks)	38 weeks+3 days	38 weeks+2 days	0.32
Fetal macrosomia[a], n (%)	6 (17%)	2 (6%)	0.17
Neonatal hypoglycemia[b], n (%)	3 (8%)	0	0.09
Developing preeclampsia[c], n (%)	3 (8%)	0	0.09
Fetal weight (g)	3,390 (2,450–5,150)	3,320 (2,850–4,210)	0.43

SD: standard deviation; GDM: gestational diabetes mellitus. Age and fetal weight are expressed as mean±SD. [a]Fetal macrosomia is defined as birth weight >4,000 g[16]. [b]According to the AAP Neonatal Hypoglycemia Guideline[17]. [c]According to the ACOG Preeclampsia Guideline 2020[18].

Table 2. Pathological features of placentas in the study groups.

Participants' characteristics	GDM (n=34)	Control (n=31)	p-value
Placental weight (g)	538 (365–750)	489 (398–577)	0.23
Umbilical cord insertion			
Central	30	28	0.13
Marginal	4	3	0.18
Villous immaturity	33/34 (97%)	18/31 (58%)	**0.0002*****
Villous edema	7 (21%)	5 (16%)	0.64
Fibrin thrombus	11 (32%)	9 (29%)	0.76
Trophoblast proliferation	30/34 (88%)	19/31 (61%)	**0.01***
Trophoblast BM thickness	27/34 (79%)	10/31 (32%)	**0.0002*****
FVM score	34.2±9.1	26.5±8.7	**0.0009*****

*p<0.05, ***p<0.001. Placental weight and FVM score are expressed as mean±SD. SD: standard deviation; GDM: gestational diabetes mellitus; BM: basement membrane; FVM: fetal vascular malperfusion. Bold indicates statistically significant values.

Table 3. Correlation of fetal vascular malperfusion score and trophoblast basement membrane thickness with gestational diabetes mellitus group variables.

GDM group	FVM score		Trophoblast BM thickness	
Variables	**ρ-coefficient**	**p-value**	**ρ-coefficient**	**p-value**
Fasting blood glucose	0.202	0.09	0.124	0.21
Neonatal hypoglycemia[a]	0.123	0.36	0.115	0.31
Preeclampsia[b]	**0.315**	**0.03***	**0.395**	**0.01***
Fetal weight	0.154	0.12	0.214	0.06
Villous immaturity	0.104	0.19	0.042	0.62
Villous edema	**0.292**	**0.41**	0.151	0.11
Fibrin thrombus	0.185	0.31	0.119	0.30
Trophoblast proliferation	**0.314**	**0.03***	**0.348**	**0.02***
FVM score			**0.433**	**0.008****

*p<0.05, **p<0.01. BM: basement membrane; FVM: fetal vascular malperfusion. [a]According to the AAP Neonatal Hypoglycemia Guideline[16]. [b]According to the ACOG Preeclampsia Guideline 2020[17]. Bold indicates statistically significant values.

DISCUSSION

Gestational diabetes mellitus is one of the most challenging endocrine disorders diagnosed during pregnancy, and it has been related to a considerably high incidence of complications such as fetal macrosomia, preeclampsia, and fetal growth restriction[19]. Even if it stayed under the statistical significance, we noticed that the GDM group had a higher incidence of fetal macrosomia, neonatal hypoglycemia, and developing preeclampsia than the control group.

Gestational diabetes mellitus is associated with the alterations in placental function and villous structure, as correlated with maternal hyperglycemia[5,20]. In line with the literature, we revealed that placental alterations, including increased villous immaturity, cytotrophoblastic hyperplasia, and thickening of the syncytiotrophoblast basement membrane, were more frequent in the GDM group [7,8,21,22]. Moreover, syncytiotrophoblast basement membrane thickness was correlated with the development of preeclampsia and FVM scores. The thickening of the syncytiotrophoblast basement membrane is a frequent histopathological alteration in GDM. It is accompanied by villous immaturity, with diminished total surface area of terminal villi and in number[5,14,23]. These alterations jeopardize maternal–fetal oxygen and nutrient transport and ultimately cause fetal macrosomia, preeclampsia, and intrauterine fetal growth restriction[9].

Fetal vascular malperfusion (formerly known as fetal thrombotic vasculopathy) is a new term and is related to the prominent chronic hypoxic placental injury that can be linked with an increased risk of perinatal morbidity and mortality[24,25]. In this study, FVM scores were correlated with the development of preeclampsia and pathological alterations of the placenta, such as villous edema and trophoblastic hyperplasia, found in the literature[23-25]. Even though the mechanism of FVM is unclear, it has been revealed that maternal hyperglycemia is the main perpetrator in the pathogenesis of endothelial cell injury in fetal vessels via oxidative stress and inflammation, which causes thrombosis and endothelial cell loss in women with GDM[24,25].

The limitations of this study need to be acknowledged. First, the low number of placental tissues could be a barrier to generalizing the study results. Second, the heterogeneity of patients with GDM in pregestational weight, body mass index (BMI), and gestational weight gain might be confounding factors for FVM scores. Third, the retrospective cohort studies provide a level 3 grade of evidence.

CONCLUSION

This study revealed that the GDM group demonstrated significantly higher villous immaturity, trophoblastic hyperplasia, FVM score, and thickening of the syncytiotrophoblast basement membrane. Additionally, syncytiotrophoblast basement membrane thickness and FVM scores were correlated with developing preeclampsia and trophoblast proliferation.

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ETHICAL STATEMENT

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AUTHORS' CONTRIBUTIONS

SA: Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **GT:** Conceptualization, Validation, Visualization, Writing – original draft. **AY:** Investigation, Methodology, Supervision. **CSU:** Investigation, Validation, Visualization, Writing – original draft. **AU:** Validation, Visualization, Writing – original draft.

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