



Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

A clinical research study on the respective relationships between visfatin and human fetuin A and pregnancy outcomes in gestational diabetes mellitus

Dan Lu ^{a,*}, Meng Yang ^b, Yanjiao Yao ^c, Yanyan Xie ^c^a Department of Gynecology and Obstetrics, The People's Hospital of North Jiangsu Province, Medical College of Yangzhou University, Yangzhou, 225001, Jiangsu, China^b Department of Gynecology and Obstetrics, Tianmen People's Hospital, Tianmen, 431700, Hubei, China^c Dalian Medical University, Dalian, 116044, Liaoning, China

ARTICLE INFO

Article history:

Accepted 26 July 2019

Keywords:

Gestational diabetes mellitus (GDM)

Visfatin (VF)

Human fetuin A (AHSG)

Maternal and foetal adverse outcome

ABSTRACT

Objective: The aim was to determine the role of visfatin (VF) and human fetuin A (AHSG) in the development of gestational diabetes mellitus (GDM) and to explore the association between these variables and adverse outcomes.**Materials and methods:** We carried out our study on 68 cases of GDM pregnant women and 42 cases of healthy pregnant women, including 56 cases with diet control and 12 cases with insulin treatment. Enzyme-linked immunoassay (ELISA) was used to test the expression levels of VF and AHSG in maternal and umbilical cord serum. Immunohistochemistry (ICH) was used to test the expression level of the VF protein in placental tissue.**Results:** The expression levels of VF and AHSG in maternal and umbilical cord serum and the expression level of VF in placental tissue in GDM pregnant women were higher than those in healthy pregnant women. The incidence of adverse outcomes in the GDM pregnant women was higher than that in healthy pregnant women, and these differences were statistically significant ($P < 0.05$). Those who had higher expression levels of VF or AHSG had a higher incidence of adverse outcomes ($P < 0.05$).**Conclusion:** The expression of VF and AHSG may participate in the development of GDM. A test of VF and AHSG in GDM pregnant women may have some predictive value for the occurrence of adverse outcomes.© 2019 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

GDM refers to the transition from normal glucose metabolism before pregnancy to different degrees of impaired glucose tolerance during pregnancy. The pathogenesis of GDM is complicated, and the aetiology is still unclear. Studies suggest that its occurrence may be the result of multiple factors. Insulin resistance (IR) and the abnormal glucose and lipid metabolism caused by IR are the important factors leading to GDM [1]. Research has shown that adipose cells and their secreted factors have an important correlation with IR. Adipose factors secreted by adipose tissue regulate insulin sensitivity [2]. Visfatin is a novel adipocytokine secreted by

visceral adipose tissue, which has the effect of an insulin-like lowering of blood glucose [2,3]. AHSG is a serum and tissue protein that participates in the development of IR and glycolipid metabolism disorders [4–6]. This study aimed to determine the role of VF and AHSG in the development of GDM and to explore the association between the level of VF and AHSG and maternal and foetal adverse outcomes.

Materials and methods

Subjects and groups

In total, 68 cases of GDM pregnant women were selected as the observation group, including 56 cases in the diet control group and 12 cases in the insulin treatment group, and 42 healthy pregnant women were enrolled as the control group among the pregnant women who underwent term birth in the People's Hospital of

* Corresponding author. Department of Gynecology and Obstetrics, Clinical Medicine School of Yangzhou University, Yangzhou, 225001, Jiangsu, China. Fax: +0514 87373255.

E-mail address: ludan1968@126.com (D. Lu).

North Jiangsu Province from January 2017 to June 2017. All met the diagnostic criteria for GDM [7] (i.e., the classification and diagnosis of diabetes from the American Diabetes Association). All the selected cases, all of which were singleton pregnancies, had no complications, and cases with type 1 and type 2 diabetes mellitus were excluded.

The experiment was reviewed by the Ethics Committee of the People's Hospital of North Jiangsu, and all subjects provided informed consent.

Information and specimen collection

The ages, delivery gestational age and maternal and foetal adverse outcomes (caesarean section, postpartum complications of the mother and child, diabetic ketoacidosis, etc.) of the selected subjects were recorded. The average age of patients in the diet control group and insulin treatment group within the observation group was 29.50 ± 4.39 and 31.83 ± 5.80 years, respectively, and the average gestational age was 39.52 ± 0.91 and 38.88 ± 1.07 weeks, respectively. The average age of the control group was 29.36 ± 4.37 years, and the average gestational age was 39.54 ± 1.00 weeks. There was no statistically significant difference in these two variables for the observation group and control group ($P = 0.23$ and $P = 0.09$, respectively).

The collection of experimental specimens was as follows. (1) Maternal blood: 5 ml of fasting venous blood from pregnant women before delivery was centrifuged at 3000 r/min for 10 min, and the supernatant was collected and stored at -80°C until used. (2) Cord blood: after the umbilical cord was cut, 5 ml of umbilical vein blood was taken and centrifuged at 3000 r/min for 10 min, and the supernatant was collected and stored at -80°C until used. (3) Placenta: immediately after delivery under aseptic conditions, the placental tissue approximately 1 cm from the maternal surface near the umbilical cord was taken, avoiding blood vessels and calcification points, and rinsed thoroughly with saline, fixed with 4% neutral formaldehyde, and after 24 h, embedded in paraffin.

Methods

(1) The expression of VF and AHSG in maternal blood and cord blood was determined by ELISA. The specific steps followed the kit (purchased from Jiangsu Feiya Biotechnology Co. LTD.) instructions. Then a standard curve was drawn, and the sample concentration was calculated. (2) The expression of VF in placental tissue was detected by IHC. The steps were dewaxing, hydration, high pressure antigen retrieval, elimination of endogenous peroxidase activity, antibody blocking, incubation of the primary antibody, incubation of the secondary antibody, DAB coloration, and haematoxylin counterstaining and returning to blue. Sealing tablets (immunohistochemical staining SP hypersensitive kit and DAB chromogenic kit (AR1022)) were purchased from Fuzhou Maixin Biotechnology Co. LTD. Hematoxylin staining solution was purchased from Nanjing Kaiji Bioengineering Co. LTD. Under high magnification, images were taken (40x using JEDAS01D morphological image system software, and 5 fields of view of each tissue were randomly taken. The images were analysed with Image-Pro-Plus 6.0. The optical density value (IOD) of the placental tissue and the actual area of the tissue (Area) was determined, and the average optical density value (MOD) was calculated ($\text{MOD} = \text{IOD}/\text{Area}$).

Statistical method

Data analysis was performed by SPSS 19.0 statistical software. Quantitative data was expressed as the mean \pm standard deviation ($\bar{x} \pm s$). The t test was used for comparison between the two groups.

One-way ANOVA was used for the difference among the three groups, and Student-Newman-Keulsa was used for comparison among the three groups. Qualitative data was expressed as a percentage (%), and the χ^2 test was performed. There was a statistically significant difference for results with $P < 0.05$, or $\alpha = 0.05$.

Results

ELISA results

Expression of VF in maternal blood and cord blood

The expression levels of VF in maternal blood and cord blood of pregnant women in three groups are shown in Table 1. Compared with the VF level in the normal control group, the level in the GDM diet control group was significantly different ($F = 33.00$, $P = 0.00$), and the result was the same in the insulin treatment group ($F = 32.09$, $P = 0.00$). However, the level was not significantly different between the insulin treatment group and the diet control group.

Expression of AHSG in maternal blood and cord blood

The levels of AHSG in the maternal blood and cord blood of pregnant women in three groups are shown in Table 2. Compared with the normal control group, the AHSG levels significantly increased in the diet control group ($F = 21.30$, $P = 0.00$) and the insulin treatment group ($F = 36.82$, $P = 0.00$). However, the level was not significantly different between the insulin treatment group and the diet control group.

IHC results

VF is mainly expressed in the cytoplasm, and the positive expression results in a yellow or brown colour in the nucleus and cytoplasm (Fig. 1A–D). The MOD values of VF in placental tissues of the GDM diet control group, insulin treatment group and the normal control group were 0.05272 ± 0.01730 , 0.05247 ± 0.00948 , and 0.03181 ± 0.00825 , respectively. The expression level of VF in the GDM group was higher than the normal control group, with a statistically significant difference ($F = 29.87$, $P = 0.00$), but the level was not significantly different between the insulin treatment group and the diet control group in the GDM group.

Comparison of pregnancy outcomes

Comparison of outcomes in pregnant women

The caesarean section rates in the diet control group (51.8%) and in the insulin treatment group (66.7%) were higher than that in the normal control group (42.9%), but the rates were not significantly different ($\chi^2 = 2.26$, $P = 0.32$). The incidence of postpartum haemorrhage in the diet control group (10.7%) and the insulin treatment group (8.3%) were higher than that in the normal control group (2.4%), but the incidence was not significantly different ($\chi^2 = 2.49$, $P = 0.28$). The incidence of postpartum fever in the diet control group (5.4%) was higher in the GDM group than that in the normal control group (2.4%), but the incidence was not significantly different ($\chi^2 = 0.64$, $P = 0.73$). The incidence of diabetic ketoacidosis in the GDM insulin treatment group (8.3%) was higher than that in the normal control group (0.0%), which was statistically significant ($\chi^2 = 8.24$, $P = 0.02$).

Comparison of neonatal outcomes

The outcomes of the newborns in each group are listed in Table 3. The incidence of neonatal hypoglycaemia and respiratory distress syndrome were significantly different between the GDM

Table 1VF levels in maternal and cord serum ($\bar{x} \pm S$).

Groups	Cases	Maternal serum VF (ng/ml)	Cord serum VF (ng/ml)
GDM group	68		
diet control group	56	50.74 \pm 14.21 ^b	35.39 \pm 10.40 ^b
insulin treatment group	12	48.47 \pm 14.49 ^b	36.70 \pm 12.00 ^b
Normal control group	42	31.07 \pm 7.47	21.01 \pm 5.80
F		33.00	32.09
P		0.00 ^a	0.00 ^a

Note: a represents a comparison among groups with $P < 0.01$; b represents a comparison with the normal control group with $P < 0.05$.

Table 2AHSG levels in maternal and cord blood ($\bar{x} \pm S$).

Groups	Cases	Maternal blood AHSG (ng/ml)	Cord blood AHSG (ng/ml)
GDM group	68		
diet control group	56	368.42 \pm 122.11 ^b	187.52 \pm 61.61 ^b
insulin treatment group	12	369.12 \pm 97.43 ^b	188.16 \pm 54.43 ^b
Normal control group	42	238.09 \pm 67.26	99.85 \pm 34.68
F		21.30	36.82
P		0.00 ^a	0.00 ^a

Note: a represents a comparison among groups with $P < 0.01$; b represents a comparison with the normal control group with $P < 0.05$.

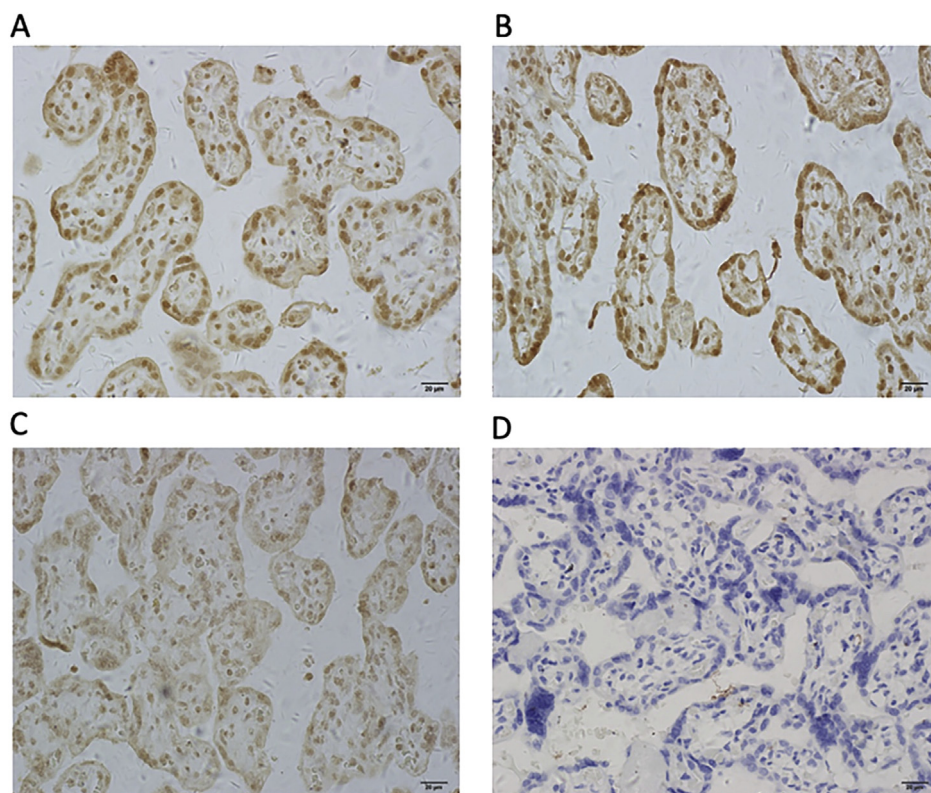


Fig. 1. Expression intensity of VF in placental tissue (40 \times): **A** is the expression of VF in the diet control group; **B** is the expression of VF in the insulin treatment group; **C** is the expression of VF in the normal control group; and **D** is a PBS negative in the control group.

group and the normal control group, with $\chi^2 = 20.13$ and $P = 0.00$ and $\chi^2 = 9.91$ and $P = 0.01$, respectively.

Relationship between VF and AHSG and pregnancy outcomes

Relationship between VF and pregnancy outcomes

According to the median VF expression level in GDM pregnant women, the GDM observation group was divided into two sub-groups (i.e., high VF group and low VF group), and the

corresponding maternal and foetal pregnancy outcomes were compared. The incidence of amniotic fluid contamination and neonatal asphyxia in the high maternal blood VF group was higher than that in the low VF group, with statistically significant differences ($\chi^2 = 3.98$, $P = 0.04$; $\chi^2 = 4.25$, $P = 0.04$, respectively). The incidence of intrauterine distress and neonatal hypoglycaemia in the high cord blood VF group was higher than that in the low VF group, with statistically significant differences ($\chi^2 = 4.25$, $P = 0.04$; $\chi^2 = 4.53$, $P = 0.03$, respectively). The incidence of intrauterine

Table 3

Comparison of neonatal outcomes [frequency (%)].

Pregnancy outcome	GDM group		normal control group (n = 42)	χ^2	P
	diet control group (n = 56)	insulin treatment group (n = 12)			
Post-term infant	3 (5.4)	0 (0.0)	4 (9.5)	1.62	0.45
Intrauterine distress	3 (5.4)	1 (8.3)	1 (2.4)	0.94	0.63
Foetal macrosomia	9 (16.1)	2 (16.7)	2 (4.8)	3.24	0.20
Small for gestational age	5 (8.9)	1 (8.3)	1 (2.4)	1.81	0.40
Amniotic fluid contamination	5 (8.9)	2 (16.7)	2 (4.8)	1.85	0.40
Neonatal asphyxia	3 (5.4)	1 (8.3)	0 (0.0)	2.81	0.25
Respiratory distress syndrome	4 (7.1)	3 (25.0)	0 (0.0)	9.91	0.01
Neonatal cyanine	3 (5.4)	0 (0.0)	0 (0.0)	2.73	0.23
Transient tachypnoea of the newborn	1 (1.8)	0 (0.0)	0 (0.0)	0.97	0.62
Neonatal infection	2 (3.6)	1 (8.3)	1 (2.4)	0.95	0.62
Neonatal hypoglycaemia	13 (23.2)	7 (58.3)	1 (2.4)	20.13	0.00 ^a
Neonatal hyperbilirubinemia	9 (16.1)	2 (16.7)	4 (9.5)	0.98	0.61

Note: a represents a comparison among groups with $P < 0.01$.

distress, amniotic fluid contamination, neonatal asphyxia, respiratory distress syndrome and neonatal hypoglycaemia in the high placental tissue VF group was higher than that in the low VF group, with statistically significant differences ($\chi^2 = 4.25$, $P = 0.04$; $\chi^2 = 3.98$, $P = 0.04$; $\chi^2 = 4.25$, $P = 0.04$; $\chi^2 = 3.98$, $P = 0.04$; $\chi^2 = 10.20$, $P = 0.00$, respectively) (Tables 4 and 5).

Relationship between AHSG and pregnancy outcomes

According to the median AHSG level of GDM pregnant women, the GDM group was divided into two subgroups (i.e., high AHSG group and low AHSG group), and the corresponding maternal and foetal pregnancy outcomes were compared. The incidence of postpartum haemorrhage, intrauterine distress, and neonatal hypoglycaemia was higher in the high maternal blood AHSG group than in the low AHSG group. There were statistically significant differences ($\chi^2 = 3.98$, $P = 0.04$; $\chi^2 = 4.25$, $P = 0.04$; $\chi^2 = 4.53$, $P = 0.03$, respectively). There was no significant difference in cord blood AHSG levels and pregnancy outcomes (Table 6).

Discussion

In recent years, the incidence of GDM has shown a clear upward trend, and GDM is one of the most common complications during pregnancy [8]. GDM can not only cause severe metabolic abnormalities of the mother and increase the rate of caesarean section but also affects the growth and development of the foetus [6]. However, the pathogenesis of GDM is still unclear, and it is believed

to be the result of multiple factors. It is thought that GDM is caused by IR and abnormal glucose metabolism.

Recent studies have shown that adipose tissue, which can secrete a large number of cytokines and biologically active substances, such as adiponectin, resistin, VF and omentin, among which VF is important in the pathogenesis of various diseases, has important endocrine functions. VF has gradually become a molecule of interest [4]. Abnormal secretion of VF leading to an increase in IR may be one of the pathogenesis mechanisms of GDM. Kaygusuz I et al. [9] found that the serum VF level was significantly elevated in pregnant women compared with non-pregnant women. Fasshauer M et al. [10] found that an abnormally high level of VF can lead to hypertensive disorders of pregnancy and GDM by increasing IR. However, there is no uniform conclusion about whether the level of VF in GDM pregnant women is lower or higher than that in pregnant women without GDM [11–13]. In this study, the ELISA results showed that the maternal blood and cord blood VF levels in the GDM group were significantly higher than those in the normal control group, with a statistically significant difference ($P < 0.01$), and the IHC results showed that the VF expression level in the placental tissues of the pregnant women in the GDM group was significantly higher than that in the normal control group, with a statistically significant difference ($P < 0.01$). Although the current results from the research on the relationship between VF and GDM are quite different [14,15], they show that VF is related to the occurrence and development of GDM.

Table 4

Relationship between maternal blood and cord blood VF levels and pregnancy outcomes in the GDM group [frequency (%)].

Pregnancy outcome	Maternal blood of GDM group (n = 68)		χ^2	P	Cord blood of GDM group (n = 68)		χ^2	P
	High VF (n = 34)	Low VF (n = 34)			High VF (n = 34)	Low VF (n = 34)		
Caesarean section	22 (64.7)	15 (44.1)	2.91	0.09	21 (61.8)	16 (47.1)	1.48	0.22
Postpartum haemorrhage	5 (14.7)	2 (5.9)	1.43	0.23	5 (14.7)	2 (5.9)	1.43	0.23
Postpartum fever	3 (8.8)	0 (0.0)	3.14	0.08	3 (8.8)	0 (0.0)	3.14	0.08
Diabetic ketoacidosis	1 (2.9)	0 (0.0)	1.01	0.31	1 (2.9)	0 (0.0)	1.01	0.31
Post-term infant	1 (2.9)	2 (5.9)	0.35	0.56	0 (0.0)	3 (8.8)	3.14	0.08
Intrauterine distress	3 (8.8)	1 (2.9)	1.06	0.30	4 (11.8)	0 (0.0)	4.25	0.04
Foetal macrosomia	6 (17.6)	5 (14.7)	0.11	0.74	6 (17.6)	5 (14.7)	0.11	0.74
Small for gestational age	4 (11.8)	2 (5.9)	0.73	0.39	4 (11.8)	2 (5.9)	0.73	0.39
Amniotic fluid contamination	6 (17.6)	1 (2.9)	3.98	0.04	4 (11.8)	3 (8.8)	0.16	0.69
Neonatal asphyxia	4 (11.8)	0 (0.0)	4.25	0.04	1 (2.9)	3 (8.8)	2.06	0.30
Respiratory distress syndrome	3 (8.8)	4 (11.8)	0.16	0.69	3 (8.8)	4 (11.8)	0.16	0.69
Neonatal cyanine	2 (5.9)	1 (2.9)	0.35	0.56	3 (8.8)	0 (0.0)	3.14	0.08
Transient tachypnoea of the newborn	1 (2.9)	0 (0.0)	1.01	0.31	1 (2.9)	1 (2.9)	1.01	0.31
Neonatal infection	3 (8.8)	0 (0.0)	3.14	0.08	2 (5.9)	1 (8.3)	0.35	0.56
Neonatal hypoglycaemia	12 (35.3)	8 (23.5)	1.12	0.29	14 (41.2)	6 (17.6)	4.53	0.03
Neonatal hyperbilirubinemia	6 (17.6)	5 (14.7)	0.11	0.74	6 (17.6)	5 (14.7)	0.11	0.74

Table 5

Relationship between expression of VF and pregnancy outcome in placental tissue of GDM group [Example (%)].

Pregnancy outcome	Placental tissues of GDM group (n = 68)		χ^2	P
	High VF (n = 34)	Low VF (n = 34)		
Cesarean section	21 (61.8)	16 (47.1)	1.48	0.22
Postpartum hemorrhage	5 (14.7)	2 (5.9)	1.43	0.23
Postpartum fever	2 (5.9)	1 (2.9)	0.35	0.56
Diabetic ketoacidosis	1 (2.9)	0 (0.0)	1.01	0.31
Post-term infant	1 (2.9)	2 (5.9)	0.35	0.56
Intrauterine distress	4 (11.8)	0 (0.0)	4.25	0.04
Fetal macrosomia	7 (20.6)	4 (11.8)	0.98	0.32
Small for gestation age	4 (11.8)	2 (5.9)	0.73	0.39
Amniotic fluid contamination	6 (17.6)	1 (2.9)	3.98	0.04
Neonatal asphyxia	4 (11.8)	0 (0.0)	4.25	0.04
Respiratory distress syndrome	6 (17.6)	1 (2.9)	3.98	0.04
Neonatal cyanine	3 (8.8)	0 (0.0)	3.14	0.08
Neonatal wet lung	1 (2.9)	0 (0.0)	1.01	0.31
Neonatal infection	3 (8.8)	0 (0.0)	3.14	0.08
Neonatal hypoglycemia	16 (47.1)	4 (11.8)	10.20	0.00 ^a
Neonatal hyperbilirubinemia	8 (23.5)	3 (8.8)	2.71	0.10

Note: a represents a comparison between groups, P < 0.01.**Table 6**

Relationship between maternal blood and cord blood AHSG levels and pregnancy outcomes in GDM group [example (%)].

Pregnancy outcome	Maternal blood of GDM group (n = 68)		χ^2	P	Cord blood of GDM group (n = 68)		χ^2	P
	High AHSG (n = 34)	Low AHSG (n = 34)			High AHSG (n = 34)	Low AHSG (n = 34)		
Cesarean section	15 (44.1)	22 (64.7)	2.91	0.09	16 (47.1)	21 (61.8)	1.48	0.22
Postpartum hemorrhage	6 (17.6)	1 (2.9)	3.98	0.04	3 (8.8)	4 (11.8)	0.16	0.69
Postpartum fever	3 (8.8)	0 (0.0)	3.14	0.08	2 (5.9)	1 (8.3)	0.35	0.56
Diabetic ketoacidosis	1 (2.9)	0 (0.0)	1.01	0.31	0 (0.0)	1 (2.9)	1.01	0.31
Post-term infant	1 (2.9)	2 (5.9)	0.35	0.56	1 (2.9)	2 (5.9)	0.35	0.56
Intrauterine distress	4 (11.8)	0 (0.0)	4.25	0.04	2 (5.9)	2 (5.9)	0.00	1.00
Fetal macrosomia	5 (14.7)	6 (17.6)	0.11	0.74	5 (14.7)	6 (17.6)	0.11	0.74
Small for gestation age	2 (5.9)	4 (11.8)	0.73	0.39	2 (5.9)	4 (11.8)	0.73	0.39
Amniotic fluid contamination	4 (11.8)	3 (8.8)	0.16	0.69	4 (11.8)	3 (8.8)	0.16	0.69
Neonatal asphyxia	2 (5.9)	2 (5.9)	0.00	1.00	2 (5.9)	2 (5.9)	0.00	1.00
Respiratory distress syndrome	4 (11.8)	3 (8.8)	0.16	0.69	4 (11.8)	3 (8.8)	0.16	0.69
Neonatal cyanine	2 (5.9)	1 (2.9)	0.35	0.56	2 (5.9)	1 (2.9)	0.35	0.56
Neonatal wet lung	0 (0.0)	1 (2.9)	1.01	0.31	0 (0.0)	1 (2.9)	1.01	0.31
Neonatal infection	2 (5.9)	1 (8.3)	0.35	0.56	2 (5.9)	1 (8.3)	0.35	0.56
Neonatal hypoglycemia	14 (41.2)	6 (17.6)	4.53	0.03	8 (23.5)	12 (35.3)	1.12	0.29
Neonatal hyperbilirubinemia	6 (17.6)	5 (14.7)	0.11	0.74	8 (23.5)	3 (8.8)	2.71	0.10

AHSG, a PP63 protein, is mainly synthesized and secreted from the liver and can be secreted from the placenta during pregnancy [16]. Recent studies have shown that AHSG is an insulin-resistant protein which plays a vital role in the formation of IR and glycolipid metabolism disorders. High levels of AHSG and human obesity are closely related to the onset of type 2 diabetes mellitus [17,18]. By measuring serum AHSG and glucose and lipid metabolism related indicators, such as triglycerides, serum total cholesterol, fasting blood glucose, and glycosylated haemoglobin, we found that AHSG levels in the serum of GDM pregnant women were closely related to IR and related to glycolipid metabolism [19]. The study found that patients with GDM have more severe IR, even reaching the level of IR in type 2 diabetes mellitus. AHSG may be associated with the formation of IR in pregnant and non-pregnant women, and IR is an important cause of GDM [20]. A number of domestic and foreign studies [21–23] have shown that maternal serum AHSG levels are higher during pregnancy and more prominent in GDM pregnant women. GDM pregnant women are more prevalent than normal pregnant women. In this study, serum AHSG levels in pregnant women with GDM were analysed. The results showed that the levels of AHSG in maternal blood and cord blood were significantly higher in the GDM group than in the normal control group, with statistically significant differences ($F = 21.30$, $P < 0.01$; $F = 36.82$, $P < 0.01$, respectively). Consistent with domestic and international

research, AHSG may be related to the occurrence and development of GDM.

At present, there are many studies on VF, AHSG and pregnancy outcomes of GDM patients, and there is no uniform conclusion. There are relatively many studies comparing the relationship between VF and intrauterine growth and development. Serum VF level is considered one of the important influencing factors for neonatal birth weight [24]. It is speculated that maternal serum VF levels may play an important role in intrauterine growth. Akturk et al. [25] did not find maternal blood VF levels related to neonatal weight; Telejko et al. [26] found that the expression of the VF protein in foetal tissue was positively correlated with neonatal birth weight. In this study, the maternal and foetal pregnancy outcomes of the subjects were collected, and the outcomes of the GDM group and the normal control group were compared. The incidence of diabetic ketoacidosis in the GDM insulin treatment group was higher than that in the GDM diet control group and the normal control group, and there was a statistically significant difference ($P < 0.05$). The incidence of neonatal hypoglycaemia and respiratory distress syndrome was higher in the GDM group than that in the normal control group, and there was a statistically significant difference ($P < 0.05$). Although the incidence of other adverse birth outcomes, such as caesarean section rate, postpartum haemorrhage, postpartum fever, foetal macrosomia, amniotic fluid

contamination, small for gestational age and foetal distress, in the GDM group as higher than that in the normal control group, there was no statistically significant difference ($P > 0.05$). It is necessary to expand the sample size to do further studies. At present, the research on VF, AHSG and GDM is limited, and there are great differences in the results. In this study the incidence of adverse outcomes in mothers and children with high expression of VF and AHSG in the GDM group was higher than that in the low expression group, and there was a statistically significant difference ($P < 0.05$). The relationship between the mechanism of VF and AHSG in GDM and their relationship with GDM pregnancy outcomes needs further study.

In summary, the occurrence of GDM is the result of multiple factors. VF and AHSG play an important role in the occurrence and development of GDM. The physiological and pathological effects and molecular biological mechanisms of VF and AHSG in the development of GDM will be gradually clarified. This study may also provide a new idea and a direction for preventing and monitoring GDM and improving maternal and foetal pregnancy outcomes for GDM.

Conflicts of interest statement

The authors declare that we have no conflicts of interest regarding the publication of this paper.

Acknowledgments

This study was supported by Traditional Chinese Medicine Science and Technology Development Plan Project of Jiangsu province (No. YB201972).

References

- [1] Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The pathophysiology of gestational diabetes mellitus. *Int J Mol Sci* 2018;19(11):3342.
- [2] Harlev A, Wiznitzer A. New insights on glucose pathophysiology in gestational diabetes and insulin resistance. *Curr Diab Rep* 2010;10(3):242–7.
- [3] Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* 2010;316(2):129–39.
- [4] Lewandowski KC, Stojanovic N, Press M, Tuck SM, Szosland K, Bienkiewicz M, et al. Elevated serum levels of VF in gestational diabetes: a comparative study across various degrees of glucose tolerance. *Diabetologia* 2007;50(5):1033–7.
- [5] Mathews ST, Singh GP, Ranalletta M. Improved insulin sensitivity and resistance to weight gain in mice null for the Ahsg gene. *Diabetes* 2002;1(8):2450–8.
- [6] Jensen DM, Korsholm L, Ovesen P, Beck-Nielsen H, Mølsted-Pedersen L, Damm P. Adverse pregnancy outcome in women with mild glucose intolerance: is there a clinically meaningful threshold value for glucose? *Acta Obstet Gynecol Scand* 2008;87(1):59–62.
- [7] American Diabetes Association. Classification and diagnosis of diabetes. *Diabetes Care* 2015;38:S8–16.
- [8] Wei YM, Yang HX. Enlightenment from the practical guide to gestational diabetes in the international obstetrics and gynecology alliance. *Chin J Prev Med* 2016;19(5):321–2.
- [9] Kaygusuz I, Gumus II, Yilmaz S, Simavli S, Uysal S, Derbent AU, et al. Serum levels of visfatin and possible interaction with iron parameters in gestational diabetes mellitus. *Gynecol Obstet Invest* 2013;75(3):203–9.
- [10] Fasshauer M, Waldeyer T, Seeger J, Schrey S, Ebert T, Kratzsch J, et al. Serum levels of the adipokine visfatin are increased in preeclampsia. *Clin Endocrinol (Oxf)* 2008;69(1):69–73.
- [11] Kuang DF, Hua SF, Han YH. Significance of VF expression in patients with gestational diabetes mellitus. *J Pract Obstetr Gynecol* 2013;(5):362–5.
- [12] Wei BX, Zou DF, An JD. Effects of VF levels in maternal serum and umbilical blood on pregnancy outcome of the cases with gestational diabetes mellitus. *Mat Child Health Care China* 2012;(2):180–2.
- [13] Fu Y, Wu NN, Ma W. Clinical value of VF and its expression in maternal serum, placenta and umbilical blood in gestational diabetes mellitus. *Chin J Diabetes* 2016;(5):431–4.
- [14] Park S, Kim MY, Baik SH, Woo JT, Kwon YJ, Daily JW, et al. Gestational diabetes is associated with high energy and saturated fat intakes and with low plasma visfatin and adiponectin levels independent of prepregnancy BMI. *Eur J Clin Nutr* 2013;67(2):196–201.
- [15] Tsiotra PC, Halvatsiotis P, Patsouras K, Maratou E, Salamalekis G, Raptis SA, et al. Circulating adipokines and mRNA expression in adipose tissue and the placenta in women with gestational diabetes mellitus. *Peptides* 2018;101:157–66.
- [16] Pedersen KO. Fetuin a new globulin isolated from serum. *Nature* 1944;154(11):575.
- [17] Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci USA* 2000;97(26):14478–83.
- [18] Reinehr T, Roth CL. Fetuin-A and its relation to metabolic syndrome and fatty liver disease in obese children before and after weight loss. *J Clin Endocrinol Metab* 2008;93(11):4479–85.
- [19] Xie M. Effect of serum fetuin A on gestational diabetes patients. *Hebei Med J* 2015;1:90–1.
- [20] Kalabay L, Cseh K, Pajor A, Baranyi E, Csákány GM, Melczar Z, et al. Correlation of maternal serum fetuin/alpha2-HS-glycoprotein concentration with maternal insulin resistance and anthropometric parameters of neonates in normal pregnancy and gestational diabetes. *Eur J Endocrinol* 2002;147(2):243–8.
- [21] He P, Wu A. Correlation between AHSG protein and gestational diabetes mellitus. *Mat Child Health Care China* 2013;(18):2889–91.
- [22] Ji B, He LC, Guo F. Effect of serum fetuin A on gestational diabetes mellitus. *Guangdong Med J* 2011;(24):3197–9.
- [23] Wang X, Li B, Shang LX. An analysis on plasma AHSG level and the relationship between AHSG and insulin resistance among pregnant women with GDM. *Zhejiang J Prev Med* 2015;(11):1090–6.
- [24] Yajnik CS. Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. *J Nutr* 2004;134(1):205–10.
- [25] Akturk M, Altinova AE, Mert I, Buyukkagnici U, Sargin A, Arslan M, et al. Visfatin concentration is decreased in women with gestational diabetes mellitus in the third trimester. *J Endocrinol Invest* 2008;31(7):610–3.
- [26] Telejko B, Kuzmicki M, Zonenberg A, Szamatowicz J, Wawrusiewicz-Kurylonek N, Nikolajuk A, et al. Visfatin in gestational diabetes: serum level and mRNA expression in fat and placental tissue. *Diabetes Res Clin Pract* 2009;84(1):68–75.