



## Evaluation of MicroRNA-16 and MicroRNA-221 in Serum and Placenta in Gestational Diabetes Mellitus: Correlation with Macrosomia



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**G**ESTATIONAL diabetes mellitus (GDM) is associated with short and long-term complications for pregnant women and their coming offspring. MicroRNAs are small noncoding RNA molecules that regulate gene expression. This study aimed to investigate the potential expression of microRNA-16 and 221 in maternal serum and their mirror expression in the placenta in GDM and their correlation with severity of GDM and Macrosomia.

This study was conducted on twenty pregnant women with GDM and other twenty healthy pregnant women who served as a control group. GDM group was further subdivided into severe and mild subgroups according to the levels of fasting and postprandial 2 hours glucose levels. MicroRNA-16 and microRNA-221 were estimated by quantitative real time polymerase chain reaction (qRT-PCR). Results revealed that microRNA-16 levels in serum and placental tissue were significantly higher in the GDM group than the control group, with high significant increase in severe GDM than in mild GDM subgroup. Significant decreases in the levels of microRNA-221 were detected in both serum and placenta in GDM group versus the control group. In GDM group, there was a direct high significant correlation of microRNA-16 in serum and placenta with fetal macrosomia, but there was no correlation of microRNA-221 in serum with fetal macrosomia. A significant increase of microRNA-16 in the severe GDM group than in the mild GDM group was observed and it could be concluded that microRNA-16 may be the noncoding RNA at the molecular level that affected the severity of GDM in mothers and the macrosomia of their offspring.

**Keywords:** GDM, Macrosomia, MicroRNA-16, MicroRNA-221, Placental tissue, Serum.

### Introduction:

Gestational diabetes mellitus is defined as impaired glucose tolerance diagnosed during pregnancy for the first time and is present in 6–7% of pregnancies with the increase in pregnancy complications (Catalano, 2014). It is one of the most common medical disorders of pregnancy. Women with GDM are at high risk for future type 2 diabetes, 50% of women with GDM subsequently develop type 2 diabetes within 25 years (England et al., 2009; O'Sullivan, 1982). Additionally, maternal diabetes during pregnancy exposes the fetus to hyperglycemia and high insulin levels, which

might be related to increased risk of childhood and adult obesity; type 2 diabetes and GDM at childbearing ages (Dabelea et al., 2000).

There are series of metabolic changes that occur during normal pregnancy, with a progressive increase in insulin resistance mainly due to placental hormones production (growth hormone, progesterone, placental lactogen, and corticotrophin) important for fetal nutrition. Gestational diabetes develops in women whose insulin production is insufficient to counteract increasing insulin resistance (Catalano, 2014) that has been correlated with increasing of maternal

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age and obesity (Lawrence et al., 2008; Ferrara, 2007).

Gestational diabetes mellitus occurs when the function of pancreatic  $\beta$  cells is insufficient to overcome the insulin resistance that occurs during pregnancy (Basina, 2012). The oral glucose tolerance test (OGTT) is still the golden standard for the identification of this disorder. However, the cut-off values established by the World Health Organization (WHO) have been modified by some international associations, generating multiple measures for the diagnosis and management of GDM (Akgöl et al., 2017).

Oral glucose tolerance test (OGTT) is commonly used to diagnose GDM in clinical practice, but this test is usually performed at 24–28 weeks of pregnancy, and sometimes is delayed until 32 weeks. This leaves little time to change diet and exercise regimens, or for medical intervention, and increases the risk and complications of GDM in later pregnancies. Hence, there is a need for prompt strategies for early diagnosis of GDM (Rodrigo & Glastras, 2018; Karcaaltincaba et al., 2017).

The expression of microRNAs has been reported to be associated with pregnancy related diseases as GDM and pre-eclampsia. Maternal-fetal cellular communication depends on factors secreted by the placenta, which could be used as markers of altered placental functions, which are detectable in the maternal circulation from the early stages of pregnancy; and therefore could have a predictive value in various pregnancy-related diseases (Ilekis et al., 2016). Several studies were conducted to identify biomarkers and to make a timely diagnosis of GDM (Karcaaltincaba et al., 2017; Powe, 2017).

The human placenta expresses >500 microRNAs, almost exclusively expressed in this organ. For this reason, the study of placental microRNAs is essential for the understanding of the regulatory mechanisms of normal and complicated pregnancies (Guarino et al., 2018).

MicroRNAs are small noncoding RNAs with 18–22 nucleotides, which have been described to control post-transcriptional gene expression through binding of the translation section, and leading to either microRNAs deprivation or translational inhibition (Krol et al., 2010). Pre-

miRNA depends on the transportation mechanism to reach the cytoplasm and then it is cut into a mature 22 nucleotides chain microRNA with the Dicer. The twofold stranded microRNA is made after the creation of the Argonaute proteins (Ago) family (Bartel, 2009), which precisely relay with small RNAs and considered to be the main of all known RNA-induced silencing complexes (RISCs). These proteins are functionally specified into distinct RNA silencing pathways. (Su et al., 2009). Mammals contain four copies of Ago genes: Ago1–Ago4, (Song et al., 2004), (*AGO1*, *AGO3* and *AGO4*) are existing as tandem copies in the similar orientation on human chromosome 1p34.3, while *AGO2* exists on its own on human chromosome 8q24.3. Only Ago2 has cleavage activity that target mRNAs directed by small-interfering RNAs (siRNAs) (Yekta et al., 2004). Ago1, Ago3 and Ago4 most likely have misplaced this cleavage function and instead exclusively depending on a non-cleavage mechanism to actuate translational restraint of target mRNAs by means of miRNAs (Wang et al., 2009).

The expression of microRNAs has been reported to be associated with pre-eclampsia and GDM (Pillar et al., 2015). In their study, Cao and colleagues reported that plasma microRNA-16-5p was significantly upregulated at different times during pregnancy in GDM compared with non-GDM women and reflected a clear separation between GDM and non-GDM patients (Cao et al., 2017).

Li et al. (2009) in their study suggested that during the development of diabetes, hyperglycemia, a malfunction of the vasculature may be induced. Exposure to high levels of glucose may alter microRNAs expression in cardiovascular cells under many pathological conditions. They identified a novel pathway by which hyperglycemic conditions impair c-kit expression and migration of human umbilical vein endothelial cells, perhaps through induction of microR-221, and they reported that manipulation of the microR-221-c-kit path might propose a new approach for management of vascular dysfunction in diabetic patients (Li et al., 2009). In several studies, the authors related upregulated microRNA-221 to asymptomatic carotid stenosis (Dolz et al., 2017), focal cerebral ischemia (Gusar et al., 2017), pulmonary artery hypertension (Nie et al., 2019) and obesity (Villard et al., 2015). Its upregulation is related to cardiac amyloidosis

(Derda et al., 2018), heart failure (Verjans et al., 2018), aortic stenosis (Zhuang et al., 2019), acute myocardial infarction (Coskunpinar et al., 2016), acute ischemic stroke (Sørensen et al., 2014) and atherosclerosis (Pereira-da-Silva et al., 2018).

Previous studies (Guarino et al., 2018) have suggested that microRNAs are involved in GDM; however, the microRNA-16 and microRNA-221 expression in serum and placental tissues from GDM patients and their significance to macrosomia of the offspring, to our knowledge, have not been evaluated. For this reason, this study was conducted aiming to determine and evaluate the association of microRNA-16 and microRNA-221 expression in gestational diabetes mellitus and to find the correlation between serum, placental microRNA-16 and microRNA-221 with macrosomia associated with Gestational diabetes mellitus.

## **Materials and Methods**

### *Study setting and study population*

The study involved forty Egyptian pregnant women who attend Ain Shams University Hospital, Obstetrics and Gynecology Department, at labor wards. 20 pregnant women who were previously diagnosed as gestational diabetic patients were recruited for the study with mean age of (29.02± 2.244) years, in addition, 20 pregnant women without GDM (control group) with mean age of (28.8± 2.375) years were also recruited. Pregnant women with GDM were further classified into mild (n= 12) and severe (n= 8) subgroups in accordance to the 2-hours oral glucose tolerance test which was initially proposed by the International Association of Diabetes and Pregnancy. Study Groups: Patients with the following criteria were excluded: (1) Pre-pregnancy diabetes mellitus; (2) Severe systemic disease; (3) Family history of known congenital or chromosomal abnormalities of the fetus; (4) Age < 18 years; (5) Twin pregnancies. All the participants were interviewed and personal data were collected and all of them agreed to be part of the study and gave informed consents preliminary to the study according to Helsinki declaration.

### *Data collection*

All participants were subjected to clinical examinations with measurements of the systolic and diastolic blood pressures. The laboratory investigations (hemoglobin, platelets, urine

albumin, ALT, AST, creatinine, urea, cholesterol, FBS, postprandial blood sugar and INR) were performed for all subjects. Blood samples were collected on admission for labor; serum was separated and collected in aliquots and stored at -80°C until further processing. All placental samples were washed with saline and stored at -80°C.

Parameters such as personal and family history of diabetes, type of delivery, complications of pregnancy, body mass index and perinatal data, as well as data regarding the infants' APGAR scores, weight and sex were taken prospectively from women joined in the trial after informed consent.

“Macrosomia” implies growth beyond an absolute birth weight, historically 4,000 gm. Excessive fetal growth is described as “large for gestational age which means a birth weight equal to or more than the 90th percentile for a given gestational age” (ACOG Practice Bulletin, 2010).

Body mass index (BMI) was calculated in this study by detecting patients weight in kilograms divided by the square of height in meters. Serum insulin concentrations were measured by radioimmunoassay (RIA) using a commercial kit, DIA source INS-IRMA kit, supplied by DIA source ImmunoAssays S.A. (Belgium). For test investigation by RIA, a porcine-specific one-site insulin RIA2 was utilized. This test is based on 125I-labeled insulin and utilized guinea pig anti-porcine insulin antibodies and goat anti-guinea pig IgG antibodies. Decontaminated human recombinant insulin preparations were utilized as guidelines for calibration. The manufacturer's procedure referred to the assay's restraint of linearity of 200 µIU/mL and prescribed dilution of the samples, which have more noteworthy concentrations with the test buffer. The limit of recognition for this measure is 1.61 µIU/mL when 100 µl of test is utilized. The cross-reactivity of the test is expressed at 100 % for porcine insulin, 100% for human insulin and 90 % for bovine insulin.

### *GDM diagnosis*

HbA1c should be used in association with other standard diagnostic tests for GDM diagnosis (Renz et al., 2019). The HbA1c analysis was performed using high-pressure liquid chromatography on a Bio-Rad Variant II instrument.

OGTT was performed after an overnight fast

of 10 h. A sample was taken at baseline and then the patient consumed a dose of 75 g glucose. The patient was required to finish the whole volume in 5 min of starting the drink. Blood samples were taken at 1 and 2 h after starting dextrose drink and measured using the Abbott glucose hexokinase method on an Architect C8000 analyzer (Abbott Australasia Pty Ltd).

Normal results for gestational diabetes:

- Fasting glucose level below 90 mg/dL
- One-hour glucose level below 130 to 140 mg/dL
- Two-hour glucose level below 120 mg/dL

Abnormal results for gestational diabetes:

- Fasting glucose level greater than 95 mg/dL
- One-hour glucose level greater than 140 mg/dL
- Two-hour glucose level greater than 120 mg/dL (Eyth et al., 2020; Li & Yu, 2019)

#### RNA extraction and Polymerase Chain Reaction

Total RNA was extracted from 25 mg of placental tissue preserved in RNA later (Ambion) followed by an enrichment procedure for small RNAs (siRNAs, microRNAs), according to the manufacturer's instructions using a mirVana microRNA Isolation kit (Ambion). Total RNAs were isolated from all samples using miRNeasy Serum/Plasma Kit (Qiagen, Germany) according to the manufacturer's instructions. Single-stranded cDNA was prepared in a reverse-transcription reaction using TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher, USA) using 5 µg of RNA, according to the manufacturer's protocol. The cycling condition for cDNA synthesis included incubation of the reaction mixture at 25°C for 10 min, 42°C for 60 min, followed by 10 min at 70°C.

PCR was carried out using cDNA, MgCl (10 mM) to improve the DNA intensification by enhancing the action of Taq DNA polymerase.,

**TABLE1. The primer sequence of the studied microRNAs**

	Primer sequence
MicroRNA-221	Forward primer :5'- TGCCTCCAAGTACTCCTAC -3 Reverse primer 5'- GCGAGCACAGAATAATACGAC CC -3
MicroRNA-16	Forward primer :5'- CGGTGTCGTAGTTGAAGTGAG -3 Reverse primer 5'- GATGTCCGATCCGTCTGAGC -3
U6	Forward :5 CTCGCTTCGGCAGCACA - 3' Reverse: 5- AACGCTTCACGAATTTGCGT -3

Taq-polymerase (5 U/µl), PCR buffer, dNTP (10 mM) and a pair of specific primers (10 µM) as shown in Table 1 in a final volume of 20 µl each tube. The PCR conditions were as follows: initial denaturation at 95°C for 5 min followed by 40 cycles, annealing at 95°C for 15s, and extension at 60°C for 20 s and final extension at 72°C for 40 s. Relative expression of studied miRNA was calculated using the comparative Ct method. All values were normalized to U6, which was used as the control housekeeping gene and reported as fold change.

#### Statistical analysis

It was performed using SPSS version 20 statistical software (SPSS, Inc, Chicago, IL) and a P value of < 0.05 was considered statistically significant. All the results were defined as mean±standard deviation. The independent sample-t test was applied to compare the microRNAs expression levels, and the other measured parameters between GDM group and healthy control group. One-way ANOVA test was applied for comparison between mild GDM, severe GDM subgroups and control group.

#### Results

Table 2 shows that there were highly significant differences in FBG, PPG, HbA1c, fasting insulin, gestational age, fetal birth weight between the two groups studied. Serum and placental microRNA-16 in GDM group increased significantly as compared to control group, serum and placental microRNA-221 in GDM group decreased significantly than control group.

Table 3 shows that in the GDM group, there was a positive significant correlation between serum and placental microRNA-16 with FBG, BMI, and fetal birth weight, while an inverse significant correlation between serum microRNA-221 and FBG, PPG was detected.

**TABLE 2.** The Demographic data and laboratory investigations of control group compared to gestational diabetes group

Parameters	Control (n= 20) Mean ± SD	Gestational Diabetes (n= 20) Mean ± SD	P values
Age (years)	28.8± 2.375	29.02 ± 2.244	0.053
Parity	1.80 ± 0.768	2.10 ± 0.960	0.227
BMI	29.63± 2.66	32.21± 4.06	0.023*
Fasting Blood glucose	81.60 ± 5.404	194.00± 60.242	0.0001**
Postprandial glucose	118.3±11.926	243.40± 63.446	0.0001**
HbA1c	4.20± 0.458	8.18± 1.58	0.0001**
Fasting Insulin (µIU/ml)	5.76± 1.89	18.93± 3.72	0.0001**
Systolic BP (mmHg)	112.00 ± 19.19	118.5 ± 16.66	0.353
Diastolic BP (mmHg)	71.30 ± 12.24	75.5 ± 9.30	0.551
Gestational age (weeks)	38.6 ± 0.94	36.1 ± 3.47	0.01*
Fetal birth weight(gm)	3260.5 ± 231.16	3955.00 ± 416.09	0.001**
Urine albumin(gm/dl)	0	1.05 ± 0.25	0.01*
Hemoglobin (gm/dl)	11.86 ± 1.721	11.70 ± 1.306	0.634
ALT(U/L)	31.40 ± 10.25	35.00 ± 7.59	0.215
AST (U/L)	34.80 ± 8.14	35.40 ± 2.79	0.918
Urea (mg/dl)	21.20 ± 4.65	25.40 ± 7.59	0.054
Creatinine(mg/dl)	0.910 ± 0.384	0.921 ± 0.339	0.855
Platelet count(×10 <sup>3</sup> /ul)	380.40± 75.37	391.20± 82.13	0.667
INR	1.14 ± 0.104	1.00± 0.194	0.07*
TLC (×10 <sup>3</sup> /ul)	6680± 2018	7010 ± 1704	0.591
Serum MicroRNA-221 (relative expression)	1.016 ± 0.019	0.550± 0.181	0.0001**
Placenta MicroRNA-221 (relative expression)	1.006± 0.012	0.210 ± 0.048	0.0001**
Serum MicroRNA-16 (relative expression)	1.014 ± 0.015	3.702± 1.474	0.0001**
Placenta MicroRNA-16 (relative expression)	1.030 ± 0.038	5.182 ± 0.940	0.0001**
Cesarean Section (%)	10 (50%)	12 (60%)	0.205

\*: Significant if (P<0.05), \*\*: High significant if (P<0.001).

Table 4 shows that in both groups, there was high a significant correlation between serum & placental microRNA-16 with fetal birth weight, FBG, PPG, and BMI, while a high significant inverse correlation between serum& placental microRNA-221 with fetal birth weight, FBG, PPG was detected.

Table 5 shows highly significant differences in mean FBG, PPG, cholesterol, fetal birth

weight, serum and placental microRNA-16 and microRNA-221 and significant difference in BMI between the mild, severe gestational diabetes and control group.

Table 6 clarifies significant differences in FBG, PPG, HbA1c, fetal birth weight, creatinine, serum & placental microRNA-16 and serum microRNA-221 between mild and severe gestational diabetes.



**TABLE 3. Correlation between Serum and placental MicroRNA-16 and MicroRNA-221 with Fetal weight, FBS, PPG, BMI in gestational diabetes group (n= 20)**

Parameter		Fetal weight	FBS	PPG	BMI
Serum MicroRNA-16	Correlation coefficient	0.435	0.508	0.428	0.865
	P value	0.05*	0.02*	0.05*	0.0001**
	GDM group N=	20	20	20	20
Placenta MicroRNA-16	Correlation coefficient	0.778	0.855	0.869	0.134
	P value	0.0001**	0.0001**	0.0001**	0.57
	GDM group N=	20	20	20	20
Serum MicroRNA-221	Correlation coefficient	-0.097	-0.650	-0.741	-0.245
	P value	.683	0.002**	0.0001**	0.298
	GDM group N=	20	20	20	20
Placenta MicroRNA-221	Correlation coefficient	-0.025	-0.327	-0.123	0.796
	P value	0.918	0.16	0.604	0.0001**
	GDM group N=	20	20	20	20

\*: Significant if (P<0.05), \*\*: High significant if (P<0.001).

**TABLE 4. Correlation between Serum and placental MicroRNA-16 and MicroRNA-221 with Fetal weight, FBS, PPG, BMI in both studied groups (n=40)**

Parameter		Fetal weight	FBS	PPG	BMI
Serum MicroRNA-16	Correlation coefficient	0.563	0.459	0.558	0.692
	P value	0.0001**	0.003**	0.0001**	0.0001**
	Studied groups N=	40	40	40	40
Placenta MicroRNA-16	Correlation coefficient	0.842	0.918	0.917	0.377
	P value	0.0001**	0.0001**	0.0001**	0.016*
	Studied groups N=	40	40	40	40
Serum MicroRNA-221	Correlation coefficient	-0.775	-0.889	-0.910	-0.393
	P value	0.001**	0.0001**	0.0001**	0.012*
	Studied groups N=	40	40	40	40
Placenta MicroRNA-221	Correlation coefficient	-0.520	-0.817	-0.804	-0.299
	P value	0.001**	0.0001**	0.0001**	0.061
	Studied groups N=	40	40	40	40

\*: Significant if (P<0.05), \*\*: High significant if (P<0.001).

**TABLE 5. Comparison between control, mild GD and severe GD groups in FBG, PPG, BMI, Cholesterol, Fetal birth weight, Serum & Placenta microRNA-16 and serum & placental microRNA-221 by using one way-ANOVA**

Parameters	control (n= 20) Mean ± SD	Mild GD(n= 12) Mean ± SD	Severe GD (n= 8) Mean ± SD	P value
FBG	81.60 ± 5.40	156.66± 35.47	250.10 ± 53.45	0.001**
PPG	118.30± 11.92	198.90±10.52	285.00±37.41	0.001**
BMI	29.62 ± 2.66	31.29± 3.95	34.27 ± 4.08	0.027*
Cholesterol	187.20± 20.26	255.33± 40.58	251.00± 12.13	0.001**
Fetal birth weight	3260.50 ± 221.82	3683.33 ± 251.13	4247.50 ± 132.9	0.001**
Serum MicroRNA-16	1.01 ± 0.015	3.04± 0.96	4.67 ± 1.62	0.001**
Placenta MicroRNA-16	1.03± 0.038	4.47± 0.38	6.25± 0.53	0.001**
Serum MicroRNA-221	1.016± 0.019	0.686± 0.077	0.345± 0.016	0.001**
Placenta MicroRNA-221	1.006± 0.012	0.213± 0.062	0.205± 0.017	0.001**

\*: Significant if (P<0.05), \*\*: High significant if (P<0.001).

TABLE 6. Comparison between mild GD subgroup (n=12) and severe GD subgroup (n= 8) in biochemical data

Parameter	Group	N	Mean	Std. deviation	P value
FBG	mild GDM	12	156.66	35.47	0.001**
	severe GDM	8	250.10	53.45	
PPG	m-GDM	12	198.90	10.52	0.001**
	s-GDM	8	285.00	37.41	
BMI	m-GDM	12	31.29	3.95	0.235
	s-GDM	8	34.27	4.08	
HbA1c	m-GDM	12	7.40	1.84	0.038*
	s-GDM	8	8.70	0.585	
Creatinine	m-GDM	12	0.65	0.307	0.001**
	s-GDM	8	1.100	0.160	
INR	m-GDM	12	0.950	0.53	0.278
	s-GDM	8	1.033	0.24	
ALT	m-GDM	12	34.05	9.54	0.398
	s-GDM	8	36.50	6.53	
Cholesterol	m-GDM	12	255.33	40.58	0.719
	s-GDM	8	251.03	12.13	
Fetal birth weight	m-GDM	12	3683.33	251.13	0.001**
	s-GDM	8	4247.50	132.9	
SerumMicro RNA-16	m-GDM	12	3.04	0.96	0.028*
	s-GDM	8	4.67	1.62	
PlacentaMicro RNA-16	m-GDM	12	4.47	0.38	0.001**
	s-GDM	8	6.25	0.53	
SerumMicro RNA-221	m-GDM	12	0.686	0.077	0.001**
	s-GDM	8	0.345	0.016	
PlacentaMicro RNA-221	m-GDM	12	0.213	0.062	.668
	s-GDM	8	0.205	0.017	

\*: Significant if (P<0.05), \*\*: High significant if (P<0.001).

### Discussion:

MicroRNAs are small noncoding ~19–24 nucleotide that play an important role in the modulation of gene expression (Bartel, 2004). It has been hypothesized that approximately 60% of genes are regulated by microRNAs; thus, these molecules are critically involved in the regulation of many biological processes (Morris & Mattick, 2014). The characterization of placental microRNAs could be essential to understand the regulatory mechanisms of normal and complicated pregnancies (Cai et al., 2017).

According to the studies done by Cao and colleagues (2017), which reported that plasma microRNA-16-5p was significantly upregulated at different times during pregnancy in GDM. On the other hand, Li et al. study in (2009) identified a novel pathway by which hyperglycemic conditions

impair c-kit expression and migration of human umbilical vein endothelial cells through induction of miR-221, and reported that manipulation of the miR-221-c-kit path may propose a new approach for management of vascular dysfunction in diabetic patients. Hence, The authors focused in this study on MicroRNA-16 and microRNA-221 expression in both serum and placental tissue with their correlation with the fetal macrosomia to detect their role in GDM and detect whether they could be used as biomarkers to identify women at risk of developing severe GDM and macrosomic infants. In the present study, in GDM group, the placental homogenate microRNA-16 levels are higher than those in the maternal serum microRNA-16, which could be explained by the fact that it originated by the placenta, and might be responsible for the occurrence of macrosomia (Filardi et al., 2020). However, it is not the case with microRNA-221 which

decreased in placental homogenate of the GDM group than in maternal serum and its level was lower than in the control group, as this might be a protective mechanism to prevent complications of its increasing levels on the placental circulation and therefore on the fetus. Another interpretation is that perhaps the upregulation in some microRNAs as the microRNA-16 in this study decreases the expression of others like the case in microRNA-221 to prevent severe vascular complications associated with high microRNA-221 (Zhao & Tao, 2019).

As regards to microRNA-16 in this study, there was a high significant increase in serum and placental microRNA-16 expression in GDM group than in the control group. This microRNA-16 whose expression increased in serum of severe GDM patients may increase diabetogenic factors in mothers. This increase in the placenta of GDM more than in the serum may potentially be linked to placental functions, development and increase of growth factors that are responsible for macrosomia and hence the increase in macrosomic fetuses in severe GDM group than in mild GDM group. This comes in accordance to Cao et al. (2017) who confirmed the results of the pilot study by Zhu et al. (2015) in a larger group of patients by analyzing the same differentially expressed microRNAs amongst which was miR-16-5p, which was progressively upregulated during pregnancy in the plasma samples of GDM women at 16<sup>th</sup>–19<sup>th</sup> week, 20<sup>th</sup>–24<sup>th</sup> week, and 24<sup>th</sup>–28<sup>th</sup> week of pregnancy (Cao et al., 2017). Their findings support the suggestion that miR-16-5p is one of the best predictors of gestational diabetes. Although some studies reported the involvement of miR-16-5p in type 2 diabetes (T2D) and in other metabolic diseases, other studies demonstrated that miR-16-5p targeted genes (*CUL4A*, *SMAD1*, *EGFR*, *ACTB*, *RRP12*, and *DAB2*) have been reported to be downregulated in T2D (Calimlioglu et al., 2015), which may be responsible for small for gestational age born infants in type 2 diabetes mellitus mothers. On the other hand, the present study differs in its results from the findings of Hocaoglu and his colleagues (2019) whose study for the first time revealed that miR-16-5p, was not related to pregnancy complicated with neither preeclampsia nor GDM as they were not differentially expressed in these pregnancy disorders as compared to healthy pregnancy. Also, in contrary to the current findings, Pfeiffer et al.

(2018) reported conflicting results, stating that the expression of plasma miR-16-5p was decreased in South African women with GDM.

In GDM group, it was found that macrosomia was directly significantly correlated with microRNA-16-5p. This means that increased levels of microRNA-16 in serum of mothers and in placental homogenate are associated with macrosomia. The present results could be explained by suggestion that the increase in microRNA-16 could be responsible for large fetuses in GDM pregnancies. These results were in agreement with the results of Ge et al. (2015) who reported that the expression of miR-16 increased in the plasma of pregnant women with fetal macrosomia compared to normal the controls. However, another study conducted by Jiang et al. (2014) did not find any significant differences in miR-16 expression levels in mothers between macrosomia group and the control group.

The present study showed that there were significant decreases in serum and placental microRNA-221 expression levels in mild GDM and severe GDM than in the control group. This could be due to pregnancy protective factors in mothers, and its decrease in the placentas of GDM patients than in their serum that may potentially be linked to protective mechanisms controlled by other factors acting upon endothelium of the vessels to maintain adequate blood supply in the placenta to the fetus. The present results came in agreement with the study of Ge et al. (2015) who reported that the expression of miR-221 decreased in the plasma of pregnant women with fetal macrosomia compared to the normal controls, further supporting the use of miRNAs as predictive biomarkers for pregnancy complications. Moreover, the results of this study come in accordance to the results of Zhao & Tao (2019) who reported that MicroRNA-221 decreased in placental homogenate of GDM rats, and its level was correlated negatively to that of the blood glucose level in GDM rats versus the controls. However, the current results are in contrary to Díaz-Pérez et al. (2016) who found that microRNA-221 to be upregulated in human fetoplacental endothelial cells isolated from third-trimester human placentas after pregnancies complicated by GDM versus healthy pregnancies. They suggested this mechanism as protection against leucocyte transmigration from blood to placenta which may worsen the inflammation



due to hyperglycemia during GDM. The first study demonstrating a specific case of placenta-blood mirroring in GDM and involving microRNAs has been performed by Xu and colleagues. They suggested the possibility of a microRNA-based specific cross talk between placenta and  $\beta$  cells in gestational diabetes (Xu et al., 2017).

Hromadnikova et al. (2020) in a recent study reported that children with a prior exposure to GDM are at higher risk of later development of diabetes mellitus and cardiovascular/cerebrovascular diseases, and would benefit from implementation of primary prevention strategies.

The role of these upregulated microRNAs in the pathogenesis of GDM are not yet fully understood. It had been well recognized that no single microRNA or signaling pathway could be responsible for the occurrence of the GDM (Monfared et al., 2018). Maintaining homeostasis in many organs, including the placenta, was established by microRNAs through targeting a large number of genes and participating in various cellular events. The identification of dysregulated microRNAs in GDM placentas and the clarification of gene networks that were regulated by those microRNAs would likely be novel steps in understanding the pathogenesis of such compromised pregnancies (Szmuiłowicz et al., 2019).

### Conclusion

The present study documented the elevation of microRNA-16 in maternal serum of GDM group and its higher level in placenta. On the contrary, the authors observed reduction of microRNA-221 in the serum of GDM group and in placenta compared to the control group. Another finding was the significant direct correlation of microRNA-16 in maternal serum and placenta with the severity of GDM and with macrosomia, which is not the case with microRNA-221. The present findings agreed with the findings, which proposed microRNA-16 as a diagnostic biomarker to identify women at risk of developing severe GDM and macrosomic infants.

*Recommendations:* Further studies are needed to clarify the role of the elevated microRNA-16 and microRNA-221 in GDM and their relation to etiology, pathophysiology, the aggravation of this condition and their role in developing macrosomia.

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