



Short Communication

Expression of Serum miRNA-137 in Women with Gestational Diabetes and its Relationship with Neonatal Hyperbilirubinemia

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ABSTRACT

Diabetes is the most common endocrine disease in pregnancy, and identifying the risk factors of gestational diabetes mellitus (GDM) helps to discover women who are at risk of neonatal jaundice in the future. The purpose of this study was to investigate the expression level of miR-137 in women with GDM and its relationship with neonatal hyperbilirubinemia. During this cross-sectional descriptive study, the serum of forty patients with GDM (13 pre-diabetic and 27 diabetic) were collected at different gestational ages. Then, the total amount of cellular RNA was extracted and the expression level of miR-137 was analyzed using real-time polymerase chain reaction (RT-PCR). The results showed that the expression level of miR-137 increased significantly in weeks 25, 26, 27 and 28 of pregnancy. This is despite the fact that the expression of this micro RNA did not show a significant change in the 24th week of pregnancy. The amount of miR-137 expression can be used as a biomarker to predict the stages of GDM. An increase in neonatal hyperbilirubinemia can be considered as an important negative consequence of GDM.

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Authors' Contribution

ML and ML conducted the experiments in this study. XS and JL contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words

miRNA-137, Diabetes, Pre-diabetic, Gestational diabetes, Neonatal hyperbilirubinemia, Real-time PCR

Gestational diabetes (GDM) is a degree of glucose intolerance that occurs during pregnancy in women without a history of diabetes and is caused by the inability of the pancreas to secrete more insulin in response to the increase in insulin resistance that occurred during pregnancy (Rasheed *et al.*, 2023; Liu *et al.*, 2021). The prevalence of GDM in different societies has been reported between 1 and 14% (Sweeney and Brown, 2001; King, 1998). Every year, more than 299,000 pregnant women in the world are affected by it, which is due to the epidemic of obesity, the increasing age of first pregnancy, a highly processed diet with high calories, consumption of saturated fats, as well as a sedentary and stressful life (Gongora and Wenger, 2015). GDM can increase the risk of type 2 diabetes, metabolic syndrome, and cardiovascular disease after pregnancy (Gongora and Wenger, 2015).

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Diagnosing patients with GDM is important because proper treatment can minimize maternal and fetal complications (Si *et al.*, 2021).

For several decades, many experiments have been carried out at the molecular level to clarify the molecular mechanisms involved in diabetic patients; In recent years, researchers have succeeded in discovering and identifying the potential of microRNAs, which not only determine the appearance of these involved mechanisms, but can be a new marker for early detection and possible prevention of diabetes in the future (Tang *et al.*, 2008). microRNAs are a large subgroup of small non-coding RNAs that are evolutionarily conserved and have a length of 18-25 nucleotides; Each microRNA can repress one or more genes at the translational or post-translational levels by binding to mRNA untranslated regions (UTRs), thereby regulating diverse processes such as cell differentiation, proliferation and apoptosis, and diseases such as diabetes (Tang *et al.*, 2008; Ambros, 2004; van Rooij and Olson, 2007).

According to epidemiological studies, GDM is directly related to the risk of neonatal jaundice (Kurjak and Chervenak, 2015). The fetus in a mother suffering from GDM is in a completely different environment from a fetus of a healthy mother. In this situation, glucose, alanine and free fatty acids are transferred from the mother

to the fetus in large amounts. Therefore, the amount of insulin in the amniotic fluid increases, which is a compensatory response of the fetus to the increase of these substances in the amniotic fluid. Overt hyperglycemia in the first three months of pregnancy increases the risk of congenital abnormalities, stillbirth, macrosomia, neonatal hypoglycemia, hyperbilirubinemia, cardiac hypertrophy, hypocalcemia, polycythemia and obesity (Widness, 1989; Kalkhoff, 1991; Schaefer-Graf *et al.*, 2000; Cunningham *et al.*, 2014). There are various reasons for the occurrence of jaundice in babies of diabetic mothers, including prematurity, polycythemia, macrosomia and poor control of maternal blood glucose level. Increasing destruction of red blood cells leads to jaundice and kernicterus (Gleason, and Devaskar, 2011). Considering this background and the high prevalence of jaundice caused by maternal diabetes, this study was conducted with the aim of investigating the expression of miRNA-137 and miRNA-508-3p in the serum of patients with GDM and its relationship with neonatal hyperbilirubinemia.

Materials and methods

This cross-sectional descriptive study was conducted on 40 women with GDM (miR-137 group) and 40 women with healthy pregnant (control group) at 24-28 weeks of pregnancy admitted to the Fourth Hospital of Shijiazhuang from 2023 to 2024. Sick and healthy samples were selected among pregnant women referring to the hospital according to the entry and exit criteria, using convenient sampling method. Patients were asked about their personal characteristics, gender and pregnancy history with their consent and following ethical principles. Blood was drawn from the subjects using regular syringes and centrifuged at 15,000 rpm for 5 min. Serum samples were collected in capped tubes and stored at 80°C.

For RT-PCR, RNA was extracted from serum samples using RNA extraction kit (DNP™) EX6071. cDNA synthesis was performed using the First Strand cDNA Synthesis Kit (Cynaclone) according to the manufacturer's instructions. The RT-PCR was performed using specific primers and the Quantitative RT-PCR kit (Cynaclone) for miR-137 and beta-actin as a reference (Table I). The reaction solution contained 10 µl of SYBR green RT-PCR master mix, 4 µl of cDNA synthesized from the template, 1 µl of primer and nuclease-free water, which was placed in the RT-PCR machine whose temperature program was set as below. The first step, which was the initial activation step, was considered for 5 min at 95 °C. Then, the denaturation step for 30 sec at 95°C, the annealing step for 30 sec at 60°C, and the elongation step for 30 sec at 72°C were considered. This step was repeated for 35 cycles.

Table I. Sequence of primers designed for tracking miR-137.

Target	Primer sequence (5'-3')
miR-NA-137	U6-F: 5'GCTCGCTTCGGCAGCACATATAC 3'
	U6-R: 5'GGTCCGAGGTATTGCACTGGATA 3'
B-actin	F: 5' CGGCCAGGTCATCACCATT 3'
	R: 5' CACAGGACTCCATGCCAG 3'

Statistical analysis of miR-137 expression data was performed using REST software. The statistical difference of miR-137 expression in different stages of pregnancy among patients and non-patients was evaluated with a significance level of $p < 0.05$. The results were measured as mean \pm standard deviation.

Results

The characteristics and biochemical findings of women with GDM in comparison with healthy pregnancies are reported in Table II. There was no significant difference between the two groups in terms of age ($P=0.131$), gestational age ($P=0.081$) and body mass index ($P=0.41$). The mean serum level of fasting blood sugar ($P=0.002$) and the value of HOMA-IR index ($P=0.004$) in GDM were significantly higher than in healthy pregnancies but for QUICKI index was significantly lower.

Table II. Comparison of individual characteristics and biochemical findings of healthy pregnant women and women with GDM.

Index	Control group (n=40)	miR-137 group (n=40)	P value
Age (years)	29.75 \pm 0.53	29.89 \pm 0.63	0.131
Gestational age (weeks)	27.55 \pm 0.23	27.89 \pm 0.14	0.081
Body mass index (kg/m ²)	26.20 \pm 0.42	26.55 \pm 0.29	0.41
Fasting blood sugar (mg/dL)	80.18 \pm 1.64	99.64 \pm 1.87	0.002
Insulin µIU/mL	7.66 \pm 0.79	9.98 \pm 1.44	0.171
HOMA-IR	1.39 \pm 0.17	2.44 \pm 0.28	0.004
QUICKI	0.38 \pm 0.01	0.35 \pm 0.01	0.037

HOMA-IR, Homeostasis model assessment of insulin resistance; QUICKI, Quantitative insulin sensitivity check index.

The results of agarose gel electrophoresis of polymerase chain reaction products showed that the RNA samples extracted on the gel had two indicator bands without smear related to 28S and 18S ribosomal RNA, which indicates the quality of the extracted RNA. Also, the calculation of absorbance at 260 to 280 nm showed a very small amount of protein and impurity. Gel electrophoresis

images of RT-PCR products also showed the presence of a specific band for miR-137 and β -actin.

The miR-137 expression analysis revealed that the microRNAs in weeks 25, 26, 27 and 28 of pregnancy in the miR-137 group has a higher cycle of threshold than the serum of the participants with healthy pregnant (control group). On the other hand, there was no significant difference in the miR-137 expression at 24 weeks of pregnancy in the serum of the mothers with GDM and the mothers with healthy pregnant ($P>0.05$). Thus, in the miRNA-137 group, increased significantly in weeks 25, 26, 27 and 28 of pregnancy, but did not show a significant change in week 24 of pregnancy (Table III).

Table III. Value of miR-137 expression Different stages of GDM (mean \pm s).

Pregnancy week	Control group (n=40)	miR-137 group (n=40)	P value
24 th week	0.94 \pm 0.29	0.95 \pm 0.5	0.139
25 th week	0.95 \pm 0.19	1.3 \pm 0.41	0.016
26 th week	0.96 \pm 0.28	1.9 \pm 0.51	0.007
27 th week	0.98 \pm 0.31	2.5 \pm 0.63	0.026
28 th week	1 \pm 0.42	2.9 \pm 0.71	0.001

Table IV. Percentage of diabetic pregnant mothers with neonatal hyperbilirubinemia and absence of neonatal hyperbilirubinemia.

Blood sugar level	Absence of neonatal hyperbilirubinemia	Neonatal hyperbilirubinemia
Pre-diabetic	27	29
Diabetic	22	36

The results obtained in the present study for 40 mothers showed that while 29% of mothers with neonatal hyperbilirubinemia and 27% of mothers without neonatal hyperbilirubinemia had pre-diabetes, 36% with neonatal hyperbilirubinemia and 22% without neonatal hyperbilirubinemia had diabetes. According to these results, most of the mothers with neonatal hyperbilirubinemia were in the diabetic stage and most of the mothers without neonatal hyperbilirubinemia were in the pre-diabetic stage (Table IV).

Discussion

Identifying predisposing factors for hyperbilirubinemia in neonates is a serious issue that plays a significant role in controlling jaundice and its primary cause (Garcia and Nager, 2002; Boskabadi *et al.*, 2010). Neonates of diabetic mothers suffer from many

problems, including prematurity and its complications, hypoglycemia, macrosomia, and jaundice. The risk factors of neonatal hyperbilirubinemia include blood incompatibilities, breastfeeding, excessive weight loss after birth, race, prematurity, use of certain drugs, polycythemia, neonatal sepsis, starvation, galactosoma, male gender and history of hyperbilirubinemia in a previous neonate of family (Behrman *et al.*, 2011; Agarwal and Deorari, 2002). Another predisposing factor for neonatal hyperbilirubinemia is the mother's problems during pregnancy, so that in cases of diabetes, twins, and rupture of the amniotic sac, the probability of neonatal hyperbilirubinemia increases (Kurjak and Chervenak, 2015).

MicroRNAs are small non-coding RNA molecules that negatively regulate gene expression by binding to the 3' transcript untranslated region (3'UTR). Identifying the primary molecular defects involved in the pathophysiology of diabetes is an important scientific and clinical goal. Clinical studies have shown that the expression of micro RNAs changes under different types of diabetes (Tang *et al.*, 2008). In the present study, the expression of miR-137 was investigated in forty patients with GDM in different degrees of diabetes. The results of this study showed that the expression level of miR-137 increased significantly from the pre-diabetic stage to the diabetic stage. Although the exact role of miR-137 in GDM is not clear, many studies have focused on finding the role of microRNAs in the process of this disease. One of the reasons for choosing miRNA-137 in this study, which has been emphasized in various studies, is its anti-functional role in GDM induced endothelial cells, which can be used as an aggressive biomarker in the serum to predict the stages of the disease and its course (Peng *et al.*, 2018).

Extensive research has shown that a group of micro RNAs are involved in the process of GDM and changes in the regulation of the expression of these factors can change the expression of other genes in patients with GDM. For example, in patients with GDM, dysfunction of vascular endothelial cells has shown a direct relationship with miRNA-137. When miRNA-137 is up-regulated by high-glucose, it has consequences in patients with GDM that include: Restriction in viability and angiogenesis, Stimulation of monocyte chemotaxis and adhesion to vascular endothelial cells and activation and inflammatory cytokine secretion of vascular endothelial cells. Peng *et al.* (2018) concluded that miRNA-137 as a novel biomarker can be used to predict dysfunction of high-glucose induced vascular endothelial cells, which can be an effective factor for investigating GDM.

In the present study, it was shown that the highest number of GDM patients in both pre-diabetic and diabetic groups had neonatal hyperbilirubinemia. In the miRNA-137

group, 29% of pre-diabetic patients and 36% of diabetic patients had neonatal hyperbilirubinemia, which according to these results, patients with GDM can be considered as the riskiest factor for neonatal hyperbilirubinemia among the studied groups. Although many factors are involved in the development of GDM and its direct relationship with neonatal hyperbilirubinemia seems very complicated, clinical studies throughout history have shown that the rate of neonatal hyperbilirubinemia caused by GDM among diabetic patients is higher than of pre-diabetic patients. However, other factors can affect the prevalence of this disease.

Conclusion

The present study showed that the increased expression of miR-137 in more advanced stages of GDM can be considered as a non-invasive marker to predict the stages of the disease and its progress. This study also showed that neonatal hyperbilirubinemia is one of the important negative consequences in the progression of GDM to higher stages, which makes the treatment of neonatal hyperbilirubinemia difficult.

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IRB approval

This study was approved by the Advanced Studies Research Board of the Fouth Hospital of Shijiazhuang, Shijiazhuang, Hebei050000, China.

Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of the Fouth Hospital of Shijiazhuang, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

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