

Featured Article

Expression and diagnostic value of peripheral blood miRNA-125b and miRNA-144 in patients with gestational diabetes mellitus

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Abstract: Objective: This study aimed to investigate the aberrant miRNA expression profiles in the peripheral blood of pregnant women and evaluate their potential diagnostic utility in gestational diabetes mellitus (GDM). **Methods:** A cohort of 62 pregnant women diagnosed with GDM (GDM group) and 62 pregnant women with normal glucose tolerance (control group) were enrolled. Peripheral blood samples were collected during late pregnancy (26–40 weeks gestation). Next-generation sequencing was employed to identify differentially expressed miRNAs, followed by validation through reverse transcription polymerase chain reaction (RT-PCR). Statistical analyses were conducted to examine the correlation between miRNA expression levels and clinical parameters, as well as their contribution to GDM risk. **Results:** A total of 157 differentially expressed miRNAs were identified in the GDM group, with 114 upregulated and 43 downregulated compared to the control group. Hierarchical clustering analysis revealed distinct miRNA expression patterns between the two groups, with 8 miRNAs significantly upregulated and 6 significantly downregulated. qPCR validation of miRNAs associated with energy metabolism confirmed that the expression levels of miRNA-125b, miRNA-543, and miRNA-144 were consistent with the sequencing data. Clinical parameters including pre-pregnancy weight, body mass index (BMI), pre-delivery diastolic blood pressure, and platelet count were significantly higher in the GDM group compared to controls. In GDM patients, miRNA-125b expression was significantly reduced ($P < 0.001$), while miRNA-144 expression was significantly increased ($P < 0.001$) compared to non-GDM pregnant women. Multivariate analysis identified miRNA-125b, miRNA-144, and pre-pregnancy BMI as independent risk factors for the development of GDM ($P < 0.05$). **Conclusion:** Dysregulated expression of miRNA-125b and miRNA-144 in peripheral blood may serve as valuable biomarkers for the early diagnosis of gestational diabetes mellitus.

Keywords: miRNA; gestational diabetes mellitus; peripheral blood

1. Introduction

Gestational diabetes mellitus (GDM) is a pregnancy-induced disorder of glucose metabolism that typically manifests in the mid-to-late stages of gestation. Its hallmark is glucose intolerance, which first arises or is diagnosed during pregnancy [1]. Over the past two decades, the incidence of GDM has increased significantly both in China and globally, posing a substantial public health threat to the well-being of pregnant women and their newborns. Recent studies estimate that the prevalence of GDM in China now ranges between 17.5% and 18.9% [2]. This rising trend is associated with multiple factors, including advanced maternal age, increasing rates of obesity, and unhealthy dietary habits, underscoring the urgency of addressing this growing health

challenge. GDM not only exerts short-term metabolic effects on pregnant women, leading to complications such as preeclampsia, fetal growth restriction, and preterm birth, but it also presents long-term health risks for both mother and child [3]. Approximately 50% to 70% of women with GDM develop type 2 diabetes postpartum, with significantly elevated risks of cardiovascular disease, metabolic syndrome, and other conditions. For the offspring, GDM markedly increases the likelihood of excessive growth, macrosomia, and the development of chronic diseases such as obesity and diabetes later in life. Given these risks, early diagnosis and intervention for GDM are critical for safeguarding maternal and neonatal health [4].

Although the precise pathogenesis of GDM remains unclear, growing evidence points to a pivotal role for microRNAs (miRNAs) in its development. miRNAs are endogenous, non-coding small RNAs that regulate gene expression by binding to target mRNAs [5]. They are involved in various biological processes, including cell proliferation, differentiation, apoptosis, and metabolism. Abnormal miRNA expression has been linked to the onset and progression of numerous diseases, including diabetes and its related complications. During pregnancy, the placenta secretes significant amounts of miRNAs, which are released into the maternal peripheral blood via exosomes or other pathways, influencing both fetal development and maternal metabolic balance [6]. Currently, the oral glucose tolerance test (OGTT) remains the standard diagnostic tool for GDM; however, it is cumbersome, and patient compliance is often suboptimal due to the associated side effects of repeated blood draws and nausea [7]. These challenges contribute to increased psychological and physical burdens on patients. As a result, recent research has focused on the development of simpler, faster, and non-invasive diagnostic methods for GDM. Due to their stability and specificity in peripheral blood, miRNAs have emerged as promising non-invasive biomarkers that offer robust support for early disease diagnosis and monitoring [8].

In this study, we concentrated on miRNA-125b and miRNA-144 as key research targets using high-throughput sequencing technology. Previous research has demonstrated that the expression levels of these miRNAs are closely associated with insulin resistance, inflammatory responses, and other diabetes-related metabolic pathways. However, systematic studies examining their specific mechanisms and diagnostic potential in GDM patients remain limited. Therefore, this study aims to assess the expression levels of miRNA-125b and miRNA-144 in the peripheral blood of GDM patients and evaluate their potential as diagnostic biomarkers. Additionally, through bioinformatics analysis, we seek to identify the potential target genes of these miRNAs and elucidate the biological processes and metabolic pathways they regulate, ultimately providing new theoretical foundations and clinical insights for the early diagnosis and treatment of GDM.

2. Methods

2.1. Study population

We enrolled 62 pregnant women diagnosed with gestational diabetes mellitus (GDM) during late pregnancy (26–40 weeks gestation), admitted to our hospital between January 2018 and February 2020. Inclusion criteria were: (1) age 24–40 years;

(2) singleton pregnancy; (3) GDM diagnosis confirmed by oral glucose tolerance test (OGTT); (4) first pregnancy; and (5) informed consent from both the patient and her family. Exclusion criteria included: (1) pre-pregnancy diabetes or hypertension; (2) multiple pregnancies; (3) complications such as threatened preterm labor, preterm birth, fetal growth restriction, placenta previa, or other pregnancy-related complications; and (4) incomplete clinical data. For the control group, 62 age-matched pregnant women with normal glucose tolerance, admitted during the same period, were selected. The GDM group received diet and exercise therapy, with subcutaneous insulin administered to those whose blood glucose levels exceeded the target range until glycemic control was achieved. The glycemic targets were fasting blood glucose <5.3 mmol/L, 1-hour postprandial blood glucose <7.8 mmol/L, and 2-hour postprandial blood glucose <6.7 mmol/L. Informed consent was obtained from all participants, and blood samples were stored at -80 °C for ultra-low temperature preservation. Clinical data were extracted from outpatient and inpatient medical records.

2.2. Next-generation sequencing (NGS)

Total RNA was extracted from peripheral blood samples of the GDM and control groups using the NanoPhotometer® spectrophotometer, Qubit®2.0 fluorometer, and Agilent 2100 RNA Nano 6000 kit. Sequencing was performed on the NextSeq platform using the SE50 program to obtain high-quality reads. Data were processed and aligned to the reference genome using Bowtie, and miRNAs were identified using miRDeep2 software. Differentially expressed miRNAs were identified using DESeq analysis, with a *Q*-value < 0.05 and $|\log_2\text{foldchange}| > 1$ as selection criteria.

2.3. RNA extraction and RT-PCR validation

Peripheral blood RNA was extracted according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using M-MuLV reverse transcriptase and loop miRNA-144/125b/543/15b/451/U6 miRNA RT primers (RiboBio, Guangzhou, China). Quantitative polymerase chain reaction (qPCR) was performed using SYBR reagent on a CFX96 real-time PCR system, with three replicates for each reaction. PCR conditions were 95 °C for 20 s, followed by 40 cycles of 95 °C for 10 seconds, 60 °C for 20 s, and 70 °C for 10 s. U6 was used for normalization of qPCR data.

2.4. Statistical analysis

Statistical analyses were conducted using SPSS 22.0 and the Weka 3.6 data mining package. The Shapiro-Wilk test was used to assess data normality. Normally distributed data were expressed as mean ± standard deviation and analyzed using *t*-tests, while non-normally distributed data were expressed as median and interquartile range and analyzed using the Wilcoxon test. Categorical data were compared using the chi-square test. Stepwise logistic regression was employed to identify factors associated with elevated blood glucose levels during pregnancy. A *P*-value <0.05 was considered statistically significant.

3. Results

3.1. Differential expression of miRNAs in peripheral blood of GDM patients

In the GDM group, 157 differentially expressed miRNAs were identified compared to the control group, with 114 miRNAs upregulated and 43 downregulated (**Figure 1a**). Hierarchical clustering analysis demonstrated distinct miRNA expression profiles between the GDM and control groups, with 8 miRNAs significantly upregulated and 6 miRNAs significantly downregulated ($P < 0.05$).

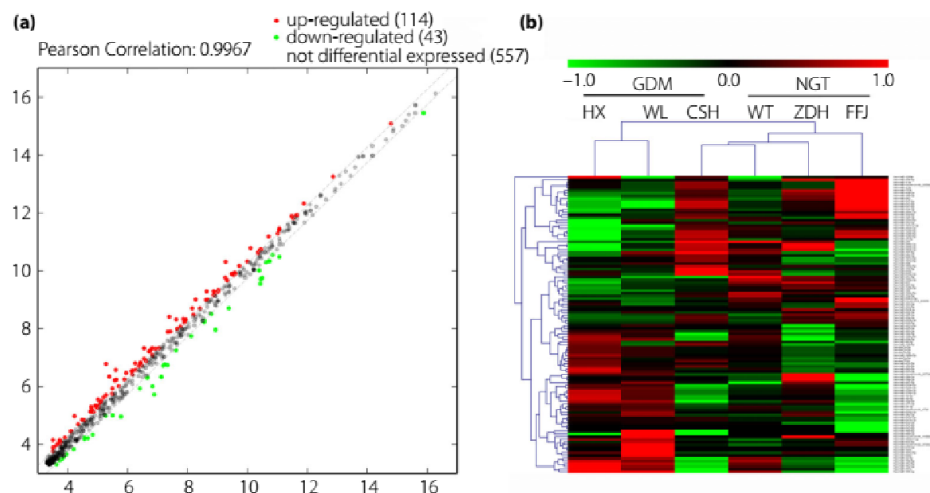


Figure 1. Differential expression of miRNAs in the peripheral blood of pregnant women from both groups analyzed by next-generation sequencing.

3.2. Validation of differentially expressed miRNAs in GDM and control groups

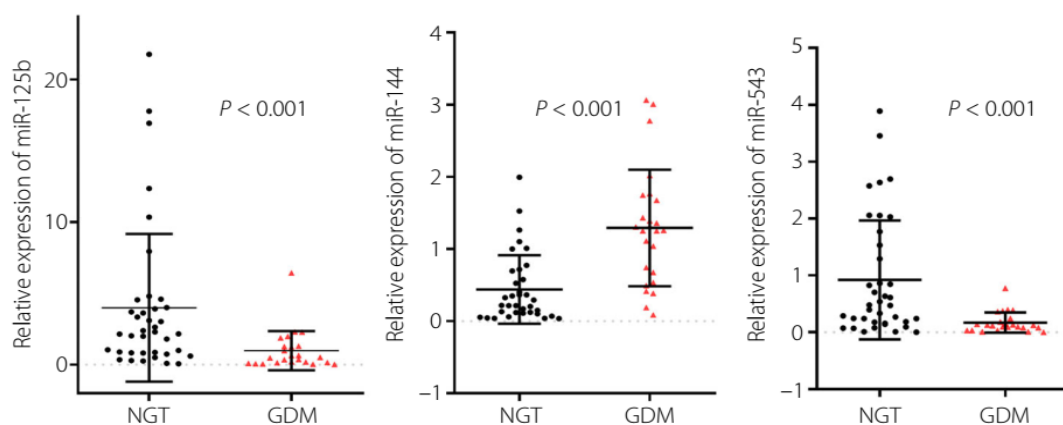


Figure 2. Differential expression of miRNAs in the peripheral blood of both groups of pregnant women detected by RT-PCR.

qPCR validation of highly expressed miRNAs associated with energy metabolism was performed. Five miRNAs (miRNA-125b, miRNA-543, miRNA-144, miRNA-15b, and miRNA-451) were selected for validation in peripheral blood samples from the GDM and control groups. qPCR results confirmed that the

expression levels of miRNA-125b, miRNA-543, and miRNA-144 were consistent with NGS findings ($P < 0.05$, **Figure 1D**). No statistically significant differences in miRNA-15b or miRNA-451 expression were observed between the groups (**Figure 2**).

3.3. Expression of miRNA-125b and miRNA-144 in peripheral blood

qPCR was used to assess miRNA-144 and miRNA-125b expression levels in the plasma of GDM and control patients. Glycated hemoglobin (HbA1c) and blood glucose levels were significantly higher in the GDM group than in the control group at all time points ($P = 0.499$). The GDM group had significantly higher pre-pregnancy weight, body mass index (BMI), pre-delivery diastolic blood pressure, and platelet levels compared to the control group. miRNA-125b expression was significantly downregulated ($P < 0.001$) and miRNA-144 expression significantly upregulated ($P < 0.001$) in GDM patients compared to non-GDM pregnant women (**Table 1**).

Table 1. Comparison of clinical characteristics between the two groups of patients.

Index	Control Group($n = 62$)	GDM($n = 62$)	t	P
OGTT 0 h(mmoll)	4.46 ± 0.40	5.23 ± 0.59	−8.505	0.000
OGTT 1 h(mmoll)	6.76 ± 2.54	11.20 ± 1.42	−12.014	0.000
OGTT 2 h(mmoll)	5.79 ± 1.12	9.23 ± 1.84	−12.574	0.000
HbA1C(%)	5.13 ± 0.36	5.69 ± 0.53	−6.882	0.000
Age	30.54 ± 5.74	32.54 ± 4.78	−2.108	0.037
Gestational time(d)	277.98 ± 9.63	279.65 ± 10.52	−0.921	0.358
Weight(Kg)	58.76 ± 11.25	65.87 ± 11.02	−3.554	0.001
Weight Gain During Pregnancy(Kg)	16.52 ± 4.43	14.23 ± 5.11	2.666	0.009
BMI(Kg/m ²)	22.32 ± 3.84	25.14 ± 4.23	−3.886	0.000
Increase in BMI During Pregnancy(Kg/m ²)	6.28 ± 1.83	5.95 ± 1.76	1.023	0.308
Systolic Blood Pressure(mmHg)	125.36 ± 9.25	128.65 ± 10.32	−1.869	0.064
Diastolic Blood Pressure(mmHg)	77.52 ± 5.63	84.32 ± 8.93	−5.072	0.000
WBC(×10 ⁹ /L)	9.46 ± 2.33	8.94 ± 2.24	1.266	0.208
Lymphocyte(%)	20.65 ± 5.24	19.65 ± 5.00	1.087	0.279
Neutrophil/Lymphocyte	4.35 ± 3.56	4.49 ± 3.02	−0.236	0.814
Hemoglobin(g/L)	115.63 ± 12.84	114.88 ± 13.04	0.322	0.747
Platelet Count(×10 ⁹ /L)	242.64 ± 53.14	213.52 ± 48.79	3.178	0.002
Abdominal Circumference(cm)	104.65 ± 8.36	105.68 ± 7.74	−0.711	0.478
Neonatal Weight(g)	3418.99 ± 436.25	3769.36 ± 536.22	30.181	0.000
miRNA-125b	2.38 ± 1.26	0.54 ± 0.79	9.742	0.000
miRNA-144	0.73 ± 0.46	2.98 ± 1.35	−12.422	0.000

3.4. Correlation between miRNA-144, miRNA-125b, and clinical parameters

miRNA-144 expression was negatively correlated with BMI during pregnancy ($r = -0.331$, $P = 0.018$) and before delivery ($r = -0.290$, $P = 0.039$), but positively correlated with platelet count ($r = 0.309$, $P = 0.028$) and 1-hour postprandial blood glucose levels ($r = 0.283$, $P = 0.044$, **Figure 3c,d**). miRNA-125b was negatively

correlated with GDM ($r = -0.906$, $P = 0.000$), while miRNA-144 and pre-pregnancy BMI were positively correlated with GDM ($P = 0.0003$ and $P = 0.0339$, respectively).

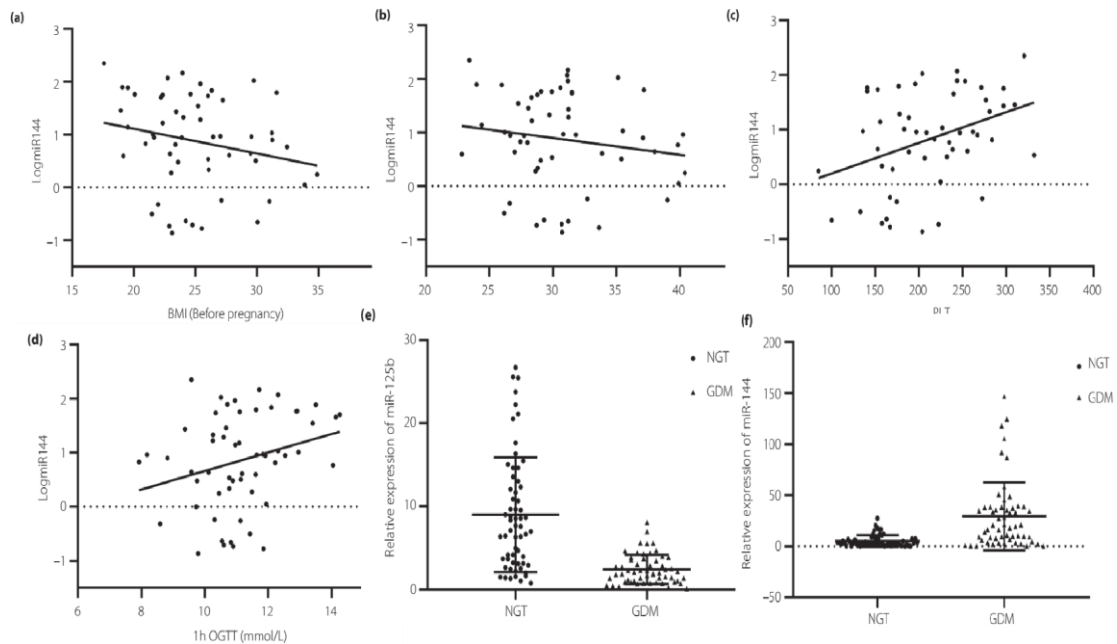


Figure 3. Correlation between miRNA-144, miRNA-125b, and clinical parameters.

3.5. Risk factor analysis for GDM development

Multivariate analysis revealed that miRNA-125b, miRNA-144, and pre-pregnancy BMI were independent risk factors for GDM development ($P < 0.05$) (See Table 2).

Table 2. Analysis of risk factors influencing the occurrence of gestational GDM.

Index	β	SE	Wald	P	OR (95% CI)
miRNA-125b	-0.9063	0.2506	13.0826	0.0003	0.404 (0.247–0.660)
miRNA-144	0.4237	0.1158	13.3970	0.0003	1.528 (1.218–1.917)
Pre-pregnancy BMI	0.1780	0.0839	4.5013	0.0339	1.195 (1.014–1.408)

4. Discussion

This study systematically analyzed, for the first time, the expression changes of miRNA-125b and miRNA-144 in the peripheral blood of patients with gestational diabetes mellitus (GDM) and their correlation with clinical parameters, revealing the potential roles of these miRNAs in GDM. The results indicate that abnormal expression of miRNA-125b and miRNA-144 is closely associated with the onset of GDM, with both serving as independent risk factors. These findings suggest that miRNA-125b and miRNA-144 could be potential biomarkers for early diagnosis and may provide new avenues for understanding GDM pathogenesis.

miRNA-125b, a key member of the miRNA-125 family, plays an essential role in the development of various diseases. Previous studies have shown its involvement in regulating pancreatic β -cell function and its strong association with insulin resistance. Our study found that miRNA-125b expression in the peripheral blood of

GDM patients was significantly downregulated, consistent with findings in type 2 diabetes, where miRNA-125b levels were also reduced [9]. However, reports on miRNA-125b expression in GDM patients have been inconsistent, possibly due to differences in study populations, lifestyle factors, sample sizes, and experimental designs. For instance, some studies collected samples only during late pregnancy, while our study included multiple stages. Factors such as age, body mass index (BMI), and insulin sensitivity also influence miRNA-125b expression, contributing to variability in findings [10]. Recent research suggests that low miRNA-125b expression may promote hyperglycemia by upregulating c-maf transcript levels. C-maf is a crucial transcription factor involved in insulin secretion from pancreatic β -cells [11]. Additionally, miRNA-125b may regulate energy metabolism and insulin sensitivity by inhibiting the expression of the deacetylase Sirtuin-7. These mechanisms provide new insights into the role of miRNA-125b in diabetes. Functional enrichment analysis of miRNA-125b target genes also suggests that it may influence GDM by regulating genes associated with energy metabolism. Future research should explore miRNA-125b's specific role in insulin signaling, particularly its dynamic changes during different stages of GDM [12].

In contrast to miRNA-125b, miRNA-144 was significantly upregulated in GDM patients, suggesting its potential role in promoting GDM development. miRNA-144 is a critical regulator of insulin signaling and has been shown to target insulin receptor substrate 1 (IRS1) and glucose transporter (GLUT), thereby affecting glucose uptake and metabolism [13]. This regulatory mechanism is consistent with the observed increase in miRNA-144 expression in diabetic patients, including those with GDM. However, conflicting reports exist regarding miRNA-144's role in various types of diabetes. For example, some studies have reported downregulation of miRNA-144 in exosomes from placental villi in GDM patients, while our study found significant upregulation in peripheral blood. This discrepancy may result from differences in sample types, as miRNA-144 may exhibit distinct biological functions in placental exosomes compared to peripheral blood, potentially related to pregnancy physiology. Variations in ethnicity, lifestyle, and sample timing may also influence miRNA-144 expression. Our study also identified significant correlations between miRNA-144 and clinical parameters. Specifically, miRNA-144 expression was negatively correlated with BMI and positively correlated with 1-hour postprandial blood glucose levels in GDM patients. This suggests that miRNA-144 may play a role in regulating glucose and lipid metabolism, particularly in insulin resistance. While the precise mechanisms of miRNA-144 in GDM remain unclear, its close association with insulin signaling warrants further investigation.

In addition to miRNA roles, this study highlighted pre-pregnancy BMI as an important predictor of GDM risk [14]. The BMI of GDM patients was significantly higher than that of the control group, consistent with existing literature. Obesity is not only an independent risk factor for GDM but may also exacerbate glucose metabolism disorders by influencing miRNA expression and metabolic pathways. Therefore, controlling pre-pregnancy weight and managing gestational weight gain may be critical for reducing GDM risk. This study validated the differential expression of miRNA-125b and miRNA-144 in GDM patients using qPCR, demonstrating their potential as diagnostic biomarkers. However, further follow-up studies are needed to

assess their predictive value in early or mid-pregnancy. Expanding sample sizes and including participants from diverse ethnic, age, and lifestyle backgrounds could improve the generalizability of these findings. Future research should focus on the regulatory roles of miRNAs in GDM pathogenesis, particularly the complex relationships between miRNAs, insulin signaling, energy metabolism, and inflammatory responses.

In conclusion, this study identified the dysregulated expression of miRNA-125b and miRNA-144 as being closely associated with GDM, providing new insights into early diagnosis and disease mechanisms. However, further mechanistic studies and large-scale clinical validation are required to translate these findings into practical clinical tools.

Author contributions: Conception and design, YW and FG; method, LS and XZ; data collection, LS and XZ; manuscript writing, YW; manuscript revision, FG; research supervision, FG. All authors have read and agreed to the published version of the manuscript.

Data availability: The data associated with this study are available from the corresponding author upon reasonable request (Email: yantai12352630@163.com).

Ethical approval: This study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Xingtai Medical College (No. XT-2023072134).

Informed consent statement: Informed consent was obtained from all subjects involved in the study.

Conflict of interest: The authors declare no conflict of interest.

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