J Surg Med. 2022;6(9):813-819.
DOI: 10.28982/josam.7347

Research article

Journal of Surgery and Medicine
e-ISSN: 2602-2079

Immunohistochemical evaluation of glucose transporter protein-1 density in the placenta in preeclampsia patients and its association with intrauterine growth retardation

Adem Yavuz [1], Mehmet Dolanbay [2], Hulya Akgun [3], Gulcan Yazici Ozgun [4], Fulya Cagli [2], Mahmut Tuncay Ozgun [2]

[1] Department of Obstetrics and Gynecology, Omer Halisdemir University Faculty of Medicine, Nigde, Turkey
[2] Department of Obstetrics and Gynecology, Erciyes University Faculty of Medicine, Kayseri, Turkey
[3] Department of Medical Pathology, Erciyes University Faculty of Medicine, Kayseri, Turkey
[4] Department of Obstetrics and Gynecology, Private Kayseri Tekden Hospital, Kayseri, Turkey

ORCID ID of the author(s)

AY: 0000-0003-4191-4004
MD: 0000-0002-8332-1568
HA: 0000-0002-9249-4153
GYO: 0000-0001-6472-126X
FC: 0000-0002-6492-3379
MTO: 0000-0003-4946-2268

Corresponding Author
Adem Yavuz
Department of Obstetrics and Gynecology, Omer Halisdemir University Faculty of Medicine, Bor Yolu Uzeri, 51240 Merkez/Nigde, Turkey
E-mail: ademyavuz@ohu.edu.tr

Ethics Committee Approval
The study protocol was approved by the Ethics Committee of Erciyes University Medical Faculty Hospital (Date of Issue/Number: 2021/258). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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

Published
2022 September 23

Abstract

Background/Aim: Preeclampsia (PE) complicates 2–8% of all pregnancies worldwide. Placental malperfusion and dysfunction are observed in PE. The supply of glucose, the main energy substrate for the fetus and placenta, is regulated by placental expression and activity of specific glucose transporter proteins (GLUTs), primarily GLUT1. GLUT1 expression is affected by uteroplacental malperfusion and oxidative stress, which are important components of PE. Very few studies have investigated GLUT1 expression in preeclamptic placentas. In this study, we aimed to compare GLUT1 staining intensity in the terminal villi of the placenta in healthy subjects and patients with E-PE or L-PE and determine whether there was a relationship between GLUT1 staining intensity and IUGR.

Methods: This case-control study was carried out in our hospital's gynecology and obstetrics clinic, a tertiary center for perinatology cases. A total of 94 placentas, 47 of which were preeclamptic and 47 were from uneventful pregnancies (controls), were included in the study. PE was diagnosed according to the American College of Obstetrics and Gynecologists 2019 diagnostic criteria for gestational hypertension and PE. Placentas in the control group were obtained from pregnancies without maternal, placental, or fetal pathology and resulted in spontaneous idiopathic preterm or term delivery. The PE group was divided into two subgroups as early onset PE (E-PE [$\leq 33^{+6}$ gestational week]) and late-onset PE (L-PE [$\geq 34^{+0}$ gestational week]), according to the gestational week of PE onset. Sections prepared from placental tissues were stained for GLUT-1 by immunohistochemical method. Slides were evaluated by light microscopy, and each slide was scored from 0 to 4 to determine the staining intensity. The results were compared between the control and PE group/PE sub-groups.

Results: GLUT1 scores were significantly higher in both early- and late-onset PE subgroups compared to controls ($P < 0.001$ for both). In the late-onset PE subgroup, GLUT1 scores were significantly higher in those with severe PE features than those without them ($P = 0.039$). While intrauterine growth restriction (IUGR) was not found in any cases in the control group, IUGR was present in 11 (23.4%) of 47 pregnant women with PE, including eight (53.3%) of the 15 pregnant women with early-onset PE and 3 (9.38%) of the 32 pregnant women with late-onset PE. GLUT1 scores were similar in placentas obtained from pregnant women who had PE with and without IUGR ($P = 0.756$). In the late-onset PE subgroup, GLUT1 scores were correlated negatively with maternal body mass index ($r = -0.377$, $P = 0.033$) and positively with placental weight-to-fetal weight ratio ($r = 0.444$, $P = 0.011$).

Conclusions: Our findings show that GLUT1 expression might be increased due to placental adaptation to new conditions in PE and, thus, is unlikely to be the main factor in PE-related IUGR.

Keywords: Preeclampsia, Placenta, Glucose transporter proteins, Glucose transporter protein-1, Intrauterine growth retardation

How to cite: Yavuz A, Dolanbay M, Akgun H, Ozgun GY, Cagli F, Ozgun MT. Immunohistochemical evaluation of glucose transporter protein-1 density in the placenta in preeclampsia patients and its association with intrauterine growth retardation. J Surg Med. 2022;6(9):813-819.

Introduction

Preeclampsia (PE) is defined as the development of hypertension with proteinuria or hypertension with thrombocytopenia, renal failure, liver dysfunction, or pulmonary edema, which begins after the 20th week of pregnancy [1]. PE complicates 2–8% of all pregnancies worldwide [1] and is responsible for 12% of all maternal deaths [2]. Although the pathophysiology of PE has not been clarified, a clinical subclassification with early-onset ($<34^{+0}$ weeks of gestation) and late-onset ($\geq 34^{+0}$ weeks of gestation) [3] has been increasingly accepted [4, 5]. Early onset PE (E-PE) has been predominantly associated with defective placentation during the first few weeks of pregnancy, indicating a pathophysiological similarity to intrauterine growth restriction (IUGR). Unlike E-PE, late-onset PE (L-PE) is suggested to develop in the presence of maternal cardiovascular risks and a discrepancy between placental supply and demand, ultimately leading to oxidative changes in the placenta [4]. Although the underlying causes and timing can differ, placental malperfusion and dysfunction are observed in both E-PE and L-PE [6].

Among the nutrients provided by the maternal circulation, glucose is the main energy substrate for the fetus [7, 8] and the placenta [9]. The main regulatory factors in the maternal-fetal glucose exchange process are the placental expression and activity of specific glucose transporter proteins (GLUTs) [8]. In the placenta, GLUT1 is the primary isoform found in syncytiotrophoblasts [10, 11], which are cells that function as the primary barrier for the transfer of nutrients from the mother to the fetus. The GLUTs that mediate glucose transfer are expressed in both the maternal-facing microvillous membrane (MVM) and the fetal-facing basal plasma membrane (BM) of syncytiotrophoblasts [9]. While GLUT1 expression remains unchanged in the MVM throughout pregnancy, its expression in the BM increases around 2-fold between the 16–22 and 27–30 gestational weeks and remains stable after this period. There is significantly higher GLUT1 expression in MVM syncytiotrophoblasts compared to BM syncytiotrophoblasts [12]. Thanks to its large surface area and high GLUT density, the MVM has a high capacity for glucose transport. This property facilitates the maintenance of a glucose gradient between the syncytiotrophoblast cells and the fetal capillary, which is necessary for net glucose transport to the fetus. In addition, this discrepancy enables the placenta to receive a sufficient amount of energy for its consumption, which corresponds to approximately one-third of total placental glucose uptake [9].

Few studies [13-15] have investigated GLUT1 expression in preeclamptic placentas or its relationship with IUGR. Considering the importance of placental adaptation for fetal and maternal health and that BM GLUT1 levels change considerably during periods coinciding with PE onset, we hypothesized that placental GLUT1 concentrations could be associated with PE or IUGR. Therefore, in this study, we aimed to compare GLUT1 staining intensity in the terminal villi of the placenta (MVM+BM) in healthy subjects and patients with E-PE or L-PE and also, to determine whether there was a relationship between GLUT1 staining intensity and IUGR.

Materials and methods

This case-control study was carried out in our hospital's gynecology and obstetrics clinic, a tertiary center for perinatology cases. The study protocol was approved by the ethics committee of the Non-invasive Clinical Research Ethics Committee of Erciyes University (date: April 7, 2021, decision no: 2021/258). All participants were informed in detail about the purpose(s) of the study, and informed consent forms were obtained before inclusion.

Study population and exclusion criteria

First, placentas in the study group were obtained according to the inclusion/exclusion criteria. Although it was noted that GLUT1 expression did not change during pregnancy in MVM and remained stable after 30 weeks of gestation in BM [12], placentas matching those complicated by PE in terms of the gestational week at birth were included in the control group. Placentas in the control group were obtained from pregnancies without maternal, placental, or fetal pathology and resulted in spontaneous idiopathic preterm or term delivery. PE was diagnosed according to the American College of Obstetrics and Gynecologists (ACOG) 2019 diagnostic criteria for gestational hypertension and PE [1]. Pregnant women with PE who had any of the following characteristics were excluded from the study: a systemic disease diagnosis before pregnancy, any complications other than PE, such as gestational diabetes mellitus or hypertension during pregnancy, smoking and alcohol use, multiple pregnancies, or fetal or placental anomalies. The final PE group was divided into two subgroups, E-PE ($\leq 33^{+6}$ gestational week) and L-PE ($\geq 34^{+0}$ gestational week), according to the gestational week of PE onset. Additionally, patients who had PE with severe features were also determined according to the presence of any of the following: severe blood pressure elevation occurring twice at least 4 h apart (systolic ≥160, diastolic ≥110 mm Hg), new-onset cerebral or visual problems, hepatic abnormality, thrombocytopenia (<100,000 per ul), renal abnormality, or pulmonary edema.

Data collection

At the first visit, a detailed anamnesis of all participants was taken, and physical and obstetric examinations were performed. Maternal age, gravidity, parity, last menstrual period (LMP), and other relevant examination results were recorded. Gestational age was primarily calculated based on the last menstrual dates reported by the participants and was confirmed by crown-rump length (CRL) measurements performed within the first trimester, whereas, in patients who did not know their last menstruation date, CRL measurements were used to calculate the gestational age. Intrauterine growth restriction (IUGR) was defined according to ACOG 2013 diagnostic criteria [16] and confirmed at postpartum.

The maternal body mass index (BMI) values were calculated before delivery. Blood and urine samples were also taken from all participants for the measurement of complete blood count and biochemical and urine analyses. Analysis results were obtained from the hospital automation system. Glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total and direct bilirubin, blood urea nitrogen (BUN), creatinine, urinary protein levels, hemoglobin (Hb), and platelet count (Plt) were recorded.

Gestational week at delivery, type of delivery, APGAR scores (1st and 5th minute), placental weight (PW), and fetal weight (FW) were obtained from newborn cards. The PW/FW ratio was calculated. In patients with PE, we recorded the mean of the last two systolic and diastolic blood pressure measurements before starting magnesium sulfate for eclampsia prophylaxis.

Placental specimen acquisition and preparation

After delivery, the placentas were randomly numbered and transferred to the Pathology Laboratory in a plastic container. All the placentas included in the study were processed in the same manner. The fetal membranes on the surface of the placenta and the umbilical cord at the insertion site were cut off. Superficial fetal vessels were drained from all blood, conjoined blood clots on the maternal surface were removed, and the placentas were weighed (gram) on a calibrated digital device. Horizontal and vertical incisions were made through the placenta to determine macroscopic intraparenchymal pathology, and a total of three tissue samples (1×1×1 cm) were taken from different parts of each placenta. Tissue samples were fixed in 10% formaldehyde for 12–24 h and embedded in paraffin blocks.

Immunohistochemical evaluation

Final tissue sections used for immunohistochemical (IHC) analysis of GLUT1 were prepared similarly to those previously described [17]. Briefly, serial 5-µm thick tissue sections of formalin-fixed, paraffin-embedded tissue samples were used. Tissue sections were deparaffinized twice in xylene (5 min applications), rehydrated through graded ethanol solutions to distilled water, and washed in 1X phosphate buffered saline (PBS; Lonza, Basel, Switzerland). Antigenic determinant or epitope retrieval was carried out by treating the slides in a PT Link (Dako, Santa Clara, CA, USA). Endogenous peroxidase was inhibited with a peroxidase-blocking solution (REAL™ Peroxidase-Blocking Solution; Dako, Denmark) for 15 min, and non-specific binding sites were blocked with an additional protein block (DAKO) for 20 min. Afterward, sections were immunostained with the anti-glucose transporter GLUT1 antibody (ref: ab15309; Abcam, Cambridge, UK) and incubated for 30 min at room temperature. Next, sections were incubated with the secondary antibody (goat anti-rabbit, Ref: P0448, Dako), and the 3,3-diaminobenzidine (DAB; Dako) chromogen was used. Finally, sections were counterstained with hematoxylin (Sigma-Aldrich, San Luis, AZ, USA), dehydrated in alcohol, cleared with xylene, and mounted for analysis. The positive control specimens for the analyses were invasive ductal carcinoma sections (breast tissue). Negative controls were obtained by using blocking serum instead of primary antibody.

Quantitative analysis and scoring of immunohistochemical staining

The staining intensity for GLUT1 was evaluated independently on coded slides by the same pathologist (HA) via light microscopy (Nikon DS-Fi2, Tokyo, Japan). Four different regions (×40 magnification) of each of the three slides prepared from the same placenta were analyzed. The intensity of GLUT1 staining in both the MVM and BM of the syncytial barrier was evaluated by a single pathologist. Staining intensity was determined as absent (0 points), trace (1 point), mild (2 points), moderate (3 points), and intense (4 points). The final score was accepted as the rounded whole number closest to the arithmetic mean of the scores obtained from four different regions of the three specimens obtained from each placenta. Microscopic images showing the presence and absence of GLUT1 staining in the trophoblastic cells of the terminal villi in a preeclamptic placenta are shown in Figure 1.

Statistical analysis

All analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). For the normality check, the Shapiro-Wilk test was used. Data are given as mean (standard deviation [SD]) or median (1st quartile - 3rd quartile) for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables. In the analysis of normally distributed variables, the independent samples t-test was used to compare two groups, and the one-way analysis of variance (ANOVA) was used between more than two-group comparisons. In the analysis of non-normally distributed variables, the Mann-Whitney U test was used to compare two groups, and the Kruskal-Wallis H-test was used to compare more than two groups. To assess directional relationships between continuous variables, Pearson's correlation coefficient was considered for normally distributed variables, and the Spearman correlation coefficient was used for non-normally distributed variables. $P < 0.05$ values were accepted as statistically significant results.

Results

A total of 94 placentas, obtained from 47 women with PE-complicated pregnancy and 47 pregnant women with a well-matched gestational week at delivery (to the PE group), were included in this study. Among the 47 pregnant women with PE, 37 (78.7%) used antenatal magnesium sulfate, and 15 (31.9%) used antenatal corticosteroids. The comparison of the PE group and the controls in terms of maternal demographic characteristics and clinical data are shown in Table 1.

Table 1: Comparison of the control and PE groups in terms of maternal demographic characteristics and clinical/laboratory data.

Parameters	Groups		
	Controls (n = 47)	Preeclampsia (n = 47)	*P*-value
MA (years), median (min - max)	28 (20 - 38)	29 (19 - 41)	**0.035**
Gravidity, median (min - max)	2 (1 - 3)	2 (1 - 4)	0.401
Parity, median (min - max)	1 (0 - 2)	1 (0 - 3)	0.594
M-BMI (kg/m^2), mean (SD)	27.66 (2.57)	30.36 (5.47)	**0.044**
SBP (mmHg), median (min - max)	110 (100 - 120)	160 (150 - 170)	**<0.001**
DBP (mmHg), median (min - max)	70 (60 - 80)	100 (90 - 110)	**<0.001**
Glucose (mg/ dL), median (min - max)	90 (76 - 111)	88 (73 - 108)	0.915
AST (U/L), median (min - max)	19 (15 - 24)	24 (19 - 49)	**0.005**
ALT (U/L), median (min - max)	10.5 (9 - 12)	15 (11 - 40)	**0.006**
LDH (U/L), median (min - max)	406.5 (369.5 - 473)	547 (437 - 754)	**0.001**
T-Bil (mg/dL), median (min - max)	0.55 (0.5 - 0.9)	0.6 (0.5 - 1)	0.562
D-Bil (mg/dL), median (min- max)	0.3 (0.2 - 0.4)	0.3 (0.2 - 0.4)	0.777
Proteinuria, n (%)	0 (0.00%)	47 (100.00%)	**<0.001**
BUN (mg/dL), median (min- max)	9 (7 - 11)	11 (9 - 16)	**0.013**
Creatinine (mg/dL), median (min- max)	0.7 (0.55 - 0.7)	0.8 (0.7 - 1)	**0.002**
Hemoglobin (g/dL), mean (SD)	12.15 (1.18)	12.77 (1.71)	0.092
Plt (×10^3 cells/mm^3), mean (SD)	220.93 (93.14)	211.99 (103.26)	0.740
GW at delivery (weeks), median (min-max)	36 (32 - 39)	36 (32 - 39)	0.967
Type of delivery			
Vaginal, n (%)	40 (85.11%)	18 (38.30%)	**0.001**
Caesarean section, n (%)	7 (14.89%)	29 (61.70%)	
IUGR, n (%)	0 (0.00%)	11 (23.40%)	**0.026**
PW/FW ratio, mean (SD)	0.20 (0.02)	0.20 (0.05)	0.656

P-values in bold indicate statistical significance ($P < 0.05$). MA: maternal age at delivery, M-BMI: maternal body mass index, SBP: Systolic blood pressure, DBP: diastolic blood pressure, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, T-Bil: total bilirubin, D-Bil: direct bilirubin, BUN: blood urea nitrogen, Plt: platelet count, GW: gestational week, IUGR: intrauterine growth retardation, PW/FW ratio: placental weight/fetal weight ratio.

Figure 1: Microscopic images showing lack of GLUT1 staining in the trophoblastic cells of the terminal villi in a preeclamptic placenta (H&E × 200, Scale bar: 5 µm).
A) GLUT-1 score 1: Placenta of a 25-year-old case diagnosed with preeclampsia at 32 weeks of gestation, with normal fetal development and severe preeclampsia features (H&E × 200).
B) GLUT-1 score 2: Placenta of a 29-year-old case diagnosed with preeclampsia at 32 weeks of gestation, with fetal growth retardation and severe preeclampsia features (H&E × 200).
C) GLUT-1 score 3: Placenta of a 31-year-old case diagnosed with preeclampsia at 37 weeks of gestation, with normal fetal development and severe preeclampsia features (H&E × 200).
D) GLUT-1 score 4: Placenta of a 30-year-old case diagnosed with preeclampsia at 36 weeks of gestation, with fetal growth retardation and severe preeclampsia features (H&E × 200).

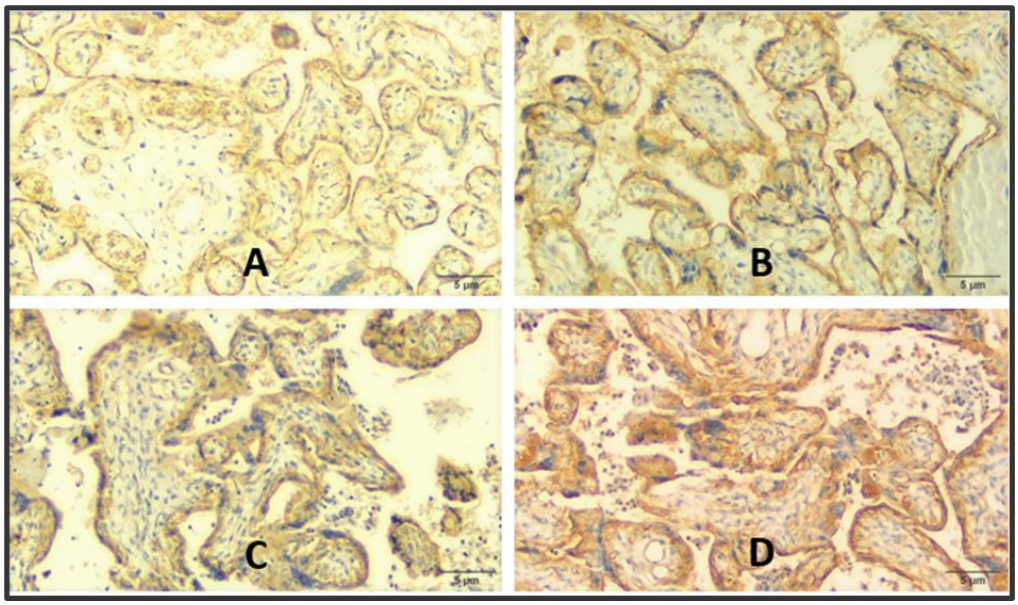


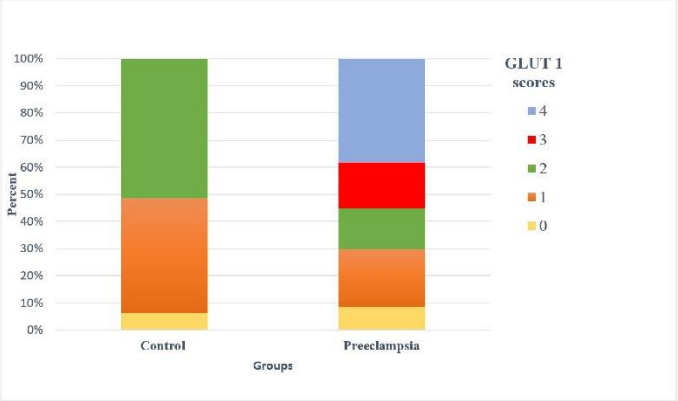
Table 2: Comparison of clinical and laboratory data between the control, E-PE, and L-PE subgroups.

	Control (n = 47) Mean (SD)	E-PE (n = 15) Mean (SD)	L-PE (n = 32) Mean (SD)	Test Statistic	*P*-value
MA (years)[1]	28 (20 - 38)	27 (20 - 39)	29 (19 - 41)	2.384 *	0.100
Gravidity	2.10 (1.17)	2.80 (1.90)	2.88 (2.32)	0.725	0.696
Parity	0.95 (0.89)	1.40 (1.68)	1.31 (1.53)	0.287	0.866
M-BMI (kg/m^2)	27.66 (2.57) [a]	29.01 (5.68) [a,b]	30.99 (5.34) [b]	6.479	**0.039**
SBP (mmHg)	113.00 (9.38)	165.67 (13.48)	158.55 (21.26)	57.520 *	**<0.001**
DBP (mmHg)	67.75 (9.80)	105.33 (13.02) [a]	98.91 (16.10) [a]	39.024	**<0.001**
GLUT1 score[1]	1.45 (0 - 4)	2.47 (0 - 4) [a]	2.72 (0 - 4) [a]	23.720	**<0.001**
Glucose (mg/ dL)	95.05 (32.21)	93.40 (22.82)	97.66 (35.59)	0.028	0.986
AST (U/L)	20.42 (7.27) [a]	184.33 (379.05) [b]	38.34 (39.90) [a,b]	10.397	**0.006**
ALT (U/L)	13.15 (8.73)	107.20 (202.90) [a]	23.88 (26.25) [a]	11.076	**0.004**
LDH (U/L)	464.9 (167.1) [a]	1192.9 (1305.5) [b]	706.5 (612) [a,b]	12.951	**0.002**
T-Bil (mg/dL)	0.69 (0.27)	1.29 (1.98)	0.74 (0.38)	0.155	0.925
D-Bil (mg/dL)	0.50 (0.83)	0.71 (1.58)	0.32 (0.16)	0.609	0.738
BUN (mg/dL)	12.86 (18.71) [a]	14.60 (6.33)	11.75 (4.53) [a]	7.982	**0.018**
Cr (mg/dL)	0.62 (0.19) [a]	0.90 (0.30) [b]	0.79 (0.23) [a,b]	10.043	**0.007**
Hb (g/dL)	12.15 (1.18)	13.02 (1.88)	12.66 (1.65)	1.357 *	0.265
Plt (×10^3/mm^3)	218.23 (99.22)	189.21 (105.96)	222.38 (102.53)	0.564 *	0.571
PW/FW ratio	0.20 (0.02) [a,b]	0.23 (0.06) [a]	0.18 (0.04) [b]	11.293	**0.004**

P-values in bold indicate statistical significance in the three-group comparison. [1] Values are presented as median (min - max). [a,b] Same letters illustrate statistical similarity between the denoted groups in post-hoc comparison(s). * One-way Analysis of Variance (ANOVA), other >2-group comparisons performed with the Kruskal-Wallis H-test. E-PE: early-onset preeclampsia, L-PE: late-onset preeclampsia, MA: maternal age at delivery, M-BMI: Maternal body mass index, SBP: systolic blood pressure, DBP: Diastolic blood pressure, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, T-Bil: total bilirubin, D-Bil: direct bilirubin, BUN: blood urea nitrogen, Cr: creatinine, Hb: hemoglobin, Plt: platelet, PW/FW ratio: placental weight/ fetal weight ratio.

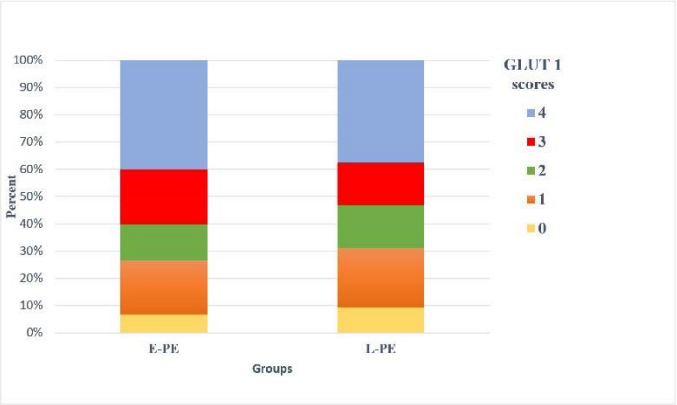
GLUT1 IHC scores were determined to be "0" in 3 (6.38%), "1" in 20 (42.55%), and "2" in 24 (51.06%) of the 47 placentas in the control group. In patients with PE, scores were determined to be "0" in 4 (8.51%), "1" in 10 (21.28%), "2" in 7 (14.89%), "3" in eight (17.02%) and "4" in 18 (38.3%) of the 47 placentas. GLUT1 scores were found to be significantly higher in the PE group compared to those with uneventful pregnancies ($P < 0.001$) (Figure 2).

Figure 2: Distribution of placental GLUT1 scores with regard to the control and preeclampsia groups.



PE was early-onset in 15 (31.9%) and late-onset in 32 (68.1%) of the 47 pregnant women in the PE group. Both antenatal magnesium sulfate and corticosteroid prophylaxis were applied to all the 15 pregnant women (100%) in the E-PE subgroup. In the L-EP subgroup, 22 (68.75%) of 32 pregnant women had received antenatal magnesium sulfate prophylaxis, while none had received antenatal corticosteroids. The frequency of those who received antenatal magnesium sulfate prophylaxis was significantly higher in the E-PE subgroup than in the L-PE subgroup ($P < 0.001$). GLUT1 IHC scores were determined to be "0" in 1 (6.67%), "1" in 3 (20%), "2" in 2 (13.33%), "3" in 3 (20%), and "4" in 6 (40%) of the 15 placentas in the E-PE subgroup. In patients with L-PE, scores were determined to be "0" in 3 (9.38%), "1" in 7 (21.88%), "2" in 5 (15.63%), "3" in 5 (15.63%), and "4" in 12 (37.5%) of the 32 placentas. GLUT1 scores were significantly higher in both the early-onset and late-onset PE subgroups compared to the control group ($P = 0.001$ and $P < 0.001$, respectively). The comparison of data between the control, E-PE and L-PE groups is shown in Table 2. However, there was no significant difference in GLUT-1 scores between the E-PE and L-PE subgroups ($P = 0.792$) (Figure 3).

Figure 3: Distribution of placental GLUT1 scores with regard to the early-onset and late-onset preeclampsia subgroups.



PE with severe features was identified in 37 (78.72) of the 47 PE patients, including all 15 (100%) women in the E-PE subgroup and 22 (68.8%) of the 32 pregnant women in the L-PE subgroup. The frequency of PE with severe features was significantly higher in the E-PE subgroup than in the L-PE subgroup (P = 0.019). GLUT1 scores were similar in placentas obtained from pregnant women with PE with and without severe features (P = 0.126). However, GLUT1 scores were significantly higher in placentas obtained from pregnant women with L-PE with severe features than in pregnant women without severe features (P = 0.039).

In our study, while IUGR was not found in any cases in the control group, IUGR was present in 11 (23.4%) of 47 pregnant women with PE, including eight (53.3%) of the 15 pregnant women with E-PE and three (9.38%) of the 32 pregnant women with L-PE. The frequency of IUGR in pregnant women with E-PE was significantly higher than in pregnant women with L-PE (P < 0.001). GLUT1 scores were similar in placentas obtained from pregnant women who had PE with and without IUGR (P = 0.756).

Finally, in each of the groups (including the E-PE and L-PE subgroups), the directional relationships between GLUT1 scores and other continuous variables, including parity, maternal age, BMI, systolic and diastolic blood pressure, blood glucose, AST, ALT, LDH, BUN, creatinine, total and direct bilirubin, hemoglobin, platelet levels, 1st and 5th minute APGAR scores, and the PW/FW ratios, were evaluated and compared. GLUT1 scores demonstrated moderate inverse correlations between maternal BMI in the L-PE subgroup (r = -0.377, P = 0.033) and moderate positive correlations with PW/FW ratio (r = 0.444, P = 0.011). A statistically significant moderate positive correlation was found between GLUT1 scores and hemoglobin levels in the PE group and the L-PE subgroup (r = 0.397, P = 0.006 and r=0.395, P = 0.025, respectively).

Discussion

In this study, the intensity of IHC staining for GLUT1 in trophoblastic cells of the terminal villi was significantly higher in PE placentas compared to the placentas of the control group, but scores were similar in the E-PE and L-PE subgroups. IHC staining intensity was similar between those with and without severe PE features in the PE group and those without IUGR; however, it was significantly higher among those with severe PE features in the L-PE group compared to those without severe PE features.

Although the pathogenesis of PE is not fully understood, the current paradigm focuses on placental malperfusion [6] and oxidative stress in trophoblasts that form the epithelial lining of placental villi in direct contact with maternal blood [18, 19]. In the perfused human placenta model, it is stated that placental glucose transport is reduced when maternal blood flow is reduced [20], but there is little reduction in glucose transfer on the fetal side until fetal blood flow rate reduces to ~100 mL/min from the maximum rate of 300 mL/min [12]. Oxidative stress has been shown to downregulate GLUT1 transcription in the human placenta, resulting in reduced glucose uptake [21], and also, it was determined to reduce glucose accumulation in syncytiotrophoblasts due to increased transepithelial permeability – without a change in the mRNA expression of GLUT1 [22]. On the other hand, it was found that hypoxia caused a significant increase in phosphofructokinase activity, one of the key enzymes of glycolysis, in the trophoblast cell line (BeWo cells), and this was associated with a significant increase in GLUT1 transcript levels [23]. *In vitro* studies have also demonstrated that hypoxia can upregulate placental glucose transporter expression via HIF-1 [24-26].

In a study by Luscher et al. [13] in which placentas were analyzed by Western blotting, it was found that, compared to normal term placentas, GLUT1 expression in preeclamptic placentas decreased by 60% in the MVM while remaining unchanged in the BM, and total membrane isolation showed a significant upregulation. In our study, the intensity of IHC staining for GLUT1 in trophoblastic cells in terminal villi (MVM+BM) was significantly higher in PE placentas compared to control placentas. Adaptation to new conditions, such as placental malperfusion and hypoxia, in PE is essential for the placenta. Considering the villous changes associated with maternal malperfusion in PE, such as distal villous hypoplasia and villous infarctions, it is not surprising that GLUT1 density is increased in the remaining villi to provide adequate glucose for the placenta and the fetus.

As established previously, E-PE is predominantly associated with defective placentation, while L-EP appears to be driven by oxidative changes in the placenta induced by a discrepancy between maternal perfusion and fetoplacental demands, particularly in the presence of maternal predisposition to cardiovascular or metabolic disease [4]. Systemic PE symptoms develop due to ischemic damage to maternal organ systems caused by soluble antiangiogenic agents released from the hypoxic placenta [27]. In a study by Dubova et al. [15], which also used IHC staining, placentas were grouped as controls, moderate PE, severe PE, and severe PE combined with IUGR. The authors found that GLUT1 syncytial expression in the terminal villi of severe PE cases (both with and without IUGR) was significantly lower than in controls. In our study, GLUT1 staining intensity in the terminal villi of PE placentas was similar between the E-PE and L-PE subgroups and those with and without severe PE features. However, GLUT1 staining intensity was significantly higher in those with severe PE features in the L-EP group than those without, and values demonstrated a moderate inverse correlation with maternal BMI. Therefore, our findings suggest that increased GLUT1 levels in the terminal villi of preeclamptic placentas may be associated

with placental and maternal changes resulting from PE rather than the development and onset of PE. In addition, GLUT1 scores were inversely correlated with maternal BMI in the L-PE subgroup. This suggests that GLUT1 upregulation in the terminal villi of the placenta in L-PE may be associated with obesity-related maternal metabolic factors.

Fetal growth depends on nutrient availability, which in turn is related to various factors, including maternal diet, uteroplacental blood supply, placental villous development, and the capacity of the villous trophoblast and fetoplacental circulation to transport these nutrients [28]. Therefore, a well-functioning placenta is essential for favorable fetal growth, and previous studies have suggested a placental weight-to-fetal weight (PW/FW) ratio as an appropriate indicator of placental function [29].

The pathophysiologies of placental-derived IUGR and PE are very similar, and placental damage is more severe in those with IUGR-related PE than those with IUGR alone, consistent with the greater degree of maternal vasculopathy [28]. In a study by Pribadi et al. [14], in which GLUT1 levels in placentas complicated with PE were measured by ELISA, it was found that GLUT1 levels were lower in the smaller-for-gestational-age (SGA) group than in the non-SGA group and showed a positive correlation with birth weight in the E-PE group. In the study by Dubova et al. [15], it was reported that GLUT1 syncytial expression in the terminal villi was significantly lower in both the severe PE and the severe PE combined with IUGR groups when compared to healthy controls, while the PW/FW ratio was similar to control group values in all three PE subgroups (moderate PE, severe PE and severe PE combined with IUGR). However, no GLUT1 expression comparisons were made between those with and without IUGR in the severe PE group. In a recent study by Shen et al. [30], researchers found that blood pressure (one of the general measures of maternal disease severity) and chronic villitis (determined in placental pathology) had a relationship with IUGR in patients with E-PE.

The former had a negative correlation, while the latter showed a positive correlation with IUGR [30]. In our study, unlike Pribadi et al. [14], GLUT1 staining in terminal villi in PE placentas was similar in patients with and without IUGR. The PW/FW ratio was similar between the PE and control groups, in line with Dubova et al.'s [15] results. In addition, there was a moderate positive correlation between GLUT1 levels and PW/FW ratio in the L-PE subgroup. Although insufficient placental glucose transport has been considered a pathophysiological mechanism in IUGR, it has been shown that GLUT1 intensities do not change in placentas with term and preterm IUGR [31] and that the GLUT1 expressions of the MVM and BM (and resultant D-glucose uptake) are not affected by IUGR [32]. In an *in vivo* study involving human term pregnancies, uterine and umbilical blood flow was determined by Doppler ultrasonography and glucose, and insulin levels were measured in the maternal radial artery, uterine vein, and umbilical artery and vein. The researchers also measured GLUT1 expression in isolated syncytiotrophoblasts from the BM and MVM. This study determined that fetal and placental glucose consumption were inversely proportional, but neither was associated with placental GLUT1 expression.

Additionally, it was stated that fetal glucose consumption was balanced against placental glucose requirements, and placental glucose consumption was a key modulator of maternal-fetal glucose transfer [33]. Of note, previous studies have shown that placenta-mediated fetal growth restriction occurs through chronic fetal hypoxia owing to poor placental perfusion [34]. Although our findings suggest that GLUT1 expression in terminal villi is not one of the main factors in the development of PE-related IUGR, it is clear that more research is needed to determine whether the GLUT1 changes observed in PE-exposed placentas have a role in the development of IUGR.

Limitations

The main limitations of the current study are that we did not determine GLUT1 staining intensities in MVM and BM separately and that we did not stain for other GLUT types. In addition, while we investigated relationships between GLUT1 expression and IUGR in PE-afflicted placentas, we did not include placentas obtained from cases with placenta-related IUGR, which PE did not complicate. This can also be cited as another limitation of the study. Finally, the low number of cases with IUGR may have reduced the study's statistical power with respect to this subgroup analysis.

Conclusion

The intensity of IHC staining for GLUT1 in trophoblastic cells in terminal villi was significantly higher in PE placentas compared to control-group placentas, but values were similar between the E-PE and L-PE subgroups. Furthermore, in the PE group, GLUT1 staining was similar among those with and without severe features and those with and without IUGR. These results suggest that the changes in GLUT1 expression result from placental adaptation to the new environment caused by PE rather than having a causative relationship. Similarly, it appears that GLUT1 expression is not a primary factor contributing to PE-related IUGR development. Nonetheless, the results of this study demonstrate the need for further research to determine whether GLUT1 expression contributes to PE development and/or its relationship with IUGR in PE-afflicted placentas.

References

1. ACOG Practice Bulletin No. 202: Gestational hypertension and preeclampsia. Obstet Gynecol. 2019;133(1):e1-25. doi: 10.1097/aog.0000000000003018.
2. Lo JO, Mission JF, Caughey AB. Hypertensive disease of pregnancy and maternal mortality. Curr Opin Obstet Gynecol. 2013;25(2):124-32. doi: 10.1097/GCO.0b013e32835e0ef5.
3. Von Dadelszen P, Magee LA, Roberts JM. Subclassification of preeclampsia. Hypertens Pregnancy. 2003;22(2):143-8. doi: 10.1081/prg-120021060.
4. Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. BMJ. 2019;366:l2381. doi: 10.1136/bmj.l2381.
5. Tranquilli AL, Brown MA, Zeeman GG, Dekker G, Sibai BM. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Pregnancy Hypertens. 2013;3(1):44-7. doi: 10.1016/j.preghy.2012.11.001.
6. Staff AC, Redman CW. The differences between early-and late-onset preeclampsia. Preeclampsia: Springer; 2018. pp. 157-172.
7. Kalhan S, Parimi P. Gluconeogenesis in the fetus and neonate. Semin Perinatol. 2000;24(2):94-106. doi: 10.1053/sp.2000.6360.
8. Stanirowski PJ, Lipa M, Bomba-Opoń D, Wielgoś M. Expression of placental glucose transporter proteins in pregnancies complicated by fetal growth disorders. Adv Protein Chem Struct Biol. 2021;123:95-131. doi: 10.1016/bs.apcsb.2019.12.003.
9. Lager S, Powell TL. Regulation of nutrient transport across the placenta. J Pregnancy. 2012;2012:179827. doi: 10.1155/2012/179827.
10. Huang X, Anderle P, Hostettler L, Baumann MU, Surbek DV, Ontsouka EC, et al. Identification of placental nutrient transporters associated with intrauterine growth restriction and preeclampsia. BMC Genomics. 2018;19(1):173. doi: 10.1186/s12864-018-4518-z.
11. Stanirowski PJ, Szukiewicz D, Pazura-Turowska M, Sawicki W, Cendrowski K. Placental expression of glucose transporter proteins in pregnancies complicated by gestational and pregestational diabetes mellitus. Can J Diabetes. 2018;42(2):209-17. doi: 10.1016/j.jcjd.2017.04.008.

12. Illsley NP, Baumann MU. Human placental glucose transport in fetoplacental growth and metabolism. Biochim Biophys Acta Mol Basis Dis. 2020;1866(2):165359. doi: 10.1016/j.bbadis.2018.12.010.
13. Lüscher BP, Marini C, Joerger-Messerli MS, Huang X, Hediger MA, Albrecht C, et al. Placental glucose transporter (GLUT)-1 is down-regulated in preeclampsia. Placenta. 2017;55:94-9. doi: 10.1016/j.placenta.2017.04.023.
14. Pribadi A, Mose JC, Achmad TH, Anwar AD. Reduced birth weight in early-onset preeclampsia might potentially be due to placental glucose transporters disorders. J Med Sci. 2020;20(1):24-8.
15. Dubova EA, Pavlov KA, Kulikova GV, Shchegolev AI, Sukhikh GT. Glucose transporters expression in the placental terminal villi of preeclampsia and intrauterine growth retardation complicated pregnancies. Health. 2013;5(7D):100-4. doi:10.4236/health.2013.57A4014
16. ACOG Practice bulletin no. 134: fetal growth restriction. Obstet Gynecol. 2013;121(5):1122-33. doi: 10.1097/01.AOG.0000429658.85846.f9.
17. Stanirowski PJ, Szukiewicz D, Pyzlak M, Abdalla N, Sawicki W, Cendrowski K. Impact of pre-gestational and gestational diabetes mellitus on the expression of glucose transporters GLUT-1, GLUT-4 and GLUT-9 in human term placenta. Endocrine. 2017;55(3):799-808. doi: 10.1007/s12020-016-1202-4.
18. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta. 2009;30(6):473-82. doi: 10.1016/j.placenta.2009.02.009.
19. Vangrieken P, Vanterpool SF, Van Schooten FJ, Al-Nasiry S, Andriessen P, Degreef E, et al. Histological villous maturation in placentas of complicated pregnancies. Histol Histopathol. 2020;35(8):849-62. doi: 10.14670/hh-18-205.
20. Illsley NP, Hall S, Stacey T. The modulation of glucose transfer across the human placenta by intervillous flow rates: an in vitro perfusion study. Cellular Biology and Pharmacology of the Placenta: Springer; 1987. pp. 535-544.
21. Lappas M, Andrikopoulos S, Permezel M. Hypoxanthine-xanthine oxidase down-regulates GLUT1 transcription via SIRT1 resulting in decreased glucose uptake in human placenta. J Endocrinol. 2012;213(1):49-57. doi: 10.1530/joe-11-0355.
22. Araújo JR, Pereira AC, Correia-Branco A, Keating E, Martel F. Oxidative stress induced by tert-butylhydroperoxide interferes with the placental transport of glucose: in vitro studies with BeWo cells. Eur J Pharmacol. 2013;720(1-3):218-26.
23. Vangrieken P, Al-Nasiry S, Bast A, Leermakers PA, Tulen CBM, Janssen GMJ, et al. Hypoxia-induced mitochondrial abnormalities in cells of the placenta. PLoS One. 2021;16(1):e0245155. doi: 10.1371/journal.pone.0245155.
24. Esterman A, Greco MA, Mitani Y, Finlay TH, Ismail-Beigi F, Dancis J. The effect of hypoxia on human trophoblast in culture: morphology, glucose transport and metabolism. Placenta. 1997;18(2-3):129-36. doi: 10.1016/s0143-4004(97)90084-9.
25. Hayashi M, Sakata M, Takeda T, Yamamoto T, Okamoto Y, Sawada K, et al. Induction of glucose transporter 1 expression through hypoxia-inducible factor 1alpha under hypoxic conditions in trophoblast-derived cells. J Endocrinol. 2004;183(1):145-54. doi: 10.1677/joe.1.05599.
26. Baumann MU, Zamudio S, Illsley NP. Hypoxic upregulation of glucose transporters in BeWo choriocarcinoma cells is mediated by hypoxia-inducible factor-1. Am J Physiol Cell Physiol. 2007;293(1):C477-85. doi: 10.1152/ajpcell.00075.2007.
27. Loussert L, Vidal F, Parant O, Hamdi SM, Vayssiere C, Guerby P. Aspirin for prevention of preeclampsia and fetal growth restriction. Prenat Diagn. 2020;40(5):519-27. doi: 10.1002/pd.5645.
28. Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. Am J Obstet Gynecol. 2018;218(2s):S745-61. doi: 10.1016/j.ajog.2017.11.577.
29. Hayward CE, Lean S, Sibley CP, Jones RL, Wareing M, Greenwood SL, et al. Placental adaptation: What can we learn from birthweight:placental weight ratio? Front Physiol. 2016;7:28. doi: 10.3389/fphys.2016.00028.
30. Shen H, Zhao X, Li J, Chen Y, Liu Y, Wang Y, et al. Severe early-onset PE with or without FGR in Chinese women. Placenta. 2020;101:108-14. doi: 10.1016/j.placenta.2020.09.009.
31. Jansson T, Wennergren M, Illsley NP. Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. J Clin Endocrinol Metab. 1993;77(6):1554-62. doi: 10.1210/jcem.77.6.8263141.
32. Jansson T, Ylvén K, Wennergren M, Powell TL. Glucose transport and system A activity in syncytiotrophoblast microvillous and basal plasma membranes in intrauterine growth restriction. Placenta. 2002;23(5):392-9. doi: 10.1053/plac.2002.0826.
33. Michelsen TM, Holme AM, Holm MB, Roland MC, Haugen G, Powell TL, et al. Uteroplacental glucose uptake and fetal glucose consumption: a quantitative study in human pregnancies. J Clin Endocrinol Metab. 2019;104(3):873-82. doi: 10.1210/jc.2018-01154.
34. Zur RL, Kingdom JC, Parks WT, Hobson SR. The placental basis of fetal growth restriction. Obstet Gynecol Clin North Am. 2020;47(1):81-98. doi: 10.1016/j.ogc.2019.10.008.

The National Library of Medicine (NLM) citation style guide has been used in this paper.