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ORIGINAL ARTICLE

Decreased Expression of miR-185 in Serum and Placenta of Patients with Gestational Diabetes Mellitus

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SUMMARY

Background: **The current study aims to investigate the expression of miR-185 in serum and placenta of patients with gestational diabetes mellitus (GDM) and its relationship with insulin resistance.**
Methods: **The levels of fasting blood glucose (FPG) and fasting insulin (FINS) were measured and the insulin resistance index (HOMA-IR) was calculated. The levels of serum and placental miR-185 were detected by real-time quantitative PCR (qRT-PCR). The relationship between serum and placental miR-185 levels and HOMA-IR was analyzed using Pearson's correlation assay. The diagnostic value of miR-185 was assessed using receiver operating characteristic curve (ROC).**
Results**: Compared with the control group, the serum and placental level of miR-185 was lowest in the severe GDM group and lower in the mild GDM group. Furthermore, the serum levels of FPG, FINS, and HOMA-IR gradually increased in the mild GDM group and the severe GDM group compared to those in the control group. Further study showed that serum and placental miR-185 levels were negatively correlated with HOMA-IR in 156 patients with GDM. ROC analysis showed that the area under the curve (AUC) was 0.927 with the sensitivity and specificity of 0.865 and 0.838, respectively, indicating serum miR-185 could differentiate patients with GDM from controls.**
Conclusions: **The down-regulation of miR-185 expression in serum and placenta of pregnant women with GDM is negatively correlated with HOMA-IR, suggesting that the decrease of miR-185 may play an important role in the occurrence and development of GDM.**
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KEY WORDS

gestational diabetes mellitus, serum, placenta, miR-185

INTRODUCTION

Gestational diabetes mellitus (GDM) is the first disorder of abnormal glucose tolerance found in women after pregnancy [1]. In China, the incidence of GDM is about 12.2%. GDM is liable to cause dangerous complications such as macrosomia, excessive amniotic fluid, dystocia, neonatal hypoglycemia and so on, which seriously threatens the life and health of mothers and infants [2]. In addition, the risk of type 2 diabetes in GDM women increased significantly within 5 to 16 years after delivery, and the risk of type 2 diabetes in their offspring in-

creased significantly in the future [3,4]. Therefore, timely diagnosis and active treatment are essential to reduce the incidence of adverse pregnancy outcomes in patients with GDM.

microRNA (miRNA, miR) is a kind of endogenous non-coding RNA [5]. By complementary matching with the 3' end of target gene, post-transcriptional regulation of genes can be realized [6]. It has been found that abnormal expression of some miRs is associated with GDM [7]. Compared with healthy pregnant women during pregnancy, the levels of miR-29a and miR-222 in serum of gestational diabetes mellitus are significantly down-regulated [8]. Additionally, the expression of miR-1268 was up-regulated in peripheral blood of GDM patients, while the expression of miR-181 was down-regulated [9]. These studies have laid the foundation for the application of miRs in the early diagnosis of GDM and provided the possibility for early intervention through dietary therapy and exercise, thereby improving the pregnancy syndrome, and ensuring the health of mothers and infants [7,10].

Abnormal expression of miR-185 has been widely reported in metabolic disorders [11,12]. In individuals with high cholesterol, the level of serum miR-185 demonstrated a 5-fold increase compared to that of control individuals [11]. In contrast, circulating miR-185 is found to be decreased in the progression of type 2 diabetes in Zucker diabetic fatty rats (ZDF rats) [12]. However, the role of miR-185 in GDM has never been explored. In this study, we detected the levels of miR-185 in serum and placenta of pregnant women with GDM and analyzed the correlation between miR-185 and insulin resistance, so as to provide a new basis for timely diagnosis and treatment of GDM.

MATERIALS AND METHODS

General information

For this study, 156 GDM pregnant women aged 22 - 43 (29.37 ± 4.81) years and 36 - 40 (38.12 ± 3.24) weeks of gestation were selected from September 2016 to September 2017 in Second Maternal and Child Health Hospital of Jinan City. According to blood sugar control, the patients were divided into mild GDM group (108 cases) and severe GDM group (48 cases). Diagnostic criteria: According to the criteria for GDM diagnosis and classification established by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) in 2010, pregnant women were given an oral glucose tolerance test (OGTT) of 75g at 24 - 28 weeks of gestation (fasting level ≤ 5.1 mM, 1 hour ≤ 10.0 mM, 2 hours ≤ 8.5 mM). The diagnosis of GDM was made as long as the plasma level was above the critical value at one time point. Inclusion criteria for mild GDM patients were as follows: fasting blood glucose < 5.8 mmol/L, 2 hours postprandial blood glucose < 6.7 mmol/L; severe GDM patients included criteria: fasting blood glucose < 5.8 mmol/L, 2 hours postprandial blood glucose > 6.7 mmol/L. Another 100 healthy pregnant women admitted to Second Maternal and Child Health Hospital of Jinan City during the same period were selected as the control group; they were 2l - 40 (28.45 + 4.67) years old, and the gestational weeks of delivery were 37 - 40 (39.04 + 1.28) weeks. All the subjects signed the informed consent. This study was approved by the Ethics Committee of Second Maternal and Child Health Hospital of Jinan City.

Inclusion/exclusion criteria

Inclusion criteria: (1) initial diagnosis, no insulin treatment; (2) single birth; (3) no stillbirth history, no history of macrosomia. Exclusion criteria: (1) patients with cardiovascular diseases such as hypertension and coronary heart disease; (2) patients with abnormal functions of heart, liver and kidney; (3) patients with endocrine diseases, tumors, severe infections, etc.

Sample collection of blood and placenta

On the morning of the cesarean section, 10 mL fasting elbow venous blood was collected from all subjects. Fasting plasma glucose (FPG) and fasting insulin (FINS) levels were measured immediately by automatic biochemical instrument (Abbott Pharmaceutical Co., Ltd). Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated as follows: HOMA-IR = FPG (mmol/L)*FINS (mlU/L)/22.5. Another tube of blood sample was stored in a - 80°C freezer for the determination of miR-185 level.

For placenta collection, within 10 minutes after delivery, appropriate placental tissue was cut near the umbilical cord and frozen in liquid nitrogen for reserve.

Total RNA Extraction

Serum and placental RNA were extracted by QIAGEN microRNA extraction kit (Germany QIAGEN Company).

Real-time quantitative PCR (qRT-PCR)

Reverse transcription of RNA was performed with QIAgen kit (QIAGEN GmbH, Hilden, Company). In brief, the reaction volume was 1 μg total RNA (1 μL), 4 μL 5 x miScript Hiflex buffer, 2 μL 10 x miScript Nucleics Mix, 2 μL miR-185 RT primer, 11 μL RNase-free water. The reaction procedure was 37°C for 1 hour and 95°C 5 minutes. The expression of miR-185 was determined by Takara qRT-PCR kit (Takara Company of Japan). The 20 μL reaction system was formulated as 6.8 μL SYBER GREEN, 0.5 μL forward primer, 0.5 μL reverse primer, 2.0 μL cDNA, and 10 μL RNase-free water. The reaction conditions were 95°C 30 seconds; 40 cycles of 95°C 5 seconds, 60°C 35 seconds. U6 was used as an internal reference.

Statistical analysis

The data are represented as the mean ± standard deviation (SD). The two-tailed unpaired Student's *t*-tests were used for comparisons of two groups. The one-way

Table 1. Comparison of clinical characteristics between the GDM group and control group.

Groups	Control	GDM
Cases	100	156
Age (years)	28.67 ± 4.91	29.46 ± 4.95
Gravida (times)	2.08 ± 0.65	2.12 ± 0.54
Para (times)	1.07 ± 0.23	1.10 ± 0.28
The weight gain during pregnancy (g)	12.78 ± 4.23	15.12 ± 4.56 ***
BMI during delivery (kg/m^2)	26.13 ± 3.02	27.86 ± 3.57 *
The gestational weeks (weeks)	39.03 ± 1.26	38.12 ± 3.12 **
Neonatal weight (g)	3,421.08 ± 375.76	3,496.08 ± 478.56
Adverse events (times)	0.24 ± 0.07	0.48 ± 0.14 ***

ANOVA multiple comparison test (SPSS 20.0) followed by Tukey post hoc test were used for comparisons of two more groups. Receiver operating characteristic (ROC) curves were used to assess miR-291-3p as a biomarker, and the area under the curve (AUC) was reported (version 20.0, IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA). Pearson's correlation analysis was carried out to analyze the relationship between serum and placental miR-185 levels and HOMA-IR. $p < 0.05$ was considered significant.

RESULTS

Comparison of clinical characteristics between the GDM group and control group

There was no significant difference between the GDM group and control group in age, number of pregnancies, parity, and neonatal weight ($p > 0.05$). In contrast, the weight gain during pregnancy, BMI during delivery, and the occurrence of adverse events in GDM group were significantly higher than those in the control group ($p < 0.05$). Meanwhile, the gestational weeks of pregnant women in GDM group were significantly smaller than those in control group ($p < 0.05$), as shown in Table 1.

Comparison of serum and placental miR-185 levels between patients with different severity of GDM and healthy controls

Compared with the control group (1 ± 0.61), the serum level of miR-185 in the mild GDM group (0.42 ± 0.22) was significantly decreased. In addition, the level of serum miR-185 in the severe GDM group (0.17 ± 0.111) was significantly lower than that in the mild GDM group (0.42 ± 0.22) and the control group (1 ± 0.61) (Figure 1A). In addition, the level of placental miR-185 in the mild GDM group (0.49 ± 0.31) was significantly lower than that in the control group (1 ± 0.68) (Figure 1B). Furthermore, the level of placental miR-185 in severe GDM group (0.12 ± 0.10) was significantly lower than that in control group (1 ± 0.68) and mild GDM group (0.49 ± 0.31) (Figure 1B).

Comparison of blood glucose and insulin levels in patients with different severity of GDM

The serum levels of FPG, FINS, and HOMA-IR in the mild GDM group were significantly higher than those in the control group (Figure 2A, 2B, and 2C). Moreover, the serum levels of FPG, FINS, and HOMA-IR in the severe GDM group were significantly higher than those in the control group and the mild GDM group (Figure 2A, 2B, and 2C).

Relationship between serum and placental miR-185 levels and HOMA-IR in patients with GDM

Serum miR-185 levels in 156 patients with GDM were negatively correlated with HOMA-IR ($r = -0.436$, $p < 0.001$), as shown in Figure 3A. Meanwhile, the level of placental miR-185 was also negatively correlated with HOMA-IR ($r = -0.601$, $p < 0.001$), as shown in Figure 3B.

Significance of miR-185 in early diagnosis and severity diagnosis of GDM

The diagnostic significance of miR-185 was analyzed by ROC curve. The results showed that the area under the curve (AUC) of miR-185 in diagnosing GDM was 0.927 (95% CI was 0.894 - 0.960). The best cutoff point of microRNA-185 was 0.60, and the sensitivity and specificity for diagnosis of GDM were 0.865 and 0.838, respectively.

DISCUSSION

The global incidence of GDM is increasing year by year. The short-term or long-term harm to mothers and infants cannot be underestimated [13]. More than half of GDM pregnant women will have abnormal glucose

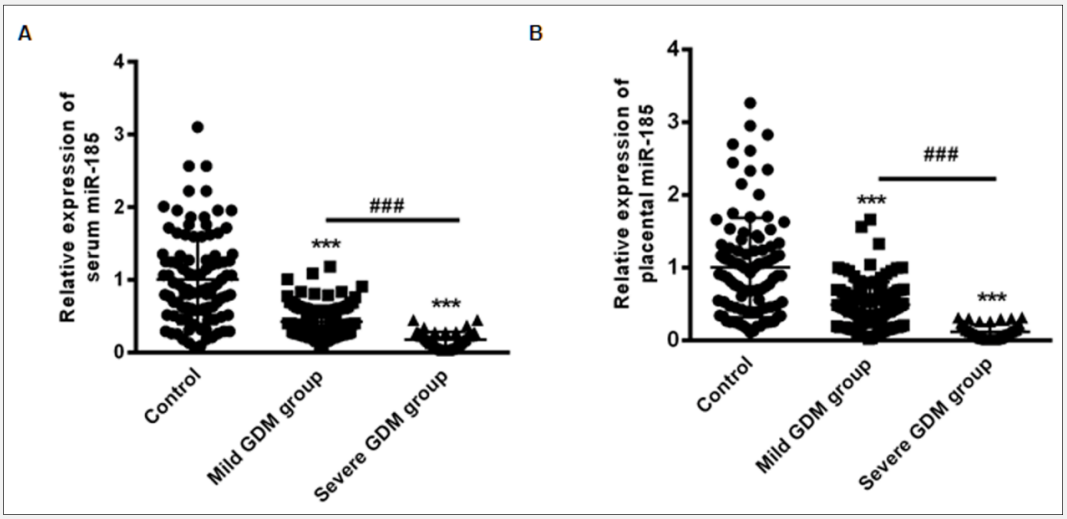

Figure 1. Real time PCR analysis of the serum and placental miR-185 levels between patients with different severity of GDM and healthy controls.

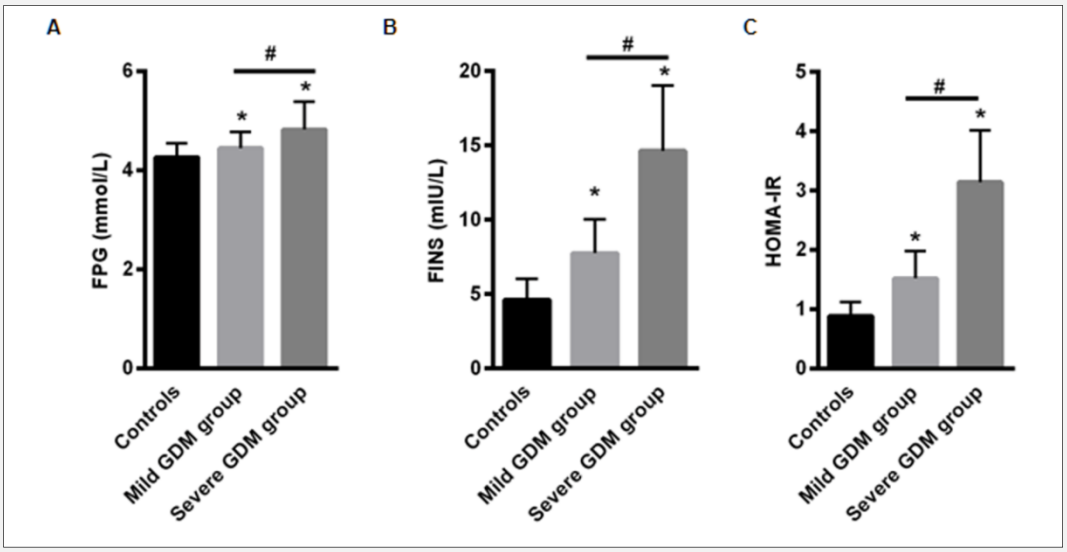

Figure 2. Comparison of blood glucose and insulin levels in patients with different severity of GDM. The serum levels of FPG (A), FINS (B), and HOMA-IR (C) were determined in the severe GDM group, mild GDM group, and control group.

tolerance in the five years after delivery and the incidence of diabetes in their offspring is also significantly increased [14]. Therefore, it is generally considered that GDM is the early stage of type 2 diabetes [3]. According to the new diagnostic criteria of GDM established by IADPSG in 2010, the population of GDM has in-

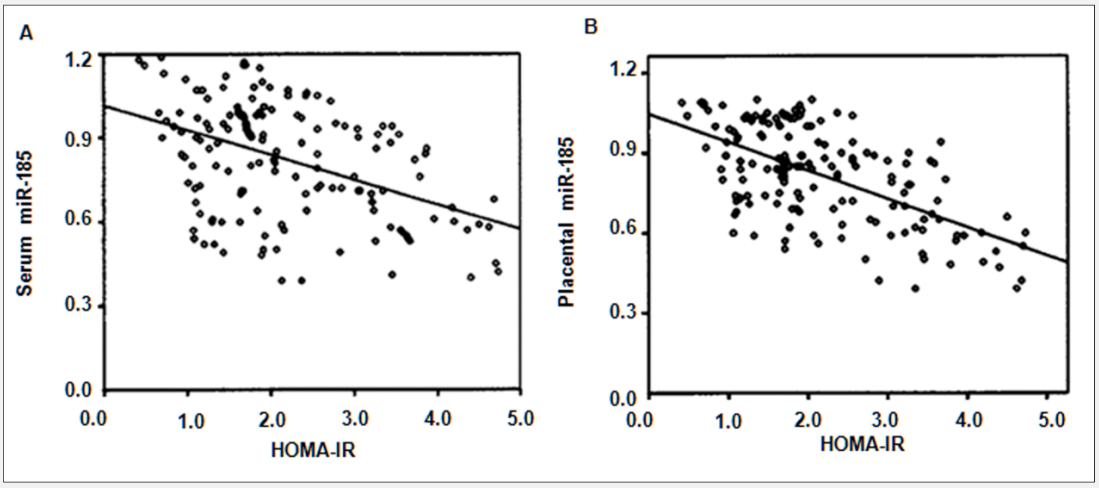

Figure 3. Person's assay analyzed the correlation between serum and placental miR-185 levels and HOMA-IR in patients with GDM. (A) Serum miR-185 levels in 156 patients with GDM were negatively correlated with HOMA-IR. (B) The level of placental miR-185 was also negatively correlated with HOMA-IR.

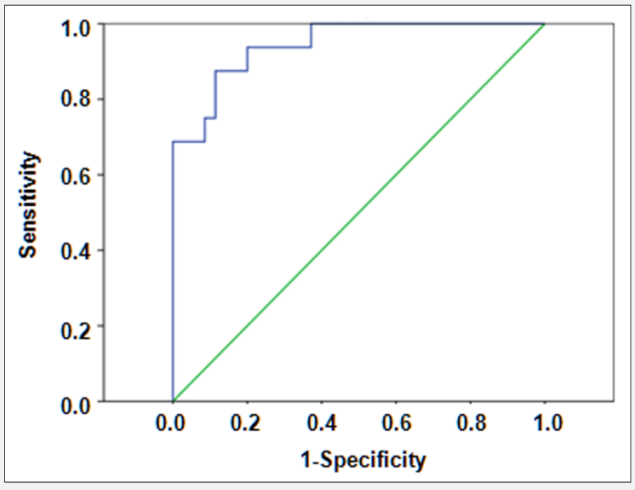

Figure 4. ROC analysis was carried out to analyze the diagnostic value of serum miR-185 in GDM patients.

creased, and attention has been paid to the prevention, early diagnosis, treatment and postpartum situation of GDM [4]. GDM pregnant women generally have high risk factors related to diabetes, such as old age, obesity, family history of diabetes, history of macrosomia childbirth, history of unprovoked abortion, stillbirth and so on [1,15]. In the present study, we found that the weight gain during pregnancy, BMI during childbirth, and the number of adverse events in GDM group were significantly higher than those in healthy control group, which indicated that the weight of pregnant women had a certain correlation with the occurrence of GDM. Because

GDM increases the risk of adverse pregnancy outcomes and increases the natural or cesarean premature delivery rate, the gestational weeks of delivery in the GDM group are significantly less than those in the healthy control group [16,17].

In recent years, the role of miRs in type 2 diabetes has attracted much attention, but the relationship between miRs and GDM has rarely been studied [10,18]. MiRs are a class of 18 - 25 nt-sized endogenous, non-coding small RNAs, which promote the degradation or translation inhibition of the target gene sequence by complementarily binding to the 3' UTR of the target gene, thereby participating in the regulation of cell proliferation, apoptosis, tumorigenesis, and many other physiological or pathological processes, including glucose metabolism [19,20]. Studies have shown that the expression of miRs in serum and placenta of pregnant women is related to infection, smoking, malnutrition, and other adverse pregnancy conditions [21,22]. MiR-185 is closely related to glycolipid metabolism [12,23,24]. In diabetic rats, the expression of miR-185 was decreased [12]. In mouse islets, high glucose significantly enhanced the level of miR-185 [23]. Moreover, miR-185 is also shown to suppress beta-cell dysfunction in diabetes via targeting SOCS3 [24].

This study found that the serum and placental levels of miR-185 in severe GDM group were significantly lower than those in the control group and mild GDM group, suggesting that the expression of miR-185 was related to the severity of GDM and might participate in the occurrence and development of GDM. Meanwhile, our data showed that the serum levels of FPG, FINS, and HOMA-IR in the severe GDM group were significantly higher than those in the control group and the mild GDM group. In addition, serum and placental levels of miR-185 in GDM patients were negatively correlated with HOMA-IR, which further demonstrated that the reduction of miR-185 may play an important role in the development of GDM. ROC analysis showed that miR-185 has high sensitivity and specificity in the diagnosis of GDM. The above results indicate that miR-185 is feasible in clinical diagnosis of GDM, but large samples are necessary for validation.

CONCLUSION

This study found that the down-regulation of serum and placenta miR-185 in pregnant women with GDM is related to the severity of GDM and negatively related to HOMA-IR, which indicates that the change of miR-185 expression is related to the occurrence and development of GDM, and may provide some reference value for the treatment and prognosis of GDM. However, the mechanism of the occurrence and development of GDM is complex, and the specific mechanism of the role of miR-185 in GDM needs to be further studied.

Declaration of Interest:
We declare no conflicts of interest.

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