Expression of intercellular adhesion molecule-1 in umbilical vascular of pregnant women with gestational diabetes mellitus and the clinical significance

FEI TENG[1], JINFANG WU[2], MIN WEI[1] and YONGXIU YANG[1]

[1]Department of Obstetrics and Gynecology, The First Hospital of Lanzhou University, Lanzhou, Gansu 730000;
[2]Department of Obstetrics and Gynecology, The Second Affiliated Hospital of
Xi'an Jiaotong University, Xi'an, Shaanxi 710004, P.R. China

Abstract. The purpose of this study was to investigate the expression of intercellular adhesion molecule-1 (ICAM-1) in umbilical vascular of pregnant women with gestational diabetes mellitus (GDM) and the clinical significance. A total of 103 pregnant women with GDM were selected in the First Hospital of Lanzhou University and the Second Affiliated Hospital of Xi'an Jiaotong University from January 2016 to December 2016 as GDM group. At the same time, 106 normal pregnant women were selected as control group. i) General information of the two groups of pregnant women including age, gestational age, gravida, parity, BMI, systolic blood pressure and diastolic blood pressure were compared; ii) the laboratory indicators of the two groups of pregnant women including fasting blood glucose, glycosylated hemoglobin (HbA1c), umbilical cord arterial pH, partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2) in umbilical artery were compared; iii) expression of ICAM-1 in umbilical vascular was detected by immunohistochemistry; iv) expression levels of ICAM-1 in umbilical vascular of the two groups of patients were compared. i) There was no significant difference in the age, smoking, gestational age, gravida, parity, BMI, systolic blood pressure and diastolic blood pressure between the two groups (p>0.05); ii) no significant differences in HbA1c, umbilical cord arterial pH, pO_2 and pCO_2 were found between the groups (p>0.05); iii) ICAM-1 was expressed in umbilical vessels of both groups of pregnant women; iv) no significant differences in expression levels of ICAM-1 in umbilical artery and umbilical vein endothelial cells were found between the groups (p>0.05). Therefore, GDM patients with good blood glucose control have no umbilical cord endothelial cell damage.

Introduction

Gestational diabetes mellitus (GDM) refers to the varying degree of abnormal glucose metabolism observed during pregnancy (1). GDM does not include diabetes existed before pregnancy. GDM can lead to macrosomia, oligohydramnios, premature birth and other complications. GDM can also cause maternal postpartum metabolic syndrome, offspring cognitive decline, abnormal glucose metabolism and other far-reaching effects. Although the pathogenesis of GDM is still unclear, genetic and environmental factors have significant effect on the development of this disease. It has been proved that insulin resistance, which can be aggregated by immune-induced chronic inflammation, is the main mechanism of GDM (2). A variety of inflammatory factors were also found to be involved in the occurrence and development of GDM (3). Vascular endothelial cells can produce a variety of inflammatory factors to participate in inflammatory defense response, such as intercellular adhesion molecule-1 (ICAM-1) and adiponectin (LPS) (4). ICAM-1 is believed to be an important indicator of endothelial dysfunction (5). Therefore, in this study, expression of ICAM-1 in umbilical artery and vein of both GDM patients and normal pregnant women was detected to explore whether GDM can induce changes in the function of umbilical cord vascular endothelium.

Patients and methods

Patients. In the GDM group were GDM patients selected in the First Hospital of Lanzhou University and the Second Affiliated Hospital of Xi'an Jiaotong University from January 2016 to December 2016. At the same time, 106 normal pregnant women were also selected to serve as control group. The study was approved by the Ethics Committee of our institute and all pregnant women or their families signed informed consent. Exclusion criteria: Pregnant women with liver and kidney dysfunctions or other complications were excluded. General

Correspondence to: Dr Yongxiu Yang, Department of Obstetrics and Gynecology, The First Hospital of Lanzhou University, 1 Donggang Xi Road, Chengguan, Lanzhou, Gansu 730000, P.R. China
E-mail: yongxiuy17@163.com

information of the groups of pregnant women (age, gestational age, gravida, parity, BMI, systolic blood pressure and diastolic blood pressure) are given in Table I. In GDM group, one case received insulin treatment and other patients were subjected to diet control and exercise therapy. GDM was diagnosed according to the recommended guidelines of the diagnosis of GDM in China established in 2014: Patients were subjected to 75 g oral glucose tolerance test (OGTT). Participants were subjected to normal diet for three days, followed by fasting for 8 h before test. Then participants were asked to orally intake 300 ml liquid containing 75 g glucose. Blood glucose level in venous blood was measured before and 1 and 2 h after the oral intake of glucose. Normal values of OGTT 0 h, OGTT 1 h and OGTT 2 h were 5.1, 10.0 and 8.5 mmol/l, respectively (1 mmol/l≈18 mg/dl). GDM was diagnosed if and higher value was detected.

Methods. Fasting peripheral venous blood (2 ml) was extracted before birth to measure the levels of glycosylated hemoglobin (HbA1c). Umbilical vessels were collected during labor stage for blood gas analysis to record pH, pO_2 and pCO_2.

After birth, umbilical cord tissue (1 ml) was collected at the position 5 cm away from placenta and washed with saline. Expression of ICAM-1 in umbilical cord blood vessels of two groups was detected by immunohistochemistry. Specific steps: Fixation; dehydration; transparency; oozing wax; embedding; slicing; dewaxing; hydration; antigen retrieval; blocking; incubation with primary and secondary antibody; hematoxylin and eosin (H&E) staining; dehydration; transparency; sealing; data analysis. All sections were analyzed by a senior pathologist. Antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and DAB kit was purchased from Vector Laboratories (Burlingame, CA, USA).

Interpretation criteria. Positive signals were yellow to brown particles in the membrane of umbilical vascular endothelial cells. Five high power visual fields were selected to record the degree of staining and the percentage of positive cells. The average degree of staining of each section was multiplied by the average percentage of positive cells to get the final score of ICAM-1 expression. Negative (-), 0 points; weak positive (+), 1-2 points; moderate positive (++), 3-5 points; strong positive (+++), 6-9 points (Table II).

Statistical analysis. Data were analyzed by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Data of the normal distribution were recorded by mean ± standard deviation (SD). Comparison of measurement data between two groups were performed by independent sample t-test. Non-normal distribution data were tested by non-parametric Mann-Whitney U test. Expression of ICAM-1 in umbilical blood vessels was analyzed by chi-square test. P<0.05 was considered to indicate a statistically significant difference.

Results

Comparison of laboratory indicators between two groups. Fasting blood glucose of GDM group and control group were 4.56±0.73 and 4.47±0.65 mmol/l, respectively. HbA1c levels of GDM group and control group were 5.87±0.51 and

Table I. Comparison of general information between two groups (mean ± SD).

General information	GDM group (n=103)	Control group (n=106)	t-value	P-value
Age (years)	29.07±4.45	28.21±5.78	1.729	0.168
Gravida (times)	2.12±0.58	2.20±0.46	1.834	0.152
Parity (times)	1.72±0.89	1.66±1.01	1.912	0.089
Gestational age (weeks)	39.25±0.58	39.03±0.72	1.867	0.115
BMI (kg/m²)	26.11±4.35	25.74±3.93	1.645	0.186
Systolic blood pressure (mmHg)	117.79±10.38	115.01±11.57	2.126	0.073
Diastolic blood pressure (mmHg)	72.79±9.38	71.36±8.64	1.981	0.094

GDM, gestational diabetes mellitus; BMI, body mass index.

Table II. Degree of staining and the percentage of positive cells.

Cell staining	0 points	1 point	2 points	3 points
Degree of staining	No color	Yellow	Yellowish-brown	Chocolate brown
Positive cells (%)	≤5	6-20	21-50	≥51

Table III. Comparison of laboratory indicators between two groups (mean ± SD).

Laboratory indicators	GDM group (n=103)	Control group (n=106)	t-value	P-value
Fasting blood glucose (mmol/l)	4.56±0.73	4.47±0.65	2.013	0.101
HbA1c (%)	5.87±0.51	5.64±0.49	2.512	0.061
Umbilical cord arterial pH	7.24±0.01	7.28±0.01	2.912	0.052
pO_2 (Kpa)	2.41±0.19	2.40±0.20	1.867	0.115
pCO_2 (Kpa)	8.43±0.22	7.57±0.20	2.645	0.056

GDM gestational diabetes mellitus; HbA1c, glycosylated hemo-globin; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon dioxide.

5.64±0.49%, respectively. Umbilical cord arterial pH values of GDM group and control group were 7.24±0.01 and 7.28±0.01, respectively. pO_2 of GDM group and control group were 2.41±0.19 and 2.40±0.20 Kpa, respectively. pCO_2 of GDM
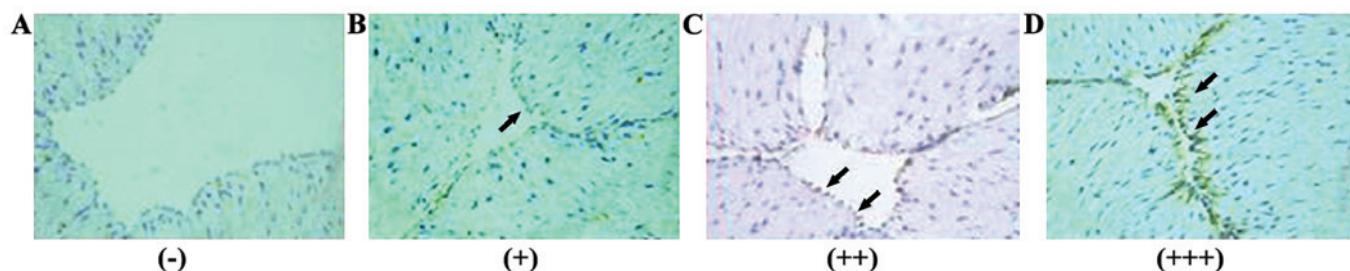
Figure 1. Expression of ICAM-1 in umbilical artery endothelial cells. (A-D) Representative results of negative (-), weakly positive (+), moderate positive (++) and strong positive (+++) expression. ICAM-1, intercellular adhesion molecule-1. Arrows indicate positive expression.
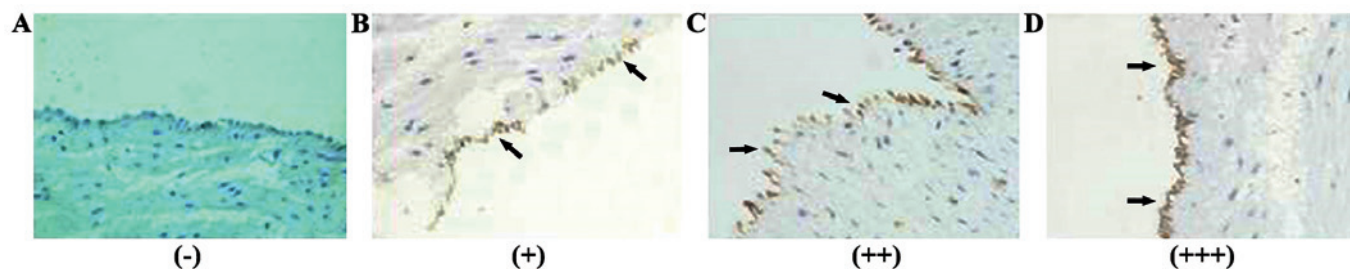

Figure 2. Expression of ICAM-1 in umbilical vein endothelial cells. (A-D) Representative results of negative (-), weakly positive (+), moderate positive (++) and strong positive (+++) expression. ICAM-1, intercellular adhesion molecule-1. Arrows indicate positive expression.

Table IV. ICAM-1 expression in GDM group and control group (mean ± SD).

ICAM-1 expression	GDM group (n=103)	Control group (n=106)	χ^2 value	P-value
Umbilical artery				
-	8 (7.77%)	9 (8.49%)	1.834	0.089
+	26 (25.24%)	27 (25.47%)	0.712	0.152
++	35 (33.98%)	36 (33.96%)	1.267	0.115
+++	34 (33.01%)	34 (32.08%)	0.645	0.186
Umbilical vein				
-	11 (10.68%)	13 (12.26%)	2.034	0.070
+	21 (20.39%)	22 (20.75%)	2.013	0.101
++	40 (38.83%)	43 (40.57%)	1.981	0.094
+++	31 (30.10%)	28 (26.42%)	2.512	0.061

ICAM-1, intercellular adhesion molecule-1; GDM, gestational diabetes mellitus.

account for 33.98 and 33.01% of all the cases, respectively. Moderately positive (++) signals in umbilical artery endothelial cells of control group accounted for 33.96% of all the cases. Moderately ICAM-1 positive (++) signals in umbilical vein endothelial cells accounted for 38.83 and 40.57% of all the cases in GDM group and control group, respectively. No significant differences in the expression of ICAM-1 in umbilical artery and umbilical vein were found between the two groups (p>0.05) (Table IV).

Discussion

The incidence of GDM is different in different races and regions (6,7). Incidence of GDM is approximately 2-6% in Europe (8) and 7% in United States (9). In addition, incidence of GDM showed an increasing trend (10,11), seriously affecting the health of mothers and children. Studies have shown that, compared with women without a history of GDM, the risk of type 2 diabetes (T2DM) within 5 to 20 years after delivery was increased by 6 times to 17-63% in women with a history of GDM (12-14). GDM is also called 'early T2DM' due to the high risk of T2DM caused by GDM (15-17). The pathogenesis of GDM is still unclear. Incidence of GDM is higher in Chinese than in blacks and whites (18). Vascular endothelial dysfunction is an important initial stage of atherosclerosis (AS) (19). Increased blood glucose caused by GDM is leading risk factor of AS and cardiovascular diseases. In view of the high mortality of cardiovascular diseases, early prevention and treatment of GDM is always needed. Studies have shown that vascular endothelial dysfunction occurs at an early stage of AS (20). Vascular endothelium can not only play a role as a physical barrier, but also can maintain the integrity and stability of blood vessels. Vascular endothelial cells can release diastolic and vasoconstrictor substances

group and control group were 8.43±0.22 and 7.57±0.20 Kpa, respectively. No significant differences in laboratory indicators were found between the groups (p>0.05) (Table III).

ICAM-1 expression in GDM group and control group. ICAM-1 expression was detected in umbilical cord blood vessels of both groups (Figs. 1 and 2).

Moderately positive (++) and strongly positive (+++) signals in umbilical artery endothelial cells of GAM group

through endocrine function and paracrine synthesis to regulate and protect vascular structure and functional integrity. Damaged vascular endothelium cannot perform the normal functions of anticoagulation, anti-platelet, anti-fibrinolysis, vasomotor and secretion and abnormal secretion of cytokines can change endothelial permeability, promote platelet aggregation, increase endothelial structural damage, which in turn promote the formation of AS (21).

Studies have shown that vascular endothelial dysfunction in patients with diabetes is expected to become a new target for prevention (22). Vascular endothelial dysfunction in patients with diabetes is caused by various factors including cytokines. Up to now, the function of human vascular endothelium can only be evaluated indirectly (21,23) and ICAM-1 is a good evaluation indicator. ICAM-1, also known as CD54, belongs to the adhesion molecule immunoglobulin superfamily. ICAM-1 single-stranded transmembrane glycoprotein of 76-114 kDa and is composed of extracellular region, transmembrane region and cytoplasmic region (24). ICAM-1 is rarely expressed in vascular endothelial cells under physiological conditions, so white blood cells cannot adhere to endothelial cells. The damaged vascular endothelium caused by external pathogenic factors (such as hyperglycemia and oxidative stress) can activate endothelial cells to secrete excessive ICAM-1, LPS and other adhesion molecules, inflammatory factors and chemokines, so as to accelerate the migration of white blood cells to the damaged region (25). Thus, studies on GDB have attracted increasing attention.

Using asymmetric dimethylarginine (ADMA) as a biochemical indicators of endothelial dysfunction and 44 pregnant women with GDB and 69 normal pregnant women (32-39 years old) as subjects, Akturk *et al* (26) found that levels of blood glucose, HbA1c and ADMA in GDB group were significantly higher than those in control group, indicating that endothelial cells in GDM patients were activated and the function was impaired. In contrast, with 32 GDM patients and 28 normal pregnant women with HbA1c lower than 6% as subjects, Kurt *et al* (27) found that there were no significant differences in the expression of ICAM-1 in umbilical cord tissue and placental tissue between the two groups. Although GDM can cause macrosomia, dystocia, eclampsia and many other adverse effects, transient and mild increases in blood glucose GDM patients do not seem to cause endothelial dysfunction and vascular dysfunction. Vastagh *et al* (28) and others (29,30) reported that there was no significant difference in AS between GDM patients with normal pregnant women with similar background (age, gestational age and BMI). In this study, no significant difference in expression level of ICAM-1 in umbilical artery and umbilical vein endothelial cells was found between two groups, indicating the good blood glucose control in GDM patients. So, umbilical cord endothelial cell damage does not seem to exist in GDM patients with good blood glucose control. The possible reasons are: i) GDM is caused by the increased insulin resistance after pregnancy, blood glucose is only increased slightly after GDM; ii) the modified cutoff score in the newly established guidelines for GDM diagnosis allows more pregnant women to receive early intervention management; iii) with the popularity of medical health education and the improvement of civic health awareness diet control and exercise therapy have been accepted by more and more people. This study is still limited by the small sample size. Future studies with greater sample sizes are needed to further confirm the conclusion of this study.

References

1. American Diabetes Association: Standards of medical care in diabetes - 2010. Diabetes Care 33 (Suppl 1): S11-S61, 2010.
2. Kim C: Gestational diabetes mellitus in korean women: Similarities and differences from other racial/ethnic groups. Diabetes Metab J 38: 1-12, 2014.
3. Ko GT, Tam WH, Chan JC and Rogers M: Prevalence of gestational diabetes mellitus in Hong Kong based on the 1998 WHO criteria. Diabet Med 19: 80, 2002.
4. Krauss T, Emons G, Kuhn W and Augustin HG: Predictive value of routine circulating soluble endothelial cell adhesion molecule measurements during pregnancy. Clin Chem 48: 1418-1425, 2002.
5. Bo S, Valpreda S, Menato G, Bardelli C, Botto C, Gambino R, Rabbia C, Durazzo M, Cassader M, Massobrio M, *et al*: Should we consider gestational diabetes a vascular risk factor? Atherosclerosis 194: e72-e79, 2007.
6. Ferrara A, Kahn HS, Quesenberry CP, Riley C and Hedderson MM: An increase in the incidence of gestational diabetes mellitus: Northern California, 1991-2000. Obstet Gynecol 103: 526-533, 2004.
7. Getahun D, Nath C, Ananth CV, Chavez MR and Smulian JC: Gestational diabetes in the United States: Temporal trends 1989 through 2004. Am J Obstet Gynecol 198: 525.e1-525.e5, 2008.
8. Kaaja RJ and Greer IA: Manifestations of chronic disease during pregnancy. JAMA 294: 2751-2757, 2005.
9. American Diabetes Association: Gestational diabetes mellitus. Diabetes Care 27 (Suppl 1): S88-S90, 2004.
10. Feig DS, Zinman B, Wang X and Hux JE: Risk of development of diabetes mellitus after diagnosis of gestational diabetes. CMAJ 179: 229-234, 2008.
11. Feng J, Li HY, Wang XL, Huo Y, Liu SX and Li L: Nicotinamide phosphoribosyltransferase enhances beta cell expansion during pregnancy. Eur Rev Med Pharmacol Sci 20: 4965-4971, 2016.
12. Bellamy L, Casas JP, Hingorani AD and Williams D: Type 2 diabetes mellitus after gestational diabetes: A systematic review and meta-analysis. Lancet 373: 1773-1779, 2009.
13. Coustan DR, Carpenter MW, O'Sullivan PS and Carr SR: Gestational diabetes: Predictors of subsequent disordered glucose metabolism. Am J Obstet Gynecol 168: 1139-1144, 1993.
14. Kjos SL, Peters RK, Xiang A, Henry OA, Montoro M and Buchanan TA: Predicting future diabetes in Latino women with gestational diabetes. Utility of early postpartum glucose tolerance testing. Diabetes 44: 586-591, 1995.
15. Bloomgarden ZT: Inflammation and insulin resistance. Diabetes Care 26: 1922-1926, 2003.
16. Carr DB, Utzschneider KM, Hull RL, Tong J, Wallace TM, Kodama K, Shofer JB, Heckbert SR, Boyko EJ, Fujimoto WY, *et al*: Gestational diabetes mellitus increases the risk of cardiovascular disease in women with a family history of type 2 diabetes. Diabetes Care 29: 2078-2083, 2006.
17. Seshiah V, Balaji V, Balaji MS, Paneerselvam A, Arthi T, Thamizharasi M and Datta M: Gestational diabetes mellitus manifests in all trimesters of pregnancy. Diabetes Res Clin Pract 77: 482-484, 2007.
18. Yue DK, Molyneaux LM, Ross GP, Constantino MI, Child AG and Turtle JR: Why does ethnicity affect prevalence of gestational diabetes? The underwater volcano theory. Diabet Med 13: 748-752, 1996.
19. Quiñones MJ, Nicholas SB and Lyon CJ: Insulin resistance and the endothelium. Curr Diab Rep 5: 246-253, 2005.
20. Iudakova TN, Girsh AO, Maksimishin SV and Mal'Kov OA: Association of cardiovascular system and endothelial dysfunction indicators in patients with hemorrhagic shock Anesteziol Reanimatol 6: 11-14, 2013 (In Russian).
21. Hartge MM, Kintscher U and Unger T: Endothelial dysfunction and its role in diabetic vascular disease. Endocrinol Metab Clin North Am 35: 551-560, 2006.
22. Caballero AE: Endothelial dysfunction, inflammation, and insulin resistance: A focus on subjects at risk for type 2 diabetes. Curr Diab Rep 4: 237-246, 2004.

23. Matsumoto T, Kobayashi T and Kamata K: Alterations in EDHF-type relaxation and phosphodiesterase activity in mesenteric arteries from diabetic rats. Am J Physiol Heart Circ Physiol 285: H283-H291, 2003.
24. Jiang Y, Jiang LL, Maimaitirexiati XM, Zhang Y and Wu L: Irbesartan attenuates TNF-α-induced ICAM-1, VCAM-1, and E-selectin expression through suppression of NF-κB pathway in HUVECs. Eur Rev Med Pharmacol Sci 19: 3295-3302, 2015.
25. Imhof BA and Dunon D: Leukocyte migration and adhesion. Adv Immunol 58: 345-416, 1995.
26. Akturk M, Altinova A, Mert I, Dincel A, Sargin A, Buyukkagnici U, Arslan M and Danisman N: Asymmetric dimethylarginine concentrations are elevated in women with gestational diabetes. Endocrine 38: 134-141, 2010.
27. Kurt M, Zulfikaroglu E, Ucankus NL, Omeroglu S and Ozcan U: Expression of intercellular adhesion molecule-1 in umbilical and placental vascular tissue of gestational diabetic and normal pregnancies. Arch Gynecol Obstet 281: 71-76, 2010.
28. Vastagh I, Horváth T, Garamvölgyi Z, Rosta K, Folyovich A, Rigó J, Kollai M, Bereczki D and Somogyi A: Preserved structural and functional characteristics of common carotid artery in properly treated normoglycemic women with gestational diabetes mellitus. Acta Physiol Hung 98: 294-304, 2011.
29. Bulzico DA, Zajdenverg L, Cabizuca CA, de Oliveira JE and Salles GF: Assessment of arterial stiffness in women with gestational diabetes. Diabet Med 29: 227-231, 2012.
30. Salmi AA, Zaki NM, Zakaria R, Nor Aliza AG and Rasool AH: Arterial stiffness, inflammatory and pro-atherogenic markers in gestational diabetes mellitus. Vasa 41: 96-104, 2012.