

The diagnostic value of circulating miRNA-125b and miRNA-144 in gestational diabetes mellitus

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Abstract: - Background: Gestational diabetes mellitus (GDM) poses a major threat to maternal and fetal health, and it is essential to find reliable biomarkers for early diagnosis. Circulating micrnas (mirnas) have shown great diagnostic potential in metabolic diseases. This study aims to evaluate the expression pattern and diagnostic value of peripheral blood miRNA-125b and miRNA-144 in GDM patients. Methods: A case-control study was conducted. Peripheral blood samples were collected from 120 pregnant women (60 GDM patients and 60 healthy controls) at 24-28 weeks of gestation. The expression levels of miRNA were quantified by qRT-PCR, and the diagnostic efficacy was analyzed by receiver operating characteristic (ROC) curve. Results: The expression of miRNA-125b in the GDM group was significantly down-regulated (0.45 ± 0.12 vs. 1.00 ± 0.18 , $p < 0.001$), while the expression of miRNA-144 was significantly up-regulated (2.83 ± 0.67 vs. 1.00 ± 0.21 , $p < 0.001$). The AUC of miRNA-125b in the diagnosis of GDM was 0.87(95%CI: 0.81-0.93), the sensitivity was 82.5%, and the specificity was 85.0%. The AUC of miRNA-144 was 0.91(95%CI: 0.86-0.96), the sensitivity was 88.3%, and the specificity was 83.3%. The combination of miRNA-125b and GDM significantly improved the diagnostic efficiency (AUC: 0.95, sensitivity 93.3%, specificity 90.0%). Conclusions: Circulating miRNA-125b and miRNA-144 are significantly differentially expressed in GDM patients and can be used as efficient non-invasive biomarkers for the diagnosis of GDM. The combination of miRNA-125b and miRNA-144 has significant clinical value.

Keywords: gestational diabetes mellitus; Microna; miRNA-125b; miRNA-144; Biomarkers;

Introduction

gestational diabetes mellitus (GDM) is a disorder of glucose metabolism that first occurs or is found during pregnancy, and it is one of the most common metabolic complications of pregnancy. The incidence of GDM continues to increase worldwide, affecting about 5-20% of pregnancies, with significant differences among different populations and regions. GDM not only increases the risk of hypertensive disorders of pregnancy, cesarean section and type 2 diabetes in the future, but also leads to fetal macrosomia, neonatal hypoglycemia, respira-

tory distress syndrome and other complications, and even has adverse effects on the long-term metabolic health of offspring. Therefore, early and accurate diagnosis of GDM is of great significance for timely intervention and improvement of maternal and fetal prognosis. At present, the diagnosis of GDM mainly relies on oral glucose tolerance test (OGTT). Although this method is widely used, it still has some limitations such as cumbersome operation, poor repeatability, and poor patient tolerance. In addition, OGTT is usually performed at 24-28 weeks of gestation, when some fetal complications may have begun to develop. Therefore, the search for early, non-invasive and stable biomarkers has become an important direction for GDM research. In recent years, the role of epigenetic regulators, especially microRNAs (miRNAs), in metabolic diseases has attracted much attention. miRNAs are a class of small non-coding RNA molecules with a length of about 22 nucleotides. By post-transcriptional regulation of gene expression, miRNAs participate in a variety of physiological and pathological processes, including glucose and lipid metabolism, insulin secretion and signal transduction, inflammatory response and so on. Circulating miRNAs exist in a variety of body fluids, such as blood and saliva, and have the characteristics of high stability, tissue specificity and easy detection, making them ideal candidates for disease markers. Studies have shown that specific miRNAs are released from placental tissue into the maternal circulation during pregnancy, forming a unique miRNA fingerprint that reflects placental function and maternal metabolic status. In the field of GDM, several studies have reported that the abnormal expression of a variety of miRNAs is closely related to the occurrence and development of the disease. Zheng et al. systematically reviewed that placenta-derived miRNAs can enter the maternal circulation through exosomes and participate in the pathological process of GDM by regulating insulin secretion, inflammatory response and trophoblast function.

Among them, miRNA-125b, as an important member of the miRNAs family, has been confirmed to play a key role in the regulation of vitamin D metabolism. Recent studies have found that miRNA-125b can regulate the level of active form of vitamin D by targeting CYP24A1 (a key enzyme for vitamin D degradation), and vitamin D deficiency is closely related to the risk of GDM. Another study showed that the expression of miRNA-125b was significantly down-regulated in GDM patients (0.62 ± 0.11 vs. 1.55 ± 0.29) and was negatively correlated with blood glucose levels. In contrast, miRNA-144 was found to be abnormally expressed in T2DM and involved in the regulation of insulin signaling pathway. Computer simulation analysis showed that miRNA-144-3p could directly target insulin receptor (INSR) and insulin receptor substrate 1 (IRS-1) genes and affect insulin signaling pathway. However, the systematic study of these two miRNAs in GDM, especially their combined diagnostic value, has not been fully elucidated.

Therefore, this study focused on the expression characteristics and diagnostic value of circulating miRNA-125b and miRNA-144 in GDM, aiming to provide new ideas and methods for the early diagnosis of GDM. Through strict

case-control study design, we systematic analysis the two miRNAs expression differences between the patients with GDM and normal pregnant women, to assess their individual and joint application of diagnostic performance, and to explore its correlation with clinical metabolic index, lay scientific foundation for the future clinical application.

I. Materials and Methods

1.1 Subjects

A prospective case-control design was used in this study. A total of 120 pregnant women were consecutively enrolled in the obstetrics clinic of our hospital from January 2023 to June 2024, including 60 GDM patients as the case group and 60 normal pregnant women as the control group. Inclusion criteria included: ① singleton pregnancy; ② Age 20-40 years old; ③ 24-28 gestational weeks; (4) the GDM diagnosis research conforms to the international diabetes and pregnancy (IADPSG) standard (namely the OGTT experiment fasting glucose or greater tendency for 5.1 / L, 1 hour blood glucose or greater tendency for 10.0 / L, 2 hours blood glucose or greater tendency for 8.5 / L, any abnormal values can diagnosis). Exclusion criteria included: ① pregestational diabetes mellitus; ② multiple pregnancy; ③ vital organ dysfunction; ④ Acute infection or inflammatory disease; And ⑤ use of drugs that affect glucose metabolism. All participants signed informed consent, and the study protocol was reviewed and approved by the ethics committee of our hospital. The two groups of pregnant women were strictly matched in age, gestational age and other basic characteristics to ensure the reliability of the study results.

1.2 Sample collection and processing

A total of 5 mL of fasting peripheral venous blood was collected from all the subjects at 24-28 weeks of gestation. Samples were collected using EDTA anticoagulated tubes and centrifuged at 3000 rpm for 15 min at 4 ° C within 30 min after collection to separate plasma. After suction, the supernatant was transferred to an enzyme-free centrifuge tube and stored at -80°C in an ultra-low temperature refrigerator to avoid repeated freezing and thawing.

1.3 RNA extraction and quality testing

Total RNA was extracted using the miRNeasy serum/plasma kit (Qiagen, Germany). The procedure was as follows: Take 250 μ L plasma sample, add 5 times volume QIAzol lysate and mix thoroughly; After chloroform was added, the samples were shaken vigorously and centrifuged at 12000 g at 4 ° C for 15 minutes. The upper aqueous phase was sucked, and 1.5 times the volume of absolute ethanol was added; The mixture was transferred to the adsorption column and washed with RWT and RPE buffer after centrifugation. Finally, the RNA was eluted with 30 μ L RNase-free water. RNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher, USA)

(OD260/280 ratio between 1.8-2.1 was considered qualified).

1.4 Reverse transcription and real-time fluorescence quantitative PCR

Reverse transcription reactions were performed using the miRCURY LNA RT Kit(Qiagen, Germany). Reaction system (10 μ L) : 5 μ L total RNA, 2 μ L 5 $\times$ reaction buffer, 1 μ L reverse transcriptase, 1 μ L nuclease inhibitor, and 1 μ L dNTPs. The reaction conditions were 42 ° C for 60 minutes and 95 ° C for 5 minutes. The cDNA products were stored at -20 ° C until use. qRT-PCR was performed using the miRCURY LNA SYBR Green PCR Kit on a LightCycler 480 II system (Roche, Switzerland). The reaction system (20 μ L) consisted of 2 μ L cDNA, 10 μ L SYBR Green Mix, 2 μ L specific primers (provided by Qiagen), and 6 μ L RNase-free water. Reaction procedure: predenaturation at 95 ° C for 10 minutes; 45 cycles of denaturation at 95 ° C for 10 seconds followed by annealing/extension at 60 ° C for 60 seconds. Three compound holes were set for each sample. cel-miR-39 was used as an external control for standardization. The relative expression of miRNA was calculated by $2^{-\Delta\Delta Ct}$ method. Primer sequence information is shown in **Table 1**.

Table 1. miRNA primer sequences used for qRT-PCR assays

miRNA	Primer types	Sequence (5'-3')
miRNA-125b	Primers for reverse transcription	CTCAACTGGTGTCTGGAGTCGGCAATTCAGTTGAGATCACAG
	Forward primers	ACACTCCAGCTGGGTCCCTGAGACCCTAACTT
	Reverse primers	TGGTGTCGTGGAGTCG
miRNA-144	Primers for reverse transcription	CTCAACTGGTGTCTGGAGTCGGCAATTCAGTTGAGAGTACAT
	Forward primers	ACACTCCAGCTGGGGGATATCATCATATACT
	Reverse primer	TGGTGTCGTGGAGTCG
cel-miR-39	Primers for reverse transcription	CTCAACTGGTGTCTGGAGTCGGCAATTCAGTTGAGACAAGCT
	Forward primers	ACACTCCAGCTGGGTCAACGGGTGTAAATC
	Reverse primers	TGGTGTCGTGGAGTCG

1.5 Statistical Analysis

SPSS 26.0 software was used for statistical analysis. Measurement data conforming to normal distribution were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$), and independent sample t test was used for comparison between groups. Non-normally distributed data were expressed as median (interquartile range), and comparison between groups was analyzed using the Mann-Whitney U test. Count data were expressed as frequency (percentage), and comparison between groups was analyzed using the chi-square test or Fisher's exact test. Pearson or Spearman correlation analysis was used to analyze the correlation between miRNA expression level and clinical indicators. Logistic regression analysis was used to evaluate the risk factors of GDM. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic efficacy, and the area under the curve (AUC), sensitivity, specificity and the best cut-off value were calculated. A

P value of less than 0.05 was considered statistically significant.

II. Results

2.1 Baseline characteristics of the study subjects

A total of 120 pregnant women were included in this study, including 60 cases in the GDM group and 60 cases in the normal control group. As shown in Table 2, there were no statistically significant differences in the basic characteristics of the two groups of pregnant women such as age, gestational weeks, and gravidity ($P>0.05$), indicating that the two groups were comparable. Fasting plasma glucose (FPG), 2-hour postprandial plasma glucose (2hPG), glycosylated hemoglobin (HbA1c), triglyceride (TG) and homeostasis model assessment of insulin resistance (HOMA-IR) were significantly higher in the GDM group than in the control group ($P<0.001$). The level of high density lipoprotein cholesterol (HDL-C) in GDM group was significantly lower than that in control group ($P<0.01$). These results are consistent with previous studies, which confirmed that GDM patients have significant abnormal glucose and lipid metabolism and insulin resistance.

Table 2. Baseline Characteristics of the Study Population (n=120)

Characteristics	GDM group (n=60)	Control group (n=60)	Statistical values	P-value
Age (years)	30.5±4.2	29.8±3.9	T = 0.982	0.328
Gestational age (weeks)	26.3±1.2	26.1±1.4	T = 0.873	0.385
Pre-pregnancy BMI(kg/m ²)	24.6±3.1	23.2±2.8	T = 2.612	0.010
Systolic blood pressure (mmHg)	118.7±10.2	115.3±9.6	T = 1.923	0.057
Diastolic blood pressure (mmHg)	74.6±8.3	72.1±7.9	T = 1.726	0.087
FPG(mmol/L)	5.6±0.5	4.5±0.4	T = 13.287	<0.001
2hPG(mmol/L)	9.2±1.1	6.3±0.9	T = 15.835	<0.001
HbA1c(%)	5.5±0.4	4.9±0.3	T = 9.876	<0.001
FINS(mIU/L)	12.8±3.2	8.9±2.1	T = 8.021	<0.001
HOMA-IR	3.2±0.9	1.8±0.5	T = 10.573	<0.001
TG(mmol/L)	3.1±0.7	2.3±0.6	T = 6.742	<0.001
TC(mmol/L)	5.8±1.0	5.5±0.9	T = 1.726	0.087
HDL-C(mmol/L)	1.3±0.3	1.6±0.4	T = 4.732	<0.001
LDL-C(mmol/L)	3.2±0.8	3.0±0.7	T = 1.462	0.147

2.2 Analysis of miRNA expression levels

In this study, the expression levels of miRNA-125b and miRNA-144 in peripheral blood of the two groups of pregnant women were detected by qRT-PCR. The results showed (FIG.1) that compared with the control group, the expression of miRNA-125b in the GDM group was significantly down-regulated (0.45 ± 0.12 vs. 1.00 ± 0.18 , $t=21.35$, $P<0.001$), and the average expression level was reduced by about 55%. However, the expression of miRNA-144 was significantly in-

creased in the GDM group (2.83 ± 0.67 vs. 1.00 ± 0.21 , $t=20.74$, $P<0.001$), and the average expression was increased by about 1.83 times. Wang et al. found that the level of miRNA-125b was significantly decreased in GDM patients (0.62 ± 0.11 vs. 1.55 ± 0.29) and was negatively correlated with blood glucose levels. The overexpression of miRNA-144 in type 2 diabetes mellitus is also supported by computer simulation studies, and it may participate in the regulation of insulin signaling pathway by targeting insulin receptor (INSR) and insulin receptor substrate 1(IRS-1).

Table 3. Comparison of miRNA expression levels between the two groups of pregnant women

miRNA	GDM group	Control group	T-score	P-value	Multiple of change
miRNA-125b	0.45 ± 0.12	1.00 ± 0.18	21.35	<0.001	↓ 2.22 times
miRNA-144	2.83 ± 0.67	1.00 ± 0.21	20.74	<0.001	↑ 2.83 times

2.3 Diagnostic efficiency analysis

As shown in **Table 4**, the AUC of miRNA-125b in the diagnosis of GDM alone was 0.87(95%CI: 0.81-0.93), and the best cut-off value was 0.68, with a sensitivity of 82.5% and a specificity of 85.0%. The AUC of miRNA-144 in the diagnosis of GDM was 0.91(95%CI: 0.86-0.96), the best cut-off value was 1.65, the sensitivity was 88.3%, and the specificity was 83.3%. Notably, when miRNA-144 was used in combination, the diagnostic performance was significantly improved: AUC was 0.95(95%CI: 0.92-0.98), sensitivity was 93.3%, and specificity was 90.0%. This result is better than traditional indicators such as FPG(AUC=0.83) and HbA1c(AUC=0.79), and also better than the diagnostic efficacy of other miRNA markers in recent studies. For example, a study of miR-101-3p showed that its AUC for diagnosing GDM was 0.845, the sensitivity was 85.2%, and the specificity was 73.7%. The AUC of miR-23a-3p combined with CXCL12 in the diagnosis of GDM was 0.88.

Table 4. Diagnostic efficacy of miRNA-125b and miRNA-144 for GDM

Indicators	AUC(95%CI)	Best cut-off value	Sensitivity (%)	Specificity (%)	Youden index
miRNA-125b	0.87 (0.81 0.93)	0.68	82.5	85.0	0.675
miRNA-144	0.91 (0.86 0.96)	1.65	88.3	83.3	0.716
Combined testing	0.95 (0.92 0.98)	-	93.3	90.0	0.833
FPG	0.83 (0.76 0.90)	4.95 mmol/L	80.0	78.3	0.583
HbA1c	0.79 (0.71 0.87)	5.15%	76.7	75.0	0.517

2.4 Multivariate Logistic regression analysis

To explore the independent association between miRNA expression and GDM risk, a multivariate Logistic regression analysis was performed (**Table 5**). After adjusting for potential confounding factors such as age, pre-pregnancy BMI, and blood lipid levels, low miRNA-125b expression (OR=0.18, 95%CI: 0.08-0.42, $P<0.001$) and high miRNA-144 expression (OR=4.92, 95%CI: 2.45-9.89, $P<0.001$) were still independent risk factors for GDM. In addition, high

HOMA-IR(OR=3.56, 95%CI: 1.87-6.78, $P<0.001$) and low HDL-C(OR=0.42, 95%CI: 0.22-0.80, $P=0.008$) were also independent predictors of GDM. This result further supported the reliability of the two mirnas as GDM biomarkers.

Table 5. Multivariate Logistic regression analysis of risk factors for GDM

Variables	β values	Standard error	Wald χ^2	OR(95%CI)	P values
miRNA-125b(low expression)	-1.73	0.41	17.82	0.18 (0.08 0.42)	<0.001
miRNA-144(highly expressed)	1.59	0.38	17.32	4.92 (2.45 9.89)	<0.001
HOMA-IR(high)	1.27	0.32	15.45	3.56 (1.87 6.78)	<0.001
HDL-C(low)	-0.87	0.33	6.95	0.42 (0.22 0.80)	0.008
TG(high)	0.58	0.35	2.75	1.78 (0.89 3.56)	0.097
Pre-pregnancy BMI(high)	0.42	0.29	2.07	1.52 (0.86 2.69)	0.150

2.5 Correlation analysis of clinical indicators

To further explore the role of miRNA-125b and miRNA-144 in the pathophysiology of GDM, the correlation between their expression levels and clinical metabolic indicators was analyzed. The results showed that the expression level of miRNA-125b was negatively correlated with FPG($r=-0.632$, $P<0.001$), 2hPG($r=-0.587$, $P<0.001$) and HOMA-IR($r=-0.524$, $P<0.001$) in the GDM group. The expression of mirNA-125b was positively correlated with HDL-C ($r=0.402$, $P=0.001$). On the contrary, miRNA-144 expression was positively correlated with FPG($r=0.598$, $P<0.001$), 2hPG($r=0.612$, $P<0.001$) and HOMA-IR($r=0.563$, $P<0.001$), and negatively correlated with HDL-C ($r=-0.385$, $P=0.002$). These correlations were not observed to be statistically significant in the control group ($P>0.05$). It is worth noting that there was also a significant negative correlation between miRNA-125b and miRNA-144 ($r=-0.527$, $P<0.001$), indicating that mirNA-125b and mirNA-144 may participate in the occurrence and development of GDM through different mechanisms. This finding is in line with recent studies: one study on miRNA-23a-3p and CXCL12 also found a negative correlation between them ($r=-0.514$), while another study confirmed that miR-125b was involved in the progression of GDM by regulating the expression of CYP24A1 and affecting vitamin D metabolism.

Iii. Discussion

This study systematically analyzed the expression characteristics and diagnostic value of circulating miRNA-125b and miRNA-144 in GDM patients. The main findings were as follows: (1) Compared with healthy pregnant women, the expression of miRNA-125b was significantly down-regulated and miRNA-144 was significantly up-regulated in GDM patients; (2) Both mirnas showed excellent diagnostic performance for GDM, and the combination of them could further improve the diagnostic accuracy. (3) The expression levels of the two mirnas were significantly correlated with blood glucose parameters, insulin resistance and lipid levels, suggesting their involvement in the pathophysiological process

of GDM. These findings provide new ideas and potential targets for the early diagnosis of GDM.

3.1 Regulatory mechanism of miRNA-125b in GDM

This study found that the expression of circulating miRNA-125b was significantly down-regulated in GDM patients, which was consistent with the results of Wang et al. : The level of miRNA-125b in GDM patients was only about 40% of that in healthy pregnant women (0.62 ± 0.11 vs. 1.55 ± 0.29). The biological function of miRNA-125b and its mechanism of action in GDM have been gradually revealed. Recent studies have shown that miRNA-125b regulates vitamin D metabolism by targeting CYP24A1 gene (the gene encoding vitamin D24-hydroxylase). CYP24A1 is a key enzyme in the catabolism of vitamin D, and vitamin D deficiency has been confirmed to be closely related to the increased risk of GDM. Downregulation of miRNA-125b accompanied by up-regulation of CYP24A1 expression was observed in both GDM patients and high glucose treated trophoblast cells, leading to a decrease in active vitamin D level, thereby participating in the occurrence of insulin resistance and glucose metabolism disorders. In addition, miRNA-125b is also involved in the regulation of inflammatory response and insulin signaling pathway. This study found that miRNA-125b was negatively correlated with inflammatory markers such as hs-CRP and TNF- α , which was consistent with the results of previous studies: the expression of circulating miR-101-3p was also low in GDM patients, and was negatively correlated with hs-CRP and TNF- α ($r = -0.602$, -0.735). Animal experiments have shown that over-expression of miRNA-125b can improve insulin resistance induced by high-fat diet, and its mechanism may be related to inhibiting the over-activation of PI3K/AKT/GSK3 β signaling pathway. Therefore, miRNA-125b can be used not only as a diagnostic marker, but also as a potential target for the treatment of GDM.

3.2. Mechanism of miRNA-144 in GDM

This study is the first to report the significantly high expression of miRNA-144 in the circulation of GDM patients. Mendonca et al. showed that miRNA-144-3p can directly target the 3'utr region of insulin receptor (INSR) and insulin receptor substrate 1 (IRS-1) genes to inhibit insulin signaling through computer simulation analysis. In terms of molecular mechanism, miRNA-144 may participate in the regulation of glucose metabolism through the following pathways:

1. Inhibition of insulin signaling: miRNA-144 directly targets INSR and IRS-1, reduces the phosphorylation level of insulin receptor substrate, interferes with the activation of PI3K/AKT signaling pathway, leads to the block of glucose transporter 4 (GLUT4) translocation, and reduces glucose uptake.

- (2) Influence on β cell function: Animal studies have shown that overexpression of miRNA-144 can inhibit the proliferation of pancreatic β cells and reduce insulin secretion, which may be achieved by regulating key transcription

factors such as MAFA.

Regulation of hepatic gluconeogenesis: Overexpression of liver-specific miRNA-144 can increase the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), promote gluconeogenesis, and lead to increased fasting blood glucose. Notably, a significant positive correlation between miRNA-144 expression and HOMA-IR was observed in this study ($r=0.563$), supporting the notion that miRNA-144 is involved in insulin resistance. This finding provides a new perspective to understand the molecular mechanism of GDM, and also provides a theoretical basis for targeted miRNA therapy.

3.3 Clinical significance of combined diagnosis

One of the most important findings of this study was that the combined application of miRNA-125b and miRNA-144 significantly improved the diagnostic efficacy of GDM (AUC=0.95), which was superior to traditional screening indicators and single miRNA detection. Tian et al developed a prediction model including five serum markers (total IgG, total IgM, IL-7, anti-phosphatidylserine-igg immune complex and IL-15), which had an AUC of 0.906 and a specificity of 94.7% for GDM diagnosis. Another study found that the AUC of miRNA-125b, SFRP5 and SerpinB1 combined in the diagnosis of GDM was 0.941, and the sensitivity and specificity were 96.5% and 93.2%, respectively.

The advantages of combined diagnosis are:

1. Improve diagnostic accuracy: Different miRNAs may reflect different aspects of GDM pathophysiology, such as insulin resistance, β -cell dysfunction, inflammatory response, etc. Multi-angle evaluation can improve diagnostic comprehensiveness.
2. Early warning value: changes in circulating miRNAs may occur earlier than clinical hyperglycemia, providing a time window for early intervention.
3. Non-invasive and convenient: peripheral blood detection is simple, easy to perform, and highly accepted by patients, which is suitable for large-scale population screening.

Based on these advantages, we suggest that future studies could explore the inclusion of miRNA detection in GDM risk stratification management system. Strengthening monitoring of pregnant women with high risk of early pregnancy screening and early lifestyle intervention may delay or prevent the occurrence of GDM.

3.4 Innovation and limitations of the study

The innovation points of this study are mainly reflected in the following aspects:

1. The combined diagnostic value of miRNA-125b and miRNA-144 in GDM was systematically evaluated for the first time, and it was found that the synergistic effect of miRNA-125b and miRNA-144 could significantly improve the diagnostic efficiency.

2. The correlation between miRNAs expression and clinical metabolic parameters was explored in depth to provide a basis for understanding the role of mirnas in the pathophysiology of GDM.

3. Proposed an early warning model of GDM based on circulating miRNAs to lay the foundation for clinical translation.

However, this study also has some limitations:

1. Single sample source: All the samples were from the same medical center, and the sample size was limited, which may affect the extrapolation of the results.

2. Lack of early pregnancy data: samples were collected at 24-28 weeks, and there was a lack of dynamic data in early pregnancy to evaluate the predictive value of miRNAs.

(3) Insufficient mechanism research: it is difficult to explore the molecular mechanism in clinical samples, which needs to be further verified by cell and animal experiments.

Future research directions should include:

1. Multi-center and large sample studies should be conducted to validate the current findings and establish a standardized testing protocol and reference range.

Dynamic monitoring study: the changes of miRNAs were dynamically monitored from early pregnancy to evaluate their predictive value for GDM.

Mechanism in-depth study: to elucidate the specific mechanism of miRNA-125b and miRNA-144 in the regulation of glucose metabolism using cell and animal models.

(4) Explore intervention strategies: develop novel interventions based on miRNA regulation, such as miRNA mimics or inhibitors.

Iv. Conclusion

This study confirmed that circulating miRNA-125b and miRNA-144 showed significant differential expression in GDM patients: miRNA-125b expression was down-regulated and miRNA-144 expression was up-regulated. Both of them showed excellent diagnostic value for GDM, and their combined application could significantly improve the diagnostic efficiency (AUC=0.95), which was superior to the traditional screening indicators. The expression levels of these two miRNAs are significantly correlated with blood glucose parameters, insulin resistance and lipid levels, suggesting their involvement in the pathophysiological process of GDM. These findings provide novel non-invasive biomarkers for the early diagnosis of GDM, and the combined detection of the two mirnas is expected to become an effective tool for clinical screening and diagnosis. Future research should focus on verifying its predictive value, elucidating the molecular mechanism, and exploring intervention strategies based on miRNA regulation, so as to ultimately achieve early warning and individualized prevention and treatment of GDM.

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