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GLUT-9 EXPRESSION IN HUMAN TERM PLACENTAS OF DIET-REGULATED GESTATIONAL DIABETES PREGNANCIES

ANDREA DAGELIĆ[1], IVANA KUZMIĆ PRUSAC[2], DAMIR ROJE[3], SANA SIMICEVIC[4], SANDRA ZEKIĆ TOMAŠ[2]

Aim: Among the 14 identified GLUT isoforms, GLUT-1, GLUT-4 and GLUT-9 are considered crucial for placental glucose transport. GLUT-9, one of the least investigated isoforms capable of transporting both glucose and fructose, is expressed in the syncytiotrophoblast and vascular endothelium. Since gestational diabetes mellitus (GDM) significantly affects both maternal and fetal health, this study aimed to analyse immunohistochemical expression of GLUT-9 in various placental compartments.

Methods: Placental samples were obtained from women with diet-regulated GDM (n=13) and healthy controls (n=11). To our knowledge, this is the first study to assess GLUT-9 expression separately in decidual cells (DC), villous trophoblast (VTB), extravillous trophoblast (EVTB), and vasculosyncytial membranes (VSM). Immunohistochemistry was performed using an anti-GLUT-9 antibody, and expression was evaluated using the HSCORE method by two blinded perinatal pathologists.

Results: There were no statistically significant differences in maternal age, gestational age, fetal weight, neonatal sex, or placental weight between the groups. Likewise, no significant differences were observed in GLUT-9 expression in DC (P=0.5620), EVTB (P=0.5620), VTB (P=0.1268), or VSM (P=0.2619).

Conclusions: The results of the study indicate that GLUT-9 expression does not differ between pregnant women with diet-controlled GDM and healthy pregnant women. Therefore, it can be concluded that GLUT-9 does not play a significant role in glucose transport in pregnancies that do not require insulin therapy; however, given the small sample size, further studies are needed.

Keywords: GESTATIONAL DIABETES MELLITUS, GLUCOSE TRANSPORTER, GLUT-9, PLACENTA, IMMUNOHISTOCHEMISTRY

Introduction

A multitude of complications during pregnancy classifies women with diabetes and their offspring as a high-risk obstetric and neonatal group. The syncytiotrophoblast surface is increased, and the villi are hypervascularized, resulting in an enlarged feto-placental endothelial surface in diabetic placentas (1). The apical plasma membrane of the syncytium faces the maternal intervillous space, whereas the basal plasma membrane faces the endothelium of the foetal capillaries. This structure constitutes the principal barrier to maternal-foetal exchange of water, nutrients, and electrolytes. A high density of GLUTs in the maternal-facing microvillous membrane (MVM), together with its large surface area, facilitates rapid glucose uptake into the syncytiotrophoblast. In contrast, GLUT expression is much lower in the foetal-facing basal membrane (BM) than in the MVM. This lower BM expression, combined with the smaller surface area, has led to the proposal that BM transport represents the rate-limiting step in placental glucose transfer (2). GLUT9, predominantly expressed in the human kidneys and liver, is one of the least well-characterised glucose transporters identified in the placenta (3). It exists in two splice variants, GLUT9a and GLUT9b, both of which function as glucose and fructose transporters (4, 5). Bibee et al. demonstrated that GLUT9 isoform expression varies considerably depending on its localisation within the syncytium. GLUT9a predominates in the cytoplasm and BM and is also present in the vascular endothelium, whereas GLUT9b is more common in the MVM. GLUT9a expression in the BM was significantly higher in all diabetes groups compared with controls and was also more prominent in the MVM. In contrast, GLUT9b predominated in both the BM and MVM only in women with insulin-dependent diabetes (6).

In one of the most recent studies, the presence of GLUT9 in the trophoblast was confirmed using computed morphometry. Consistent with previous findings, a significant increase in transporter expression was observed in placentas from patients with insulin-dependent diabetes (7).

[1]Department of Obstetrics and Gynaecology, Split University Hospital Centre
[2]Pathology Department, Split University Hospital Centre
[3]Policlinic Hormona
[4]Norra Älvsborgs Länssjukhus, Trollhättan, Sweeden

Corresponding author:
Andrea Dagelić, MD. PhD.
Department of Obstetrics and Gynecology, Split University Hospital Centre
21000 Split, Spinčićeva 1, Croatia
E-mail: andrea.dagelic@gmail.com

Subjects and methods

The placental samples were obtained from 24 women after vaginal or caesarean delivery at the Department of Obstetrics and Gynaecology, Split University Hospital Centre. The inclusion criteria were as follows: maternal age >18, singleton pregnancy, and gestational age of >37 weeks. Exclusion criteria were foetal malformations, intrauterine foetal growth restriction, maternal chronic or pregnancy-induced hypertension, and maternal or fetal inflammatory response. The placentas were divided into two groups: a study group comprising women with diet-regulated GDM (13 placentas) and a control group (11 placentas) comprising women with uncomplicated pregnancies. GDM was diagnosed based on the 75 g oral glucose tolerance test (OGTT) performed between 24 and 28 gestational weeks, in accordance with the criteria defined by the Hyperglycaemia and Adverse Pregnancy Outcome study (8). Placentas were collected within 20 minutes of delivery, fixed in 10% buffered formalin, and sent to the Pathology Department for further analysis. For the study, one full-thickness placental section was taken from a macroscopically normal placental disc, close to the umbilical cord insertion. All placental samples were examined by two perinatal pathologists blinded to the assigned clinical category. Immunostaining was performed on the same serial section of each placental sample, as follows: paraffin sections were mounted on Superfrost slides (Thermo Scientific, Dreieich, Germany) and processed in an automatic stainer (Ventana BenchMark Ultra autostainer, Ventana Roche, Tucson, AZ). For the detection of GLUT-9, a primary "ready-to-use" polyclonal rabbit antibody (ab104623, Abcam, USA, dilution 1:100) was applied. The Ultra View Universal DAB Detection Kit (RRID: AB_2753116, Ventana, Tucson, Arizona, USA) was used as a secondary antibody. Brown staining of the cell membrane and/or cytoplasm was considered positive. Endothelial cells of fetal blood vessels within chorionic villi served as a positive control. Expression of GLUT-9 was determined separately for DC, VTB, EVTB, and VSM by the HSCORE method using the equation: HSCORE = Pi(i+1), where is the intensity of staining, with a value of 1 (weak), 2 (moderate), or 3 (strong), and Pi is the percentage of stained trophoblast cells of each intensity (9). All measurements were performed manually using a ×40 objective (Olympus BX41 microscope). Each placental sample was analysed throughout 10 high-power fields (HPFs).

Table 1.
Comparison of maternal, fetal, and placental characteristics between study groups

	Diabetic group n=13	Control group n=11	P
Maternal age (years)	33 (22-43)	30 (20-36)	NS*
Gestational age (weeks)	38 (37-41)	38 (37-40)	NS*
Fetal weight (g)	3670 (2660-5140)	3350 (2210-3710)	NS*
Gender (male/female)	8/5	3/8	NS**
Placental weight (g)	587 (428-902)	537 (413-620)	NS*
DC	4 (3,4-4)	4 (3,5-4)	NS*
EVTB	4 (3,4-4)	4 (3,5-4)	NS*
VTB	3,4 (2,4-4)	3,9 (3,1-4)	NS*
VSM	3,6 (2,4-4)	3,9(3,1-4)	NS*

*Mann-Whitney U test, presented as median with highest and lowest values
**Chi-square test

Results

There was no statistically significant difference in maternal age (Z=1.565; P=0.1177), gestational age (Z=0.148; P=0.8821), fetal birth weight (Z=1.448; P=0.1475), neonatal gender (χ^2=2.701; DF=1; P=0.1003), or placental weight (Z=0.782; P=0.4341). Expression of GLUT-9 was determined separately for DC, VTB, EVTB, and VSM by the HSCORE method described previously. There was also no statistically significant difference in GLUT-9 immunohistochemical expression in DC (Z=0.580; P=0.5620), EVTB (Z=0.580; P=0.5620), VTB (Z=1.527; P=0.1268), or VSM (Z=1.122; P=0.2619) (Table 1).

Discussion

Although many authors (1, 10, 11) have found that women with gestational diabetes have newborns with higher fetal and placental weight, no such differences were observed in our study. One possible explanation is that 84% of women with gestational diabetes were hospitalised during pregnancy in our hospital; they were under a strict protocol, their blood glucose levels were measured regularly, which probably increased their awareness of the problem, and they strictly followed their diet. Likewise, labour was induced before completion of the 40th week (12). Women with GDM were older than those in the control group, as older age has been described as one of the risk factors for GDM (13). Male neonates were more frequently born to GDM mothers, as described by Dagelic et al., but with no statistical significance (12). Unlike Stanirowski et al., who used morphometric software to assess immunostaining, we used the HSCORE method, performed twice by two independent perinatal pathologists (7). Expression of GLUT-9 was determined separately for decidual cells, villous trophoblast, extravillous trophoblast, and vasculosyncytial membranes, which has not been reported previously, to our knowledge. There was no statistically significant difference between diet-regulated GDM pregnancies and healthy control groups; these findings are in accordance with the research by Stanirowski et al. (7).

Conclusion

Our findings suggest that GLUT9 does not play a significant role in placental glucose uptake if there is no need for insulin treatment of GDM and if it can be managed by diet alone, as described by Stanirowski et al. (7). Proper follow-up, diet control, and earlier pregnancy

termination minimize the risks of macrosomia, increased placental weight, and increased expression of GLUT-9 in diet-regulated GDM.

Abbreviations:

GDM - Gestational diabetes mellitus
DC - Decidual cells
VTB - Villous trophoblast
EVTB - Extravillous trophoblast
VSM - Vasculosyncytial membranes
MVM - Microvillous membrane
BM - Basal membrane
OGTT - Oral glucose tolerance test

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Sažetak

EKSPRESIJA GLUT-9 U POSTELJICAMA TRUDNICA S DIJETOM REGULIRANIM GESTACIJSKIM DIJABETESOM

Andrea Dagelić, Ivana Kuzmić Prusac, Damir Roje, Sana Simicevic, Sandra Zekić Tomaš

Cilj: GLUT-9 je jedan od manje proučenih transportera glukoze i fruktoze prisutan u sinciciotrofoblastu i endotelu posteljice. Cilj ovog istraživanja bio je analizirati imunohistokemijsku ekspresiju GLUT-9 u različitim strukturama posteljice trudnica s dijetom reguliranim gestacijskim dijabetesom (GDM).

Metode: U studiju je uključeno 13 posteljica trudnica s dijetom reguliranim GDM-om i 11 kontrolnih posteljica. Ekspresija GLUT-9 procijenjena je korištenjem HSCORE metode u decidualnim stanicama (DC), viloznom trofoblastu (VTB), ekstraviloznom trofoblastu (EVTB) i vaskulosincicijalnim membranama (VSM). Analizu su provela dva neovisna perinatalna patologa.

Rezultati: Nije pronađena statistički značajna razlika između skupina u demografskim i kliničkim parametrima, kao ni u ekspresiji GLUT-9 u DC, EVTB, VTB ili VSM.

Zaključak: Rezultati istraživanja ukazuju na to da se ekspresija GLUT-9 ne razlikuje između trudnica s dijetom reguliranim GDM-om i zdravih trudnica. Time e može zaključiti da GLUT-9 nema značajnu ulogu u prijenosu glukoze u trudnoćama koje ne zahtijevaju inzulinsku terapiju, ali, s obzirom na mali uzorak, potrebna su daljnja istraživanja.

Ključne riječi: GESTACIJSKI DIJABETES, GLUT-9, POSTELJICA, TRANSPORT GLUKOZE, IMUNOHISTOKEMIJA

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