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Correlation of VCAM-1 expression in serum, cord blood, and placental tissue with gestational hypertension associated with fetal growth restriction in women from Xingtai Hebei, China

H.G. Zhang[1], W. Guo[1], H.F. Gu[1], S.B. Chen[2], J.Q. Wang[1], Z.X. Qiao[3], H.S. Ma[3] and S.X. Geng[1]

[1]Department of Neonatology,
Affiliated Xingtai People's Hospital of Hebei Medical University, Xingtai, Hebei, China
[2]Department of Radiology,
Affiliated Xingtai People's Hospital of Hebei Medical University, Xingtai, Hebei, China
[3]Obstetric, Affiliated Xingtai People's Hospital of Hebei Medical University, Xingtai, Hebei, China

Corresponding author: W. Guo
E-mail: guowei_xt@126.com

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ABSTRACT. The aim of this study was to investigate the expression of vascular adhesion molecule (VCAM)-1 in the maternal serum, cord blood, and placental tissue of pregnant women from Xingtai, Hebei, with gestational hypertension (GH) combined with fetal growth restriction (FGR). A total of 108 patients with GH combined with FGR (GH-FGR), 60 patients with GH alone (GH), and 50 healthy

pregnant women (control) were recruited to this study. VCAM-1 expression was detected in the maternal serum and cord blood by enzyme-linked immunosorbent assay, and in the placental tissue by immunohistochemistry. VCAM-1 expression was significantly higher in the maternal serum of patients with GH-FGR (164.38 ± 60.35) and GH alone (103.85 ± 54.47) than in the serum of the control population (46.70 ± 21.79; $P < 0.05$). On the other hand, VCAM-1 expression in the cord blood of GH-FGR (163.19 ± 69.46), GH (149.82 ± 58.20), and control (128.89 ± 43.59) subjects was not significantly different ($P > 0.05$). Moreover, the VCAM-1 expression rates were significantly higher and lower in the vascular endothelial and trophoblastic cells of the placenta of patients with GH-FGR (74.71 and 56.1%) and GH (72.98 and 55.36%), respectively, compared to those in the control subjects (46.48 and 95.11%). Therefore, we concluded that VCAM-1 plays an important role in the development and generation of GH. Additionally, the low VCAM-1 expression in the trophoblastic cell could be correlated to the pathogenesis and progression of GH.

Key words: Vascular adhesion molecule-1; Serum; Cord blood; Placental tissue; Gestational hypertension; Fetal growth restriction

INTRODUCTION

Hypertensive disorders complicating pregnancy (HDCP), or pregnancy-induced hypertension syndrome, is a common gynecological complication (with an incidence rate of 6-8%) responsible for disease and death in pregnant women and newborns (Poon et al., 2010; Angeli et al., 2011). HDCP was the second leading cause of death in pregnant women in the USA during 1996, with a mortality rate of 15%, behind thrombosis (1996). The incidence of HDCP in China is subject to regional differences. A national investigation into oral epidemic diseases conducted in 1996 revealed that the incidence of HDCP was higher in the eastern and south-central regions of China; in fact, the incidence and mortality rates of HDCP have been reported to be 1.59 and 77%, respectively, in the Guangdong Province during 2001 (accounting for 10% of the total death) (Qian and Jiang, 1991). Gestational hypertension (GH), preeclampsia, eclampsia, chronic hypertension combined with preeclampsia, and chronic hypertension are the five types of HDCP, among which the incidence of preeclampsia (*de novo* hypertension after mid-pregnancy, combined with new-onset proteinuria) and GH (*de novo* hypertension after mid-pregnancy, but without proteinuria) are the highest (Villar et al., 2006).

Normal fetal growth depends on several maternal, fetal, placental, genetically predetermined growth potential, and external factors (Gardosi et al., 1992). Placental dysfunction impacts multiple systems, and is also responsible for increased mortality and morbidity (Baschat et al., 2000; Bernstein et al., 2000). Fetal growth restriction (FGR) is a relatively common phenomenon resulting in disproportionately high perinatal morbidity and mortality (Knutzen and Sher, 1982; Newton et al., 1987). FGR is characterized by a birth weight below the tenth percentile according to the gestational age, or a fetal body weight below two standard deviations of the mean of a normal newborn; several factors, including gene defects, chromosomal abnormalities, poor placental function, maternal smoking status,

maternal alcohol or drug abuse, and altered maternal substrate concentrations, have been implicated in FGR incidence Churchill et al., 2000).

Previous studies (Cines et al., 1998; Higgins et al., 1998) have reported that injuries to the vascular endothelial cell could induce leukocyte activation, as vascular adhesion molecules (VCAM) present on the leukocyte and endothelial cell surface regulate the release of inflammatory mediators, thereby aggravating the injury to the vascular endothelial cell. Current research into adhesion molecules has primarily focused on the association between these molecules and cardiovascular disease and organ transplantation; however, the relationship between adhesion molecules and GH remains controversial.

Few studies have investigated the VCAM-1 expression in women with GH and those with GH combined with FGR. In this study, we analyzed VCAM-1 expression in the maternal serum, cord blood, and placental tissue of women with GH, and GH combined with FGR from Xingtai, Hebei, in order to investigate the role of VCAM-1 in the development and generation of GH.

MATERIAL AND METHODS

Patients

A total of 108 women clinically diagnosed with GH combined with FGR, 60 women with GH, and 50 healthy pregnant women (control subjects) who delivered their babies in the Obstetrics Department of Xingtai People's Hospital from January 1, 2012 to October 31, 2014 were recruited to this study. The control subjects were selected at random. The general clinical data of all patients are summarized in Table 1. The selected patients and controls did not differ significantly in terms of the median age and gestational age. The patients were recruited during their first pregnancy and delivered by Caesarean section (without any associated infection), and had no history of other complications or tumors.

Table 1. Clinical data of patients with gestational hypertension associated with fetal growth retardation (GH-FGR) and GH alone, and healthy control subjects (means ± standard deviation).

Group	Cases (N)	Median age (years)	Gestational age (days)
GH-FGR	108	29.64 ± 3.2	248.74 ± 22.40
GH	60	30. 93 ± 3.9	260.61 ± 27.93
Control	50	27.91 ± 3.5	275.82 ± 810.58

Tissue samples were obtained from all patients and controls after obtaining an approval from the medical Ethics Committee of Xingtai People's Hospital, as well as informed consent from the study subjects.

Diagnostic criteria

GH and FGR were diagnosed according to the criteria detailed by Le (2000). The women in the GH-FGR and GH groups showed the following symptoms: blood pressure ≥140/90 mmHg, negative urine protein, upper abdominal discomfort or thrombocytopenia, and recovery after 12 weeks of delivery.

Collection of maternal serum and cord blood samples and ELISA

Maternal serum samples were collected prior to the Caesarean section and cord blood samples were extracted from the root of the umbilical cord after placental expulsion. In both cases, 4 mL venous blood was collected in tubes containing ethylenediaminetetraacetic acid, and centrifuged at 3000 *g* for 10 min. Subsequently, the supernatant was collected and stored at -80°C.

VCAM-1 expression in the maternal serum and cord blood was detected by ELISA using a standard kit (Takara Bio Inc., Dalian, China).

Collection of placental tissue samples and immunohistochemistry (IHC)

Three 1.0 cm x 1.0 cm x 0.6-cm tissue sections were obtained from the maternal surface of the placenta (without calcification) after placental expulsion. The tissues were fixed in 10% paraformaldehyde (Thermo Fisher Scientific, Waltham, MA, USA) for 8-12 h and embedded in paraffin (Sigma-Aldrich, St. Louis, MO, USA), which was subsequently cut into 4-μm sections. VCAM-1 expression was detected by IHC using a rabbit anti-VCAM-1 antibody (1:200; Proteintech Group Inc., Wuhan, China) and a standard (rabbit) Histostain-Plus IHC kit (Thermo Fisher Scientific) according to the manufacturer protocols.

Scoring and evaluation of samples

IHC sections were observed under an Olympus microscope (Olympus Imaging Co., Ltd., Beijing, China) and the images analyzed by a high-pathological image analysis system-1000 (HPIAS-1000, Olympus). Five images of each section were selected at random and the average absorption value was determined. The positively stained cells were scored according to the presence of yellow particles as follows: 0, positive staining ≤5%; 1, 5 < positive staining ≤25%; 2, 25 < positive staining ≤50%; 3, 50 < positive staining ≤75%; and 4, positive staining ≥75%.

Statistical analysis

The measured data are reported as means ± standard deviations and analyzed on the SPSS 17.0 software platform (IBM, Armonk, NY, USA). The variations between the three groups (between-group comparisons) were analyzed by the *t*-test or the Kruskal-Wallis rank sum test. Variations within each group were analyzed by one-way analysis of variance (ANOVA). A P value <0.05 was considered to indicate a significant difference.

RESULTS

VCAM-1 expression in maternal serum and cord blood

VCAM-1 expression was observed to be significantly higher in the maternal serum of patients with both GH associated with FGR and GH only compared to that in the control group ($P < 0.05$). Moreover, VCAM-1 expression was significantly higher in the women with GH associated with FGR than that in women with GH alone ($P < 0.05$). On the contrary, VCAM-1 expression in the cord blood did not differ significantly among the three groups (Table 2).

Table 2. Vascular cell adhesion molecule (VCAM)-1 expression in maternal serum and cord blood of patients with gestational hypertension and healthy controls (means ± SD).

Groups	Cases (N)	VCAM-1 (μg/mL)	
		Maternal serum	Cord blood
GH-FGR	108	164.38 ± 60.35*#	163.19 ± 69.46
GH	60	103.85 ± 54.47*	149.82 ± 58.20
Control	50	46.70 ± 21.79	128.87 ± 43.59

GH = gestational hypertension; FGR = fetal growth retardation. *P < 0.05 compared to the control group. #P < 0.05 compared to patients with GH alone.

VCAM-1 expression in placental tissue

VCAM-1 expression was significantly higher in the vascular endothelial cells and significantly lower in the trophoblastic cells of women with GH associated with FGR, as well as GH alone, than that in cells from the control group (P < 0.05 and 0.01, respectively). Additionally, VCAM-1 expression was higher in the vascular endothelial and trophoblastic cells of patients with GH-FGR than that in patients with GH alone; however, this difference was not statistically significant (Table 3).

Table 3. Positive intensity (means ± SD) and rate (%) of vascular cell adhesion molecule (VCAM)-1 in the placental cells of patients with gestational hypertension and healthy control subjects.

Groups	Cases (N)	Vascular endothelial cell		Trophoblastic cell	
		Intensity (means ± SD)	Rate (%)	Intensity (means ± SD)	Rate (%)
GH-FGR	108	0.63 ± 0.22*	74.71*	0.41 ± 0.16*	56.10*
GH	60	0.61 ± 0.32*	72.98*	0.40 ± 0.24*	55.36*
Control	50	0.39 ± 0.25	46.48	0.62 ± 0.18	95.11

GH = gestational hypertension; FGR = fetal growth retardation. *P < 0.05 compared to the control group.

DISCUSSION

HDCP is a complicated syndrome with multiple risk factors. A number of scholars have theorized that genetic defects result in immune function imbalance in the maternal body, which in turn induces hypoxic-ischemia in the placenta or generates toxic factors that injure the endothelial cells and result in spasms in the arteriole (Yamaji et al., 2012). In fact, the possible correlation between these genetic defects and scathing of the vessel endothelium and placental shallow bed is of critical importance.

VCAM-1 (CD106; molecular weight: 100-110 kDa) is a member of the immunoglobulin superfamily distributed on the surface of endothelial cells that mediates the adhesion between leukocytes and activated endothelial cells (Dietmaier et al., 1999), through a ligand-receptor interaction with the very late appearing antigen-4 on the leukocyte (Elices et al., 1990). Higgins et al. (1998) reported that the expression of VCAM-1 was disrupted and its serum concentration was positively related to the severity of clinical symptoms. Budak et al. (1998) reported higher levels of serum VCAM-1 in patients with preeclampsia (VCAM-1 >450 mg/L) and eclampsia than those in healthy pregnant women; that is, the increase in VCAM-1 levels appeared to be indicative of endothelial cell injury and leukocyte activation (Sibai et al., 2005), similar to the results noted by Kim et al. (2004). In this study, VCAM-1 expression was found to be higher in the maternal serum of patients with GH associated with

FGR and GH alone than that in the control subjects; moreover, VCAM-1 expression was found to be significantly higher in the GH-FGR group than that in the GH group ($P < 0.05$).

High VCAM-1 expression is responsible for the attachment of a large number of leukocytes to the endothelial cells, thereby causing endothelial cell injury and vasodilatory dysfunction via a series of pathological mechanisms, which in turn induces hypertension, proteinuria, edema, and other clinical symptoms of preeclampsia (Zeisler et al., 2001). VCAM-1 plays an important role in early placental development and trophoblast invasion; however, women with preeclampsia undergo adverse remodeling of the uterine spiral arteries, inducing abnormal trophoblast invasion, and consequently, induced hypoxic-ischemia in the placenta. Low placental perfusion induces the up-regulation of VCAM-1 expression, which in turn leads to degeneration, necrosis, and exfoliation of the endothelial cell, thereby aggravating hypoxic-ischemia in the placenta. Low perfusion in the placenta is also responsible for placenta maldevelopment and FGR. Djurovic et al. (1997) reported that VCAM-1 detection could help elucidate preeclampsia development and intrauterine growth retardation (IUGR) in patients with preeclampsia.

Very few studies have analyzed and reported the expression of VCAM-1 in fetal circulation. Krauss et al. (1998) reported that the expression levels of VCAM-1 and the intercellular adhesion molecule in the cord blood did not differ significantly between women with preeclampsia and those with late pregnancy ($P > 0.05$). In this study, the expression of VCAM-1 in the cord blood of patients with GH-FGR and GH, and in healthy controls did not differ significantly. Therefore, we assumed fetal circulation to be independent of maternal circulation. However, the role of VCAM-1 in the pathological process of endothelial cell injury in patients with GH requires further investigation.

Previous studies have indicated that VCAM-1 induces and aggravates the invasion of syncytiotrophoblast (Du et al., 2013), enhances the adhesion of the placenta to the uterine wall (Papayannopoulou et al., 1995), and facilitates and positively influences the exchange of materials between the mother and the fetus (Wilczyński et al., 2003), among others. Endothelial cell adhesion molecules (ECAMs) play an important role in several mechanisms responsible for vascular endothelial injury. For example, ECAM-1 facilitates in the adhesion of trophoblast to the vascular endothelium during early pregnancy and mediates trophoblastic vascular invasion during the remodeling of placental vasculature; mediates the adhesion between the placental villi, cells in the decidual tissue, and peripheral blood leukocytes; and sustains the non-activation of decidual lymphocytes, preventing the immunological rejection of the fetus by the mother.

Madazli et al. (2000) reported a significant increase in the fibronectin and VCAM-1 levels in women with GH, which was correlated with the diastolic blood pressure. Krauss et al. (1997) theorized that the increase in serum VCAM-1 levels reflected the activation of endothelial cells, but not cell injury, the factor responsible for predicting the development of GH and IUGR. In this study, the positive intensity and rate of VCAM-1 expression in the vascular endothelial cells of patients with GH-FGR and GH alone were both greater than values in the control subjects (0.63 ± 0.22, 0.61 ± 0.32, and 0.39 ± 0.23, and 74.71, 72.98, and 46.48%, respectively). This result indicated that the negative effect of VCAM-1 on (causing imbalances in) endothelial function could play a role in the development and progression of GH, as reported previously by Heimrath et al. (2000). The positive intensity and rate of VCAM-1 expression in the trophoblastic cells of women with GH-FGR and GH alone were significantly lower than the levels seen in the controls (0.41 ± 0.16, 0.40 ± 0.24, and $0.62 \pm$

0.18; and 56.1, 55.36, and 95.11%, respectively), as seen previously by Zhou et al. (1997) in the cytomorphology of the placental bed. The results indicated that the trophoblastic cells of women with GH lacked the expression of adhesion molecules such as a5b3/a1b1, VCAM, and platelet endothelial adhesion molecule-1, resulting from transforming defects in the genotypes of adhesion molecules or the formation of invasion-specific genotypes. However, cytotrophoblastic cells and capillary endothelial cells showed little to no VCAM-1 expression in this study.

In conclusion, the VCAM-1 secretion was disrupted in the maternal serum, cord blood, and placental tissue of women with GH or GH combined with FGR, compared to healthy pregnant women. However, the pathogenesis and progression of GH, the mechanism of VCAM-1 expression, and their correlation requires further investigation.

Conflict of interest

The authors declare no conflict of interest.

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