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Is there any relationship between glycodelin levels and perinatal outcomes?

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Abstract

The aim of this study was to assess the correlation between uterine artery Doppler parameters examined from 20-24 weeks of pregnancy with glycoledin level in maternal blood. The study included 80 patients attending our clinic from September 2019 to June 2020. Participants were divided into two groups according to uterine artery findings. Group 1 included pregnant patients who had abnormal uterine artery Doppler USG at 22–24 weeks of pregnancy, and Group 2 consisted of healthy pregnant patients with uterine artery Doppler USG performed during the same period of pregnancy. Serum glycodelin levels were analyzed at 24 weeks of gestation of two goups and their perinatal outcomes were compared. No significant difference were seen between the groups regarding age, body mass index, blood pressure, gestational age, and blood test sampling time for serum glycodelin analysis. The mean pulsatility index values detected with Doppler ultrasonography were 1.92±0.41 and 0.82±0.25 for Groups 1 and 2, respectively. Comparative analysis revealed that the mean glycodelin level was significantly higher in Group 1 than Group 2 (521.75±21.68 -475.67±41.09; p< 0.001). Glycoledin may assist in predicting negative perinatal outcomes especially preeclampsia. However, many more comperative studies with larger series from multicenter are need to support this conclusion.

Keywords: Glycodelin, ultrasonography, doppler ,pregnancy outcome, pre-eclampsia, uterine artery, fetal growth retardation

Introduction

Currently screening from the first weeks of pregnancy has many purposes, and one of these is to predict possible poor obstetric outcomes as early as possible and to take precautions linked to this and prevent these situations [1]. From this aspect, many methods are used and one of these is uterine artery Doppler assessment [2]. Color Doppler assessment of the uterine artery is reported to be an easy, cheap and noninvasive method to predict poor obstetric outcomes [3]. Glycodelin (GdA), known as placental protein 14, is included in the lipocalin family and is an immunomodulatory agent released from the decidua and playing a role in pregnancy implantation [4]. After implantation, it plays a role in sustaining pregnancy [5]. Additionally, a role in angiogenesis was revealed with the identification that it increased angiogenic response in human umbilical cord vein endothelial cells (HUVEC) after birth [6]. This study aimed to assess the correlation of uterine artery Doppler assessment results used for prediction of poor obstetric outcomes with glycoledin protein levels.

Additionally, to the best of our knowledge, there is no study in the literature comparing these two parameters.

Material and Methods

A total of 80 women at 22–37 weeks of pregnancy were included in this study between September 2019 and July 2020. Participants were divided into two equal groups as patients with abnormal uterine artery and with normal uterine artery findings from 22-24 weeks of pregnancy, respectively. Group 1 included pregnant patients who had abnormal uterine artery Doppler USG at 22–24 weeks of pregnancy, and Group 2 consisted of healthy pregnant patients with uterine artery Doppler USG performed during the same period of pregnancy. Serum glycodelin was analyzed at 24 weeks of gestation in the two groups and perinatal outcomes were compared.

All obstetric and gynecological examinations were performed by the same gynecologist, while Doppler checks were performed by a specialist in the perinatology department. Patients with chronic hypertension, diabetes, and multiple pregnancy were excluded

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from the study. Both groups were followed up to delivery.

Gestational age was calculated based on the estimated first day of the last menstrual period or obstetric ultrasonograms performed during the first trimester of pregnancy.

The variables collected for this study included age, body mass index (BMI), gravidity, parity, gestational age during uterine artery Doppler USG, week of gestation during labor, labor type, uterine artery pulsatility index (PI), and maternal serum glycodelin level. Doppler ultrasonograms were performed using Voluson 730 (General Electric Company, Fairfield, CT) with a 3.5-MHz probe. Imaging of interval flow Doppler were performed with insonation angle below 50 degrees and sampling interval of 2 mm to cover the whole vein. The uterine artery pulsatility index was measured when repeated similar wave forms were obtained, with mean uterine artery PI calculated for the right and left uterine arteries. Bilateral notching in the uterine artery or high resistance (mean PI >95th percentile for both uterine arteries) were accepted as abnormal uterine artery findings.

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Doppler USG and the levels were recorded. Blood samples were collected in tubes and centrifuged at 3000 g for 15 minutes. The supernatants were then stored at −80 °C until the experiments started. Samples were thawed, and the enzyme-linked immunosorbent assay (ELISA) method using human serum pp14 ELISA kit (Sunlong, Catalog number: SL1395Hu) was utilized for the quantitative measurement of glycodelin levels in serum samples. Biochemical analyses were performed using a plate reader (Thermo Scientific Multiscan FC, 2011-06, USA) with ELISA of serum samples. Laboratory data are given as ng/mL. Body mass index was calculated as weight divided by the square of height (kg / m²).

Pregnancy outcomes and obstetric complications of all study patients were retrospectively retrieved from patient files. Complications including preeclampsia, fetal growth restriction (FGR), small for gestational age (SGA), in utero ex fetus, placental abruption, and preterm labor were recorded in the database. Preeclampsia was diagnosed as blood pressure levels of higher than 130/80 mmHg measured on two separate occasions more than 6 hours apart with or without the presence of protein in urine after 20 weeks of gestation. Patients with prenatal bleeding and pelvic pain who were detected to have retro-placental blood clots were diagnosed with placental abruption. In utero ex fetus was defined as pregnancy loss after 20 weeks of gestation. FGR was defined as fetal weight at least 10% lower than the standard weight corresponding to the gestational age. Preterm labor was defined as labor between the 22nd and 37th weeks of pregnancy.

This study was conducted at the Istanbul Medipol University Department of Obstetrics between September 2019 and July 2020 under the Principles of the Helsinki Declaration. It was approved by the Medipol University Ethical Review Committee (25.09.2019/663).

Power analysis

Sampling power was calculated using the G-power 3.1.9.4 program. Based on the mean for glycodelin in the first group (x=521.75, SD=21.68) and second group (X=475.67, SD=41.09), the effect size was found to be 1.42. In order to find significant differences between measures, for α=0.05, 1- β=0.95 and power of the test=0.96, the results of power analysis was determined as at least 15 for each sample group.

Statistical Method

Analysis of data used SPSS version 22 (IBM Corp released 2012. IBM SPSS statistics for windows, IBM Corp, Armonk, NY). Data are given as mean ± SD or interval (min-max), case number and percentage. P values <0.05 were accepted as significant. The distribution of variables was checked with the Kolmogorov–Smirnov test. The t-test and Mann-Whitney U-test were used in the analysis of quantitative data for independent samples. The chi-square test was used for the analysis of qualitive data. The Spearman rho correlation analysis was used to assess the correlation between obstetric outcomes and serum glycoddelin levels. Receiver operating characteristic (ROC) analysis was used to determine the performance of glycodelin level for the diagnosis of uterine artery resistance and the cut-off points for sensitivity and specificity were calculated. A two-tailed p value of less than 0.05 was considered statistically significant.

Results

Mean patient age was 30.99±4.87 years and 29.86±4.58 years, while the mean gestational age at birth was 37.99±1.42 and 38.30±1.22 for Groups 1 and 2, respectively. No difference was found between the groups in terms of BMI, gravidity, and parity. A review of the delivery types of the study participants found there were 23 (51.1%) normal deliveries and 17 (48.6%) cesarean sections in Group 1, whereas there were 18 (51.4%) normal deliveries and 22 (48.9%) cesarean sections in Group 2 (Table 1).

The average pregnancy week of blood sampling was 24.60±1.81 and 24.55±1.05 for Groups 1 and 2, respectively. The mean PI value in Group 1 (1.92±0.41) was significantly higher than in Group 2 (0.82±0.25; p< 0.001) (Table 1).

Similarly, the mean serum glycodelin level of Group 1 (521.75±21.68) was significantly higher than Group 2 (475.67±41.09; p< 0.001) (Figure 1).

A statistically significant positive correlation was found between serum glycodelin and PI values (p< 0.001) (Table 2). The PI value was the only clinical parameter that positively correlated with the serum glycodelin levels.

Linear regression analysis showed that the obstetric outcomes were not associated with serum glycodelin levels (Table 3).

The area under the curve (AUC) and Youden index were calculated to determine the cut-off value for maternal serum pp14 level in predicting increased uterine artery resistance. According to this analysis, the AUC was 0.841 (95% CI: 0.753–0.929; sensitivity 97.5% and specificity 50%) with the cut-off value of 475.34 ng/ml (Figure 2).

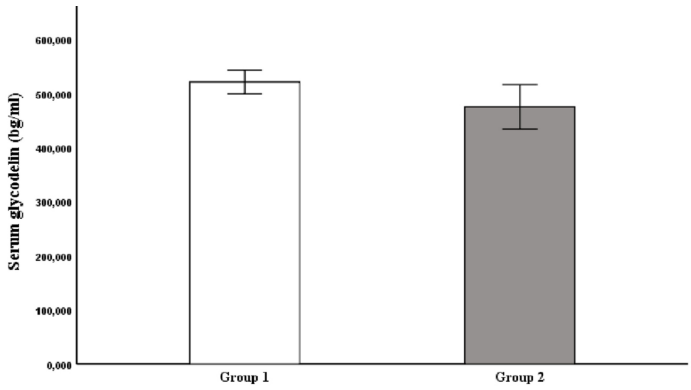

Figure 1. Mean serum human placenta glycodelin values in the study groups. *p <0.05 indicated statical significance

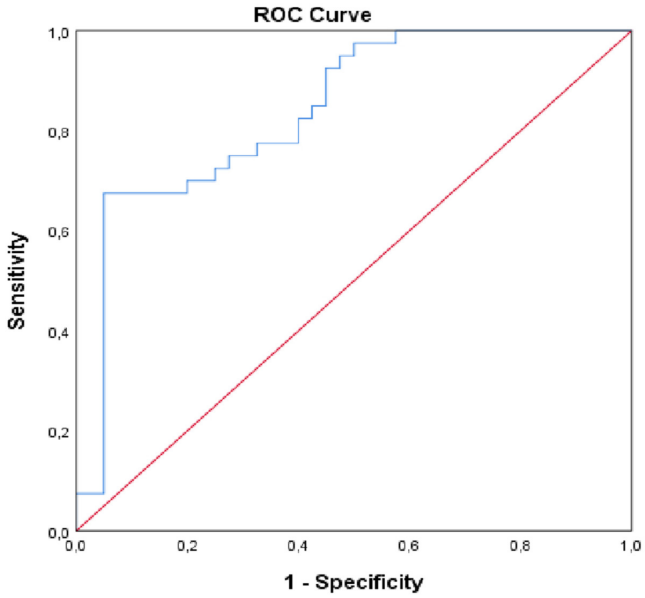

Figure 2. Receiver operating characteristic (ROC) curve analysis of the glycodelin (GdA) value determined to differentiate group 1 and group 2

Table 1. Clinical characteristics and laboratory results of the patients

	Group 1 (n = 40)	Group 2 (n = 40)	p
Age (years)	30.99±4.87	29.86±4.58	0.205
BMI (kg/m2)	28.97±1.11	28.90±1.09	0.965
Gravidity	1.79±0.88	1.62±0.65	0.445
Parity	0.95±0.83	0.70±0.66	0.221
Gestational age at Doppler ultrasound (weeks)	22.60±1.81	22.55±1.05	0.708
Gestational age at blood test (week)	22.70±1.07	22.91±0.77	0.511
Delivery			
Normal delivery	17 (48.6%)	18 (51.4%)	0.822
Cesarean section	23 (51.1%)	22 (48.9)	
Weight of the baby (g)	3096.88±477.03	3210.25±404.28	0.292
Smoking			
No	30 (48.4%)	32 (51.6%)	0.592
Yes	10 (55.6)	8 (44.4%)	
PI value	1.92±0.41	0.82±0.25	<0.001
Systolic (mmHg)	106.52±10.76	105.76±10.11	0.769
Diastolic (mmHg)	63.43±15.96	63.82±17.10	0.938
Gestational age at delivery (week)	37.99±1.42	38.30±1.22	0.374
Glycodelin (GdA)	521.75±21.68	475.67±41.09	<0.001

BMI: body mass index, GdA: Glycodelin PI: pulsatility index; boldfaced p values are statistically significant

Table 2. Analysis of the correlation between glycodelin and clinical parameters

	Serum Glycodelin	
	r	p
Age (years)	0.041	0.715
BMI (kg/m²)	0.015	0.892
Gravidity	0.078	0.493
Parity	0.024	0.833
Gestational age at Doppler ultrasound (week)	−0.098	0.389
Gestational age at blood test (week)	0.055	0.626
Weight of the baby (g)	−0.102	0.370
PI	0.560	<0.001*
Systolic (mmHg)	−0.056	0.619
Diastolic (mmHg)	−0.006	0.957
Gestational age at delivery (week)	−0.107	0.343

BMI: body mass index, PI: pulsatility index, GdA: Glycodelin *p < 0.005 indicated statistical significance (Spearman correlation test); Boldfaced p values are statistically significant.

Table 3. Univariate regression analysis results for the serum glycodelin levels and obstetric outcomes of the patients

		Univariate model		
Variables	**B**	**Lower limit**	**Upper limit**	**p**
Delivery	8.70	−10.38	27.79	0.366
Smoking	15.04	−6.93	37.10	0.177
FGR	18.25	−48.58	85.09	0.588
Preeclampsia	0.95	−67.05	68.96	0.978
SGA	−5.67	−138.43	127.09	0.932
IUMF	27.21	−55.01	109.45	0.512
Preterm Labor	29.51	−76.53	135.57	0.581
		R = .24, R2 = .05, F = 0.64, p = 0.719		

FGR: Fetal growth restriction; SGA: small for gestational age; IUF: in utero ex fetus

Discussion

Glycodelin is one of the members of the lipocalin glycoprotein family with a 28 kDa molecular weight and it plays a role in cellular proliferation, differentiation, adhesion, and motility [7]. It can contribute to placental genesis at the implantation site via its modulatory effect on immunity cells and cytotrophoblasts [8].

The immunosuppressive activity of GdA is believed to be associated with fetomaternal defense [9]. In this way, strong variations were observed in GdA expression in early pregnancy losses and molar pregnancies [10]. Especially in decidua tissue of patients resulting in miscarriage, glycodelin expression was observed to be significantly reduced compared to normal pregnancies [11].

In patients with FGR and HELLP, decidual tissue samples in paraffinized placenta tissues were observed to have less glycodelin expression when incubated with polyclonal or monoclonal antibodies against glycodelin A, preeclamptic decidual tissue was not identified to have significant degree of difference compared to normal placental tissue controls [12].

Abnormal uterine wave forms detected in fetal Doppler ultrasound imaging performed between 22nd and 37th weeks of pregnancy are associated with defective trophoblastic invasion of the spiral arteries [13]. It is known that insufficient uteroplacental arterial blood flow is the underlying mechanism for several obstetric complications, including preeclampsia and FGR [14].

Abnormal placentation, systemic inflammatory response, an imbalance between pro- and anti-angiogenic factors, and oxidative stress are predicted to take part in this process by impairing endothelial function [15]. It is widely accepted that resultant placental ischemia and increased resistance in placental blood inflow leads to unfavorable obstetric outcomes, including preeclampsia [16]. Studies regarding the predictive value of

uterine artery Doppler USG findings and the risk of preeclampsia indicated that the presence of a diastolic notch or high resistive index led to higher risk of preeclampsia [17].

The hypothesis that the background etiology of many negative perinatal outcomes, led by preeclampsia, is disrupted endothelial function disorder, vascular dysfunction and resulting oxidative stress and linked increasing uterine artery resistance has been proven many times [18].

Several studies investigated the potential biomarkers for early detection of uterine artery resistance [19]. These studies focused on placental microvesicles, anti-angiogenic factors, and autoantibodies. Accordingly, inhibin A, activin A, and pregnancy-associated plasma protein-A levels were analyzed by a double-antibody sandwich method [20]. The primary purpose of these endeavors is the prediction of obstetric complications, including eclampsia, and improvement in early treatment strategies [21,22]. In the literature, there is very little data about the role of glycodelin, also known as human placental protein 14, in obstetric and gynecological practice [23]. GdA participates in early placenta development due to the modulatory effect on immune cells and cytotrophoblasts [24] In addition to this structuring effect, glycodelin was shown to have the potential to regulate a variety of processes including immunosuppression, fertilization and implantation [25]. Compared with infants born at normal weight, SGA infants were observed to be predicted by glycodelin examined in the first trimester [26].

The main hypothesis of this study was to compare the level of glycodelin, a glycoprotein with a role in the placenta, in maternal serum of pregnant cases with abnormal uterine artery Doppler findings with levels in pregnant cases with normal Doppler findings and to assess the obstetric outcomes in these patients.

Notably, similar studies in the literature identified increased placental glycodelin levels in some women with preeclampsia compared to women with healthy pregnancies, which were significantly higher compared to normal pregnancies [27]. However, another study found that the sensitivity of glycodelin was 59% and specificity was 93.3% at a threshold of >83.97 ng/ml for patients with severe preeclampsia [23]. It was reported that GdA may be a predictive marker for preeclampsia due to excess expression of GdA linked to increased placental necrosis [28]. In conclusion, it is considered to act as an important endometrial factor during pregnancy [29].

According to the results of our study, patients with abnormal uterine artery Doppler findings had significantly increased serum glycodelin levels ($p<0.001$). This supports the hypothesis that this process prepares the way for placental ischemia which can lead to poor obstetric outcomes. However, there was no correlation identified between serum glycodelin level and perinatal outcomes. The low number of patients and the parallel inadequate poor obstetric outcomes are factors limiting the power of the study.

Conclusion

In our study, there was a correlation between serum glycodelin and perinatal outcomes. A variety of studies have been performed in order to predict poor outcomes in the obstetric field and unfortunately effectivity is limited. For this reason, identification of more sensitive and specific serum markers is important to identify possible risks during pregnancy in terms of preventive medicine and the ability to minimize complications

Conflict of interests
These authors declare that there are no conflicts of interest.

Financial Disclosure
We did not receive financial support for this study.

Ethical approval
This observational prospective study was carried out in line with research regulations, including the approval of the Ethics Committee of our institute dated 25/09/2019 and numbered 663 and according to the principles of the "World Medical Association Helsinki Declaration".

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