Determination and comparison miR135a in the serum between women with GDM, non- pregnant type 2 diabetes , healthy pregnant and control group

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Abstract

Objectives: Diabetes is one of the most important endocrine diseases caused by complex reactions between genetic and environmental factors. Recent studies have shown that microRNAs play an important role in the production, inhibition, and secretion of insulin. Identifying the relationship between key miRNAs that control the genes involved in the pathogenesis of diabetes is clinically important because it provides a way to identify preventive methods or treatments. In the present study, the expression of miR135a in serum samples between women with Gestational diabetes mellitus (GDM), non-pregnant type 2 diabetes, and healthy pregnant women were compared with the control group.

Materials and methods: This study was a case-control study and non-random sampling method was used. The present study was conducted among four groups (healthy non-pregnant women (control), non-pregnant Diabetes type 2, GDM, and healthy pregnant). After serum separation, expression of miR-135a was measured using QRT-PCR technique and the results were analyzed by Stata and SPSS21 software.

Results: The results show that the mean expression of miR-135a gene in control group was 0.9 ± 0.06, control of pregnancy was 1 ± 0.1, GDM group was 1.7 ± 0.3 and non-pregnant diabetic type 2 group was 6 ± 6 / 3. The results of analysis of variance showed that the mean difference of miR-135 gene expression was significant higher in the non- pregnant type 2 diabetes than GDM group ($F = 2776.3$, $P < 0.001$).

Conclusion: The widespread role of miRNAs as post-transplantation gene regulators in gestational diabetes mellitus suggests that miR135a may act as a potential indicator of the prevention, treatment, and management of gestational diabetes .

Key words: miR135a, non- pregnant type 2 diabetes, gestational diabetes mellitus, QRT-PCR

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Introduction

Diabetes is one of the most important endocrine diseases that can cause insufficiency or lack of insulin and glucagon production. (1,2) Diabetes is caused by complex reactions between genetic and environmental factors (2, 3). According to the World Health Organization (WHO), diabetes is the seventh cause of death in the world, with the number of diabetic patients estimated to reach 520 million in 2030 (4, 5). Diabetes, includes three types of type 1 , type 2 diabetes and gestational diabetes . Gestational diabetes is diagnosed for the first time during pregnancy. This type of diabetes is usually transient and improves after pregnancy. But these women are at risk of developing type 2 diabetes. (6) Gestational diabetes has many effects on the mother and the fetus, the most common of which are fetus macrosomia, problems during childbirth, cesarean section, polyhydroamenia, preeclampsia and neonatal metabolic disorders (hypoglycemia, hyperglycemia), fetus jaundice and an increased prevalence of pregnancy deaths(7-9). In recent years, various studies have shown that miRNAs are one of the most important genetic factors in the development of diabetes. (10,11)

miRNAs are small intrinsic origin RNAs (19 to 25 nucleotides) that regulate the gene expression by targeting the 3'UTR of the mRNA gene and preventing its transcription (6, 10, 11). miRNA as a regulator of glucose homoeostosis by targeting the genes involved in making insulin, the differentiation of β-pancreatic cells and the agents involved in insulin exocytosis, can play an important role in the development of diabetes can be used as a primary marker for diagnosing gestational diabetes as well as preventing its progress (12). The results of a study on a group of genes expressing the microRNAs involved in pregnancy disorders on chromosomes number 19 and 14 showed that the expression of these microRNAs has a direct relationship with pregnancy disorders, including gestational diabetes (13). Iran is a developing country with limited economic resources and a young population. About 11 million people are women of reproductive age who are at risk of developing the disease (8, 9). As recent studies have shown that the role of miR135a in inhibiting the INSR gene, which targets and suppresses the expression of IRS2 gene, has been shown to reduce glucose uptake in the cell and cause diabetes (14-16). Therefore, the present study aimed to study the effect of miR135a on women with gestational diabetes mellitus(GDM) and non- pregnant type 2 diabetes compared with healthy pregnant women and control group.

Materials and Methods

Characteristics of patients

This study was case-control, It was done on 120 human cases in four groups of 30 (healthy non pregnant (control), healthy pregnant , gestational diabetes mellitus and non-pregnant diabetes type 2). The non-randomized and available sampling method, based on the introduction of the endocrinologist and metabolism specialist, as well as those referring to the Shahid Blindian Diabetes Screening Center, was based on the 2016 health and medical diagnostic laboratories of Qazvin province with a code of ethics REC.1394.191.

Laboratory examination

Peripheral blood samples (2 mL) were collected from women at 16–19 weeks of pregnancy. At 24–28 weeks of pregnancy, a 50-g glucose challenge test (GCT) was conducted. Women with an abnormal 1-hour post-GCT glucose level (≥7.8 mmol/L) were recommended to undergo a 3-hour, 75-g OGTT, during which the blood glucose level would be tested four times after intake of a cola-like drink (at 0, 1, 2, and 3 hours). Women were deemed to have GDM when at least two of the four concentrations measured were above the cutoff values (10.3 mmol/L at 0 hours, 8.6 mmol/L at 1 hour, 6.7 mmol/L at 2 hours, and 5.8 mmol/L at 3 hours.(17)

RNA isolation and Quantitative Real-time PCR

Peripheral blood was obtained by tube containing anti coagulation EDTA and then centrifugation at 2,000 g for 6 minutes. It was then aliquoted and stored at -80C until miRNA detection. Extracted total RNA from 250µl of plasma using TRK- 1001 (LC-Bio, USA) was done according to the protocol 5 µl of plasma RNA containing miRNA and was reverse transcribed to cDNA, reverse transcription solution system that includes 5 µl total RNA, 1 µl M-MLV Buffer, 1 µl M-MLV, 0.5 µl dNTP, and 0.5 µl RRI (Reverse Transcriptase M-MLV (RNase H-), TAKARA, China). Reverse transcription reaction was done in Real Time PCR instrument (MyGene L96G, LongGene, China). Real-time PCR was performed using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen: 11733-038). RNU6B stable stay in serum and changeless in different people was used as internal control gene according to the Applied Biosystems Application Note ,and it has been widely used in different fields of research. All reactions were run in triplicate.

To analyze the data, software (Version 14.1: College Station, Texas, USA) STATA, REST 2009 and SPSS 21 were used at the significant level ($P < 0.05$).

Results

This study was performed on 120 human samples including four groups (non-pregnant healthy, non-pregnant type 2 diabetes , healthy pregnant and gestational diabetes mellitus) for measuring the expression of miR135a gene as a diagnostic marker in GDM subjects. At first, the mean and confidence interval of miR-135a gene expression was evaluated by different groups (Table 1). The mean of expression of miR-135a gene in healthy control group 0.9 (confidence interval 95%, 0.8, 0.9) in pregnant control group was 1.0 (confidence interval 95%, 0.9, 1.0) in gestational diabetes group 1.7 (confidence interval 95, 1.6, 1.9) and was reported in the diabetes mellitus group 3.6 (confidence interval 95% 3.4, 3.9).

Table 1: Mean expression of miR-135a gene in healthy control, pregnancy control, gestational diabetes and non pregnant type 2 diabetes

group	mean	confidence interval 95%
Healthy control	0.9	0.9,0.8
Pregnant control	1.0	1.0,0.9
Gestational Diabetes mellitus	1.7	1.9,1.6
non pregnant type 2 diabetes	3.6	3.9,3.4

To determine the difference between the mean expression of miR-135a gene in the studied groups, analysis of variance was used (Table 2). Based on the results of ANOVA, there was a significant difference in the expression of miR-135a gene among the groups (F (0.05, 119) = 276.3, P value <0.001). The results of Bonferroni's post hoc test for comparing the two groups showed that expression of miR-135a gene in the healthy control group was significantly lower than the gestational diabetes groups (mean difference of group = 0.8, P value <0.001) and type 2 diabetes (Mean difference of two groups = 2.7, P value <0.001).

Table 2. Evaluation of the difference between mean expression of miR-135a gene in the studied groups using the variance analysis table

Difference Type	SS	Degrees of release	MS	F	P VALUE
The difference between the groups	149.3	3	49.7	276.3	0.001>
Intra-group difference	20.8	116	0.1		
Total	170.2	119	1.47		

Comparison of the mean gene expression in the control group of pregnant women and other groups showed that women in this group compared with GDM groups (mean differences = 0.7, P value <0.001) and non- pregnant type 2 diabetes (mean difference = 2.6, P value <0.001) had lower gene expression and the observed difference was statistically significant. In addition, the difference between gestational diabetes mellitus and non- pregnant type 2 diabetes showed that women with type 2 diabetes significantly had higher gene expression (mean difference = 1.8, p value <0.001) (Table 3).

Table 3: Bonferroni post hoc test result table for comparing two and two differences in the mean expression of miR-135a gene in healthy control, pregnancy control, gestational diabetes and type 2 diabetes

	Healthy control	Pregnant control	Gestational Diabetes
group	Mean difference (P value)	Mean difference (P value)	Mean difference (P value)
Pregnant control	0.09(0.1)		
Gestational Diabetes	0.8(0.001)	0.7(0.001)	
non pregnant Type 2 diabetes	2.7(0.001)	2.6(0.001)	1.8(0.001)

Discussion

Diabetes is now considered one of the most important diseases that has many complications. Despite various treatments, no definitive treatment of this disease has yet been found (18). Gestational diabetes is a major concern for mental health; recent findings suggest that GDM may have long-term implications for mother and child (19).

The pair produces several different microRNAs, some of which are released in the mother's circulation. These genes have been observed in the plasma of GDM-positive women with pregnancy outcomes (21). Based on the results of this study, there was no significant difference between the control and the healthy pregnant control group. However, the mean expression of miR-135a gene in GDM cases was significantly higher than of the healthy pregnant and control group. This illustrates the impact of this Micro RNA in pregnancy-related disorders, such as gestational diabetes, which coincides with the study of Diana M. Morales-Prieto and colleagues to demonstrate the role of microRNAs in the development of gestational diabetes. A study conducted in 2011 with the aim of examining pregnant microRNAs concluded that maternally circulating microRNAs could be a new diagnostic tool for the diagnosis of pregnancy disorders (22); also shown in a study by Nir Pillar and his

colleagues in 2015. It has been concluded that microRNAs play a major role in the pathogenesis of GDM and can be used as a primary bio marker for diagnosis of gestational diabetes, as well as for preventing progression (23). Yanan Zhu and colleagues in a preliminary study concluded that the expression of five positive miRNAs (HSA-MIR-16-5p, HSA-MIR-17-5p, HSA-MIR-19A-3P, HSA-MIR-19B-3P, HSA -MIR-20A-5P) was higher in the GDM group than in the control group and there was a higher amount of these RNAs in these subjects than non-diabetic subjects, which correlated with the expression of miRNA expression. Like the present study, Yanan Zhu's study also identified miRNA in plasma, which predicted GDM in the second quarter. The strengths of the study included the use of a high sequencing platform and the validation of MSRNA results by qRT-PCR. (24)

In the present study, the expression of miR135a in diabetic patients with up-regulate pregnancy was higher than that of healthy pregnant women and control group and according to a study by Chun Zhao and colleagues in 2011 on 16 women with gestational diabetes and 16 non-diabetic (control) subjects. This study examined the expression of microRNAs and their implications in gestational diabetes, and concluded that the expression of miR-132, miR-29a and miR-222 in pregnant women was higher than that of normal and control subjects and prevention of expression. These microRNAs lead to an increase in insulin gene expression (25).

Since the GDM group had a higher gene expression than healthy pregnant group and had less gene expression than non-pregnant type2 diabetes, also considering that studies have shown that women with gestational diabetes are at increased risk for diabetes later in life, (6), and one third of mothers who have gestational diabetes during pregnancy later develop Type 2 diabetes (20). It seems that pregnant women should have diabetes after termination of pregnancy and recovery. Gestational diabetes is monitored for expression of miR135a.

Chen and colleagues compared serum miRNA expression in type 2 diabetic patients with healthy subjects and showed that the serum miRNA expression profile was significantly different between the control and the healthy pregnant group (26). Zampetaki and colleagues performed micronutrient screening and qRT-PCR methods to determine the plasma miRNA expression profile in type 2 diabetes, and concluded that the expression of miR-20b, miR-21, miR-24, miR-15a, miR-126, MiR-191, miR-197, miR-223, miR-320 and miR-486 were more common in type 2 diabetes (10).

Honardoost and her colleagues in their studies have found that miR135a is effective in inhibiting the INSR gene and creating a diabetic state in mouse muscle cells. The goal of this miR is the IRS2 gene, which reduces glucose uptake in the cell. Studies at the molecular level also showed that the suppression of this miRNA reduces hyperglycemia (14, 16, 17, 27).

Conclusion

In general, available data suggest that the serum level of miR135a can be a promising new diagnostic tool for diagnosing gestational diabetes mellitus. However, more research is needed to investigate the mechanism of the effect of mir135a on the incidence of gestational diabetes mellitus.

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