# Relationship Between Serum microRNA-372-3p and Glucose Transporter 4 Levels and Insulin Resistance in Gestational Diabetes Mellitus

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**Abstract:** *Objective:* To observe the changes in insulin resistance in patients with gestational diabetes mellitus (GDM) based on the detection of serum microRNA-372-3p and glucose transporter protein 4 (GLUT4) levels. *Methods:* We conducted a retrospective cohort study of 42 patients who were diagnosed with GDM and hospitalized in our hospital during the period from January 2017 to December 2021 and another 42 patients who had normal pregnancy during the same period by collecting their clinical data. We analyzed their serum microRNA expression profiles and miR-372-3p levels to study the relationship between GDM and insulin resistance. *Results:* The relative expression of miR-372-3p in the serum of patients in the GDM group was significantly higher than that of patients in the control group, but the GLUT 4 level of the GDM group was significantly lower than that of the control group ($P < 0.05$). Compared with the control group, the GDM group had significantly higher levels of fasting blood glucose (FBG), fasting insulin (FINS), 2-hour postprandial blood glucose (2h-BG), total cholesterol (TC), triglyceride (TG), and homeostatic model assessment for insulin resistance (HOMA-IR) index but significantly lower homeostasis model assessment of β-cell function (HOMA-β) index ($P < 0.05$). The relative expression of miR-372-3p in serum was independently and positively correlated with HOMA-IR, while the level of GLUT4 was independently and negatively correlated with HOMA-IR ($P < 0.05$). *Conclusion:* Glycosylated hemoglobin test in the early stages of pregnancy (12–13 weeks of gestation) is important to ensure the health of pregnant women and fetuses. The screening and intervention for elevated glucose in pregnant women act as a guideline for the treatment of GDM. Patients with insulin resistance and related complications such as hyperinsulinemia and hypoglycemia should be given priority.

**Keywords:** Gestational diabetes mellitus; microRNA-372-3p; Glucose transporter; Insulin resistance

## 1. Introduction

Gestational diabetes mellitus (GDM) is a disease state in which diabetes occurs during pregnancy. The prevalence of GDM ranges from 7.4% to 16.6%. For women of childbearing age, having GDM increases the risk of type 2 diabetes mellitus, preterm births, miscarriages, babies with low birth weight and congenital malformations, and neonatal mortality; moreover, the risk of perinatal mortality also increases with GDM. Therefore, the prevention and treatment of GDM is of great importance to women's health. The prevalence of GDM in the Chinese population is about 12%–18% [1-6]. In recent years, with the increased incidence of glucose metabolism abnormalities in pregnancy, GDM has become a major factor affecting the health of both mother and child. Elevated glucose in pregnancy is closely associated with insulin resistance, which, in turn, leads to metabolic abnormalities in the body and subsequently affects placental

function. The relationship between insulin resistance and the development of GDM is still inconclusive, and no clear mechanism has been proposed in the study of the pathogenesis of GDM. Serum glucose transporter 4 (GLUT4), as a novel glucose transporter, plays an important role in the regulation of glucose homeostasis, and studies have demonstrated its pivotal role in obesity and metabolic syndrome [7-10]. In recent years, an increasing number of studies have shown that abnormally elevated serum GLUT4 levels ($\geqslant$ 10 ng/mL) and significantly increased insulin resistance in patients with GDM correlate with obesity, hypertension, and other metabolic syndromes. The aim of this study was to investigate the relationship between elevated glucose levels and insulin resistance during pregnancy and its associated mechanisms.

## 2. Data and methods
### 2.1. General information
We conducted a retrospective cohort study of 42 patients who were diagnosed with GDM and hospitalized in our hospital during the period from January 2017 to December 2021 and another 42 patients who had normal pregnancy during the same period. Information on gender, age, body mass index (BMI), height, waist circumference, body mineral density (BMD), and microRNA (miRNA) expression results was collected. Inclusion criteria: (i) patients with a confirmed diagnosis of GDM; (ii) patients with BMI $\geqslant$ 30 kg/m$^2$; (iii) patients with previous GDM before 28 weeks of gestation; (iv) patients with type 1 diabetes, other autoimmune diseases, or a family history of diabetes. A pre-pregnancy fasting glucose test was done for all the patients, and their blood was collected at the 3rd, 4th, and 6th week of gestation. Their plasma calcitonin gene-related peptide (CGRP), S100 calcium binding protein A4 (S100A4), and miR-372-3p levels were measured by real-time quantitative polymerase chain reaction (RT-PCR). Their serum microRNA expression profiles and miR-372-3p levels were analyzed to investigate the relationship between GDM and insulin resistance.

### 2.2. Design
#### 2.2.1. Detection of metabolism-related indexes
6mL of venous blood was collected from patients in the GDM group on the day after admission and from the control subjects at physical examination; the blood was centrifuged at 3000 RPM for 10 min (centrifugation radius, 8 cm), and the supernatant was taken and stored at -80°C. Serum total cholesterol (TC) and triglyceride (TG) levels were measured using BS-450 automatic biochemistry analyzer (Shenzhen Myriad Biomedical Electronics Co., Ltd.). The glucose oxidase method was used to detect fasting plasma glucose (FPG) levels, while radioimmunoassay was used to detect fasting insulin (FINS) levels; glucose tolerance test was performed to obtain their 2-h postprandial blood glucose (2h-BG) levels.

#### 2.2.2. Detection of the relative expression of serum miR-372-3p and GLUT4 levels
Total serum RNA was extracted using TRIzol Total RNA Extraction Kit (Shanghai Rongwida Industrial Co., Ltd.), the concentration and purity were detected by ultraviolet (UV) spectrophotometer, and the integrity was verified by agarose gel electrophoresis. The RNA was reverse-transcribed to cDNA using TaKaRa Reverse Transcription Kit (Shanghai Yanhui Biotechnology Co., Ltd.) and stored in -20°C refrigerator.

### 2.3. Statistical analysis
SPSS 24.0 was used for data analysis. Measurement data were expressed in mean ± standard deviation and tested by t-test, while count data were expressed in percentage and tested by chi-square test. $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Relative expression of miR-372-3p in serum and GLUT4 level

The relative expression of miR-372-3p in the serum of patients in the GDM group was significantly higher than that of patients in the control group, and the level of GLUT4 of the GDM group was significantly lower than that of the control group ($P < 0.05$). The results are shown in **Table 1**.

**Table 1.** Comparison of the relative expression of miR-372-3p and GLUT4 levels in the serum of patients in both groups

| Group | miR-372-3p | GLUT4 (μg/L) |
|---|---|---|
| Control group (n = 42) | 1.24 ± 0.33 | 5.26 ± 1.62 |
| GDM group (n = 42) | 2.38 ± 0.69 | 2.47 ± 0.51 |
| t | 8.961 | 3.694 |
| *P* | < 0.05 | < 0.05 |

### 3.2. Metabolism-related indexes

The levels of FBG, FINS, 2h-BG, TC, TG, and HO-MA-IR of the GDM group were significantly higher than those of the control group; however, HOMA-β was significantly lower in the GDM group compared to the control group ($P < 0.05$), as shown in **Table 2**.

**Table 2.** Comparison of metabolism-related indexes between the two groups

| Indicator | Control group (n = 42) | GDM group (n = 42) | t | *P* |
|---|---|---|---|---|
| FBG | 4.52 ± 0.50 | 6.34 ± 0.89 | 6.398 | < 0.05 |
| FINS | 7.87 ± 1.64 | 13.41 ± 2.37 | 4.256 | < 0.05 |
| 2h-BG | 6.03 ± 1.35 | 8.33 ± 1.09 | 5.362 | < 0.05 |
| TC | 4.31 ± 1.02 | 5.94 ± 1.71 | 4.369 | < 0.05 |
| TG | 1.00 ± 0.32 | 2.21 ± 0.60 | 3.568 | < 0.05 |
| HOMA-IR | 2.52 ± 0.87 | 3.99 ± 0.96 | 6.982 | < 0.05 |
| HOMA-β | 1.91 ± 0.60 | 1.13 ± 0.22 | 4.699 | < 0.05 |

Abbreviations: 2h-BG, 2-hour postprandial blood glucose; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR homeostatic model assessment for insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; TC, total cholesterol; TG, triglyceride.

### 3.3. Multiple linear regression analysis of the relative expression of miR-372-3p in serum and the relationship between GLUT4 level and HOMA-IR in patients with gestational diabetes mellitus

Multiple linear regression analysis was conducted with the relative expression of serum miR-372-3p and GLUT4 level as independent variables and HOMA-IR as dependent variable. The results showed that the relative expression of serum miR-372-3p was positively and independently correlated with HOMA-IR. GLUT4 level, on the other hand, was found to be negatively correlated with HOMA-IR ($P < 0.05$), as shown in **Table 3**.

**Table 3.** Multiple linear regression analysis of the relative expression of miR-372-3p in serum and the relationship between GLUT4 and HOMA-IR in patients with gestational diabetes mellitus

| Indicator | β | Standard error | t | *P* |
|---|---|---|---|---|
| miR-372-3p | 0.612 | 0.201 | 2.987 | < 0.05 |
| GLUT4 | -0.246 | 0.265 | 2.698 | < 0.05 |

## 4. Discussion

Insulin resistance exists in patients with GDM and is one of the main risk factors for GDM. Insulin resistance refers to decreased sensitivity of the body to insulin and is often manifested as disorders of glucose and lipid metabolisms. In this study, 81.9% of pregnant women with normal gestation had reduced insulin sensitivity (fasting glucose ≤ 5.1 mmol/L), while only 15.8% of GDM patients had high fasting glucose levels. In addition, there is a special group of pregnant women with normal gestation who have decreased insulin sensitivity but do not have high blood glucose levels [11-13]. Studies have shown that, the incidence of glucose metabolism disorders, dyslipidemia, lipoprotein metabolism disorders, and vascular inflammation was higher in patients with GDM compared with controls. This may be due to the inaccuracy of conventional detection methods used in determining serum glucose and lipid levels in patients with GDM, which may lead to false positive results.

With lifestyle changes and the further aging trend of the society, the number of people suffering from diabetes mellitus is increasing. Studies have shown a close correlation between GDM and insulin resistance and the importance of early diagnosis of GDM during pregnancy or postpartum for the prevention and treatment of diabetes. In this study, the relationship between elevated blood glucose and insulin resistance in pregnant women was explored by comparing the serum microRNA and GLUT4 levels in healthy pregnant women and those with GDM. The results of this study showed the presence of gestational prediabetes status in pregnant women with normal fasting glucose and insulin resistance in patients with gestational diabetes. In addition, studies have found markedly increased glucose challenge test, insulin receptor substrate 1 (IRS-1), and GLUT1 levels in GDM patients, indicating the presence of insulin resistance in GDM patients [14,15]. However, the present study only analyzed the serum microRNA and GLUT4 levels in patients with GDM combined with IR. Therefore, more studies are needed to prove the relationship between these two indicators and GDM combined with IR. We believe that the relationship between these two indicators and GDM combined with IR will become clearer with subsequent studies.

HOMA-IR is an independent risk factor to predict the risk of diabetes. In the present study, this index was detected in all pregnant women during fasting glucose examination in late pregnancy. Hemoglobin A1c (HbA1c) was significantly higher in the GDM group compared to the non-diabetic group. The study also found that pregnant women in the first trimester of pregnancy were more likely to have abnormal insulin resistance and HOMA-IR indices. In addition, both HbA1c and HOMA-IR index were associated with glucose intake, obesity, or overweight. The correlation between serum GLUT4 levels during pregnancy and blood glucose levels in late pregnancy was not significant, probably because of its low concentration during pregnancy and little effect on disease in pregnancy. By adjusting the factor analysis, a significant correlation ($P < 0.05$) was observed between late pregnancy glucose level, BMI, fasting glucose level, and blood GLUT4 concentration; fasting insulin resistance index during pregnancy was also positively correlated with serum GLUT4 concentration. Therefore, there is still a need to further investigate the mechanism of action of GLUT4 in the regulation of glucose homeostasis in the blood of pregnant women with GDM and its use as a biomarker for predicting the risk of developing pregnancy-related diseases.

In the present study, the patients were not subjected to glucose loading test as there were insufficient data to verify whether or not it has an effect on glycemic changes; moreover, the serum samples used were obtained from women only during pregnancy. In assessing pregnant women with GDM using the GDM risk score, clinical examination indicators were not used to determine whether or not a pregnant woman has GDM. However, because the present study used non-clinical indicators to assess the differences between GDM patients and healthy pregnant women during pregnancy and 3 months after delivery, it was not possible to make a judgment about the relationship between it and insulin resistance. In addition, although clinical tests were used to assess the relationship between glucose elevation and insulin resistance in pregnant women during pregnancy, these clinical tests showed no correlation in the normal population. Therefore, there is a need for more in-depth studies on different populations in the future.

In conclusion, glycosylated hemoglobin examination in early gestation (12–13 weeks of gestation) is important to ensure the health of pregnant women and fetuses. The screening and intervention for elevated glucose in pregnant women act as a guideline for the treatment of GDM. Patients with insulin resistance and related complications such as hyperinsulinemia and hypoglycemia should be given priority.

## Disclosure statement

The authors declare no conflict of interest.

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