

Original Article

Efficacy of metformin combined with insulin lispro on gestational diabetes and effects on serum miR-16

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Abstract: Objective: This study was designed to explore the efficacy of Metformin combined with insulin lispro on gestational diabetes (GDM) and the effects on serum miR-16. Methods: In total, 117 GDM patients admitted to our hospital from March 2018 to March 2019 were included in this study and divided into the Study Group (SG) and the Control Group (CG) based on the therapeutic regimen. The CG (n=55) adopted insulin lispro, and the SG (n=62) insulin lispro combined with Metformin. Before and after treatment, the 2 groups were compared for treatment efficacy, delivery outcome and adverse outcome of newborns, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c) and 2h-Postprandial Plasma Glucose (2hPPG), tested for serum miR-16 by fluorescent quantitative PCR, analyzed for the contribution of miR-16 in the prediction of GDM by ROC, and the effective hazards in GDM treatment by logistic regression. Results: The SG reported a higher total effective rate, and lower incidences of premature birth, hydramnion, C-sect, as well as less adverse outcomes of newborns as compared with the CG (P<0.05); treatment led to a decrease in the FPG, HbA1c, and 2hPPG after three meals in both groups (P<0.05), after which, those indicators were at a far lower level in the SG as compared with the CG (P<0.05); after treatment, both groups attained a decrease in serum miR-16 (P<0.05), which was more dominant in the SG (P<0.05); the AUC, critical level, sensitivity and specificity of miR-16 in the prediction of GDM were 0.711, 35.01, 66.13, and 67.27 respectively; according to logistics regression, age, BMI, Stein-Leventhal syndrome and parity are the effective and independent hazards in the GDM treatment. Conclusion: The combination of Metformin and insulin lispro has better efficacy and safety in treatment of gestational diabetes. It can reduce the incidence of adverse outcomes of newborns and serum miR-16; deserving popularization in clinical treatment for GDM.

Keywords: Metformin combined with insulin lispro, gestational diabetes, efficacy, serum miR-16

Introduction

Gestational diabetes (GDM) refers to glucose intolerance diagnosed for the first time during pregnancy, which increases the risk of maternal and infant complications in the same period [1, 2]. According to statistics, the GDM rate was 10.8% in Switzerland, 9.2% in the United States, and 6.8% in China which is elevating [3]. GDM is an important but convertible hazard leading to adverse gestational outcomes such as giant baby and preeclampsia. In addition to the adverse outcomes during gestation and delivery, its adverse impacts also extends to other fields beyond gestation, as evidenced by an investigation of glucose function in the puerperants after delivery, that in the 1st year, the case rate of type 2 diabetes was 38%, and in 60% of the cases, the disease continued for 16 years [4-6].

The absence of effective preventions in this field leads to different suggestions on the application of diagnosis and treatment methods of GDM [7]. Insulin lispro is a special and non-pathogenic insulin analog synthesized by laboratory strains, and a class B gestational drug applied in GDM. Though no risk was revealed in the studies of animal reproduction, no strictly controlled studies were carried out in pregnant women, and some adverse reactions such as glucopenia and kaliopenia were observed in some cases [8]. According to associated reports, Metformin is an effective and safe alternative to insulin in the treatment of GDM patients. Instead of causing glucopenia and increasing weight, Metformin can reduce hepatic gluconeogenesis, which is suggested to be advantageous in the preservation of β cell function. Metformin is also related to a transplantation rate between 10-16% for fetuses, which

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Table 1. Clinical materials

| | CG (n=55) | SG (n=62) | χ^2/t | P |
|----------------------------------|------------|------------|------------|-------|
| Age (y) | 27.41±3.21 | 27.63±2.96 | 0.386 | 0.701 |
| BMI (kg/m ²) | 25.12±2.33 | 25.51±2.67 | 0.837 | 0.404 |
| Smoking [n (%)] | | | 0.117 | 0.733 |
| Y | 17 (30.91) | 21 (33.87) | | |
| N | 38 (60.09) | 41 (66.13) | | |
| Drinking alcohol [n (%)] | | | 0.001 | 0.972 |
| Y | 22 (40.00) | 25 (40.30) | | |
| N | 33 (60.00) | 37 (59.68) | | |
| Domicile [n (%)] | | | 1.122 | 0.289 |
| Urban | 32 (58.18) | 30 (48.39) | | |
| Rural | 23 (41.82) | 32 (51.61) | | |
| Previous medical history | | | | |
| Menses normal or not [n (%)] | | | 0.932 | 0.334 |
| Normal | 35 (63.64) | 34 (54.84) | | |
| Abnormal | 20 (36.36) | 28 (45.16) | | |
| Fibroid [n (%)] | | | 0.344 | 0.557 |
| Y | 15 (27.27) | 14 (22.58) | | |
| N | 40 (72.73) | 48 (77.42) | | |
| Stein-Leventhal syndrome [n (%)] | | | 0.132 | 0.717 |
| Y | 24 (43.64) | 25 (40.32) | | |
| N | 31 (56.36) | 37 (59.68) | | |
| Gravidity (time) | 2.52±0.31 | 2.46±0.28 | 1.100 | 0.274 |
| Parity (time) | 1.33±0.27 | 1.29±0.26 | 0.816 | 0.416 |

may account for fetal abnormality or potential adverse impacts on the mother and the baby [9, 10]. According to the studies of Mogensen UM, Andersson C, Fosbøl EL, et al., the combination of Metformin and insulin is characterized by low total case rate (TCR), low incidences of glucopenia and angiopathies, and low mortality rate [11].

However, few studies were carried out concerning the combination of Metformin and insulin lispro in the treatment of GDM. Therefore, this study was performed to investigate the efficacy of this combination in GDM and the effects on serum miR-16, in order to provide references for clinical treatment.

Materials and methods

General materials

In total, 117 GDM patients admitted to our hospital from March 2018 to March 2019 were included, and divided into the Study Group (SG) and the Control Group (CG) based on the therapeutic regimen. The CG (n=55) adopted insulin lispro, and the SG had (n=62) insulin lispro

combined with Metformin. The patients were aged between 21 and 35 years with the mean age of (27.34±3.14).

Inclusion and exclusion criteria

Inclusion criteria: patients diagnosed with GDM according to IADPSG [12], without diabetes before pregnancy, unable to control their blood sugar by simply diet control and enhanced exercise, highly compliant to the doctor's advice with little chance of withdrawal during the study, were included. Exclusion criteria: some patients were excluded as they were infected or suffering from other serious medical diseases and malignant tumors, or they had previous medical history of mental diseases or with multiple births or in

early pregnancy. The study has been approved by the Ethics Committee of the hospital, and we obtained informed consent from all patients and their family members.

Methods

Both groups were provided with healthy diets and guide about exercise. Patients in the CG were injected with insulin lispro (Lilly France, registration standard of imported drugs: JS20-110021) subcutaneously half an hour before their 3 meals in a fasting status at the initial dose of 0.5 U/ (kg·d) and subsequent doses adjusted based on the patient's real-time blood sugar level (BSL); while patients in the SG received the same treatment but also were orally administered with deltamine (Sino-American Shanghai Squibb Pharmaceuticals Ltd., GYZ Zi No.: H20023370) at a dose of 0.25 g after their supper each day. The treatment in both groups continued until delivery.

Observation indicators

Before and after treatment, the 2 groups were compared for treatment efficacy, delivery out-

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Table 2. Clinical efficacy [n (%)]

| | CG (n=55) | SG (n=62) | χ^2 | P |
|----------------------|------------|------------|----------|-------|
| Markedly effective | 28 (50.91) | 34 (54.84) | 0.181 | 0.671 |
| Effective | 16 (29.09) | 25 (40.32) | 1.615 | 0.204 |
| Ineffective | 11 (20.00) | 3 (4.84) | 6.360 | 0.012 |
| Total effective rate | 44 (80.00) | 59 (95.16) | 6.360 | 0.012 |

Table 3. Delivery outcome [n (%)]

| | CG (n=55) | SG (n=62) | χ^2 | P |
|-----------------|------------|------------|----------|-------|
| Premature birth | 9 (16.36) | 22 (35.48) | 5.471 | 0.019 |
| Hydramnion | 7 (12.73) | 1 (1.61) | 5.652 | 0.017 |
| C-section | 34 (61.82) | 21 (33.87) | 9.138 | 0.003 |

Table 4. Adverse outcome of newborns [n (%)]

| | CG (n=55) | SG (n=62) | χ^2 | P |
|--------------------------|------------|-----------|----------|-------|
| Premature birth | 9 (16.36) | 2 (3.23) | 5.906 | 0.015 |
| Glucopenia | 4 (7.27) | 1 (1.61) | 2.282 | 0.131 |
| Jaundice | 3 (5.45) | 1 (1.61) | 1.303 | 0.254 |
| Giant baby | 1 (1.82) | 1 (1.61) | 0.007 | 0.932 |
| Fetal distress in uterus | 1 (1.82) | 1 (1.61) | 0.007 | 0.932 |
| Total | 18 (32.73) | 8 (12.90) | 6.627 | 0.010 |

come and adverse outcome of newborns, FPG, HbA1c and 2hPPG, were tested for serum miR-16 by fluorescent quantitative PCR, and analyzed for the contribution of miR-16 in the prediction of GDM by ROC, along with the effective hazards in GDM treatment by logistic regression.

Test methods

Evaluation criteria of efficacy: Markedly effective: the blood sugar level is controlled in the normal range; effective: the blood sugar level is stabilized; ineffective: the blood sugar level fluctuates. Total effective rate = (markedly effective + effective)/total number of patients * 100%.

Testing of blood sugar level: Both groups were tested for FPG, HbA1c and 2hPPG after their 3 meals before and after treatments relying on a blood sugar monitor purchased from Sanuo Biosensor Co., Ltd. by the glucose oxidase method. All operations were done by professional technicians.

miR-16 extract of serum RNA and fluorescent quantitative PCR testing: Serum samples collected were mixed with TRIZOLLS and 200 μ L

chloroform. The evenly distributed mixture was then let stand for 15 min, and centrifuged at 4°C and 12,000 rpm for 10 min. The aqueous phase was removed from the superstratum to another centrifuge tube, mixed with isopropanol for centrifugation at 4°C and 12,000 rpm for 10 min. Next, the supernatant was abandoned, and the remaining mixture was mixed with ethanol and centrifuged at 7500 rpm for 5 min, after which, 100 μ L 0.1% DEPC ddH₂O was added to purify and collect the RNA according to the instructions on the RNA recovery kit. miR-16 was tested by reverse transcription fluorescent PCR with U6 as the internal reference, primer and probe from ABI, the United States. The test was carried out in strict accordance with the steps.

Statistical analysis

Statistical analysis was performed with SPSS 19.0 (Asia Analytics Formerly SPSS China). Numerical data were expressed as Mean \pm Standard

Deviation, and nominal data expressed as [n (%)]. Comparison studies were carried out through Students' t-test, and the value of miR-16 in the prediction of GDM was calculated with ROC.

Results

No differences in baseline data between the two groups

The two groups had no difference in terms of age, BMI, history of smoking and drinking alcohol, domicile and previous medical history ($P > 0.05$, **Table 1**).

Study group showed higher clinical efficacy

The total effective rate was 80.00% in the CG, which is significantly lower than 95.16% in the SG ($P < 0.05$, **Table 2**).

Study group showed lower incidences of complications

The SG reported lower incidences of premature birth, hydramnion and C-sect as compared with the CG ($P < 0.05$, **Table 3**).

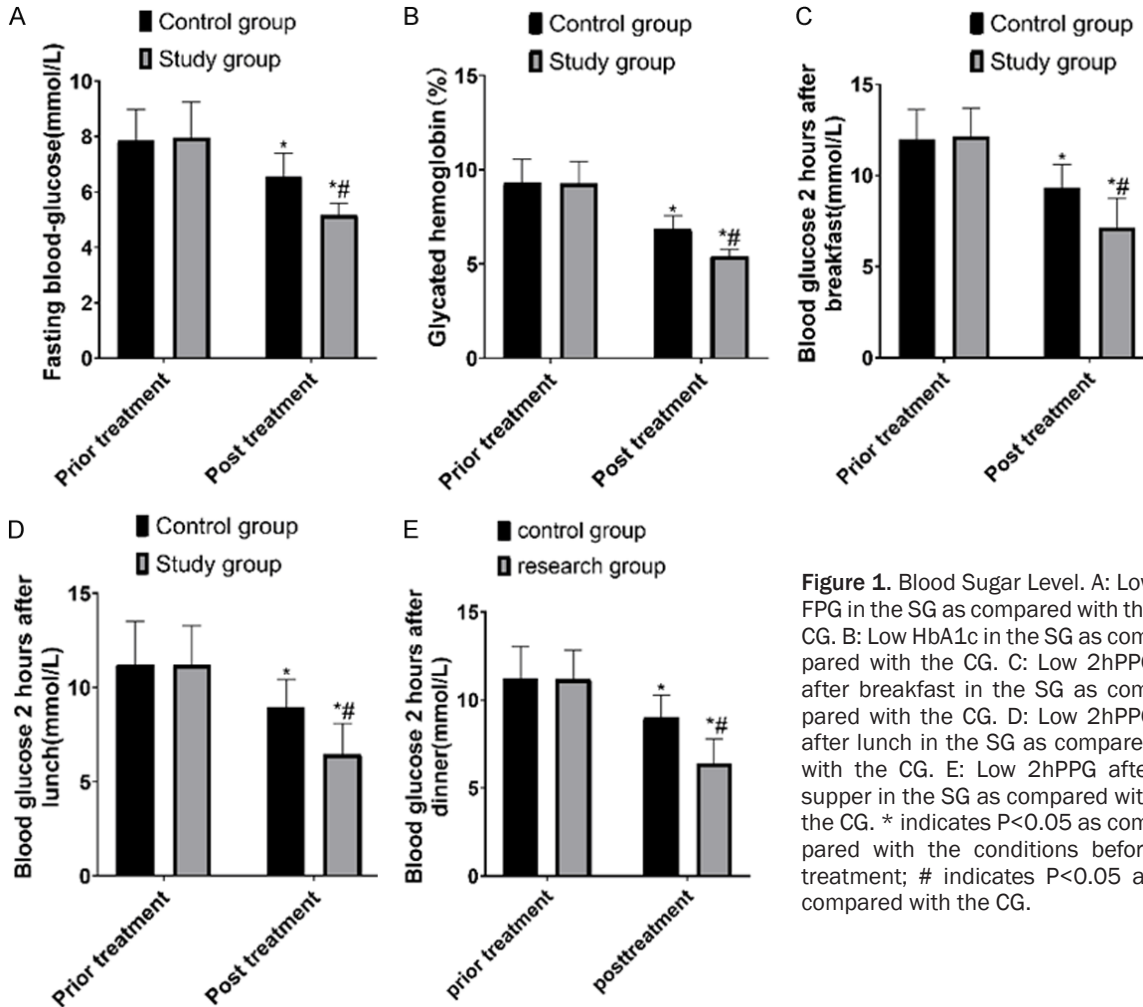


Figure 1. Blood Sugar Level. A: Low FPG in the SG as compared with the CG. B: Low HbA1c in the SG as compared with the CG. C: Low 2hPPG after breakfast in the SG as compared with the CG. D: Low 2hPPG after lunch in the SG as compared with the CG. E: Low 2hPPG after supper in the SG as compared with the CG. * indicates P<0.05 as compared with the conditions before treatment; # indicates P<0.05 as compared with the CG.

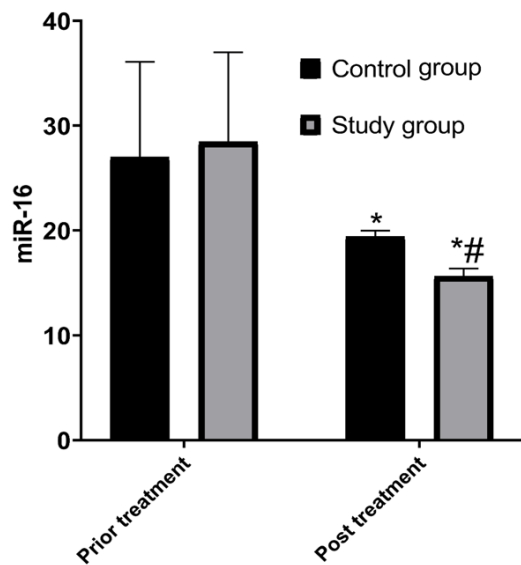


Figure 2. Serum miR-16. * indicates P<0.05 as compared with the conditions before treatment; # indicates P<0.05 as compared with the CG.

Study group showed lower adverse outcomes of newborns

The incidence of adverse outcomes of newborns was 32.73% in the CG and 12.90% in the SG (P<0.05, Table 4).

Blood sugar level

After treatment, SG reported a value lower FPG and HbA1c levels than the CG's (P<0.05); Reduction of 2hPPG level was observed in both groups (P<0.05), and a far lower value in the SG as compared with the CG (P<0.05, Figure 1).

Study group showed lower serum miR-16 levels

Before treatment, the serum miR-16 was (27.01±9.08) and (28.46±8.55) in the CG and the SG respectively; after treatment, it dropped

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Table 5. Value of serum miR-16 in the prediction of GDM

| | AUC | Critical Level | 95% CI | Sensitivity % | Specificity % |
|--------|-------|----------------|------------------|---------------|---------------|
| miR-16 | 0.711 | 35.01 | 0.6173 to 0.8038 | 66.13 | 67.27 |

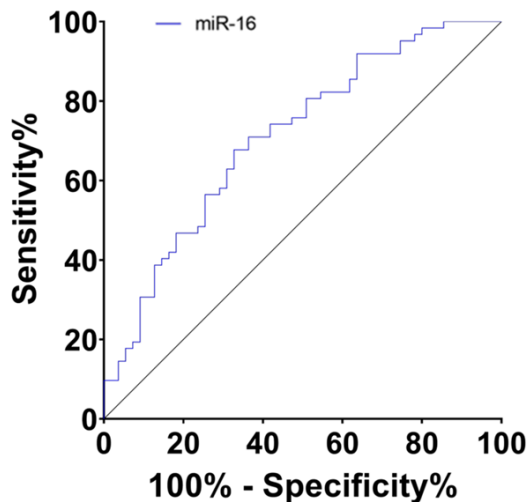


Figure 3. Value of Serum miR-16 in the Prediction of GDM. The AUC of miR-16 in the prediction of GDM was 0.711.

to (19.43±0.56) and (15.64±0.74) respectively (P<0.05, **Figure 2**).

Value of serum miR-16 in the prediction of GDM

The AUC, critical level, sensitivity and specificity of miR-16 in the prediction of GDM were 0.711, 35.01, 66.13, and 67.27 respectively (**Table 5** and **Figure 3**).

Monofactor analysis

According to the effective rate, patients were divided into the effective group (n=103) and ineffective group (n=14). Their clinical materials were collected for monofactor analysis. Results showed no difference in smoking, drinking alcohol, normal menses or not, fibroid and gravidity (P>0.05), but did show differences in age, BMI, Stein-Leventhal syndrome and parity (P<0.05, **Table 6**).

Multifactor analysis of effective rate

Indicators showing difference according to monofactor analysis were included and valued (referring to **Table 7**), and analyzed by logistic regression. Results indicated that age, BMI Stein-Leventhal syndrome and parity are the

effective and independent hazards in the GDM treatment (**Table 8**).

Discussion

GDM reflects the demands relative to the stage before gestation, the damaged maternal insulin secretion, and the temporary metabolic pressure from the placenta and the fetus [13]. GDM will impose major risks on the instant and long-term health of the mother and her baby, and accelerate the growth of the baby in the uterus, leading to oversized or overaged newborns and a higher risk of adverse perinatal outcomes. Therefore, effective clinical interventions must be adopted to maintain and control blood sugar, and control the growth of the fetus according to the normal parameters. However, knowledge of this aspect, and a therapeutic regime with good guiding significance are in shortage [14, 15]. According to related studies, the addition of insulin lispro in the regime of oral antihyperglycemia can effectively improve or recover the control over blood sugar [16].

In this study, the SG reported a far higher effective rate and more outstanding control of blood sugar based on the monitoring results of blood sugar. Studies by Levit et al. [17] proved that the mixed injection of insulin lispro and Metformin 3 times a day was effective and safe for patients with type 2 diabetes by clearly reducing glucopenia, which is consistent with the findings in this study and contributes to its higher confidence. Besides, patients were monitored for HbA1c during treatment, which was an important indicator of gestational diabetes whose changes may affect the outcome of gestation significantly according to the reports by Zhao [18]. A higher HbA1c level corresponds to a higher incidence of adverse gestational outcome. In this study, the HbA1c was lower, the delivery outcome and adverse outcome of newborns were better in the SG as compared with the CG, further demonstrating the more ideal efficacy and safety of metformin combined with insulin lispro. According to the reports by Kim et al. [19], GDM is an independent hazard for large gestational age infant (LGA), which may

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Table 6. Monofactor analysis

| | Effective (n=103) | Ineffective (n=14) | χ^2 | P |
|----------------------------------|----------------------|-----------------------|----------|--------|
| Age (y) | | | 14.350 | <0.001 |
| >30 | 34 (33.01) | 12 (85.71) | | |
| ≤30 | 69 (66.99) | 2 (14.29) | | |
| BMI (kg/m ²) | | | 6.725 | 0.010 |
| <25 | 60 (58.25) | 3 (21.43) | | |
| ≥25 | 43 (41.75) | 11 (78.57) | | |
| Smoking [n (%)] | | | 0.781 | 0.377 |
| Y | 32 (31.07) | 6 (42.86) | | |
| N | 71 (68.93) | 8 (57.14) | | |
| Drinking alcohol [n (%)] | | | 0.639 | 0.424 |
| Y | 40 (38.83) | 7 (50.00) | | |
| N | 63 (61.17) | 7 (50.00) | | |
| Domicile [n (%)] | | | 0.110 | 0.740 |
| Urban | 54 (52.43) | 8 (57.14) | | |
| Rural | 49 (47.57) | 6 (42.86) | | |
| Menses normal or not [n (%)] | | | 0.185 | 0.667 |
| Normal | 60 (58.25) | 9 (64.29) | | |
| Abnormal | 43 (41.75) | 5 (35.71) | | |
| Fibroid [n (%)] | | | 0.096 | 0.757 |
| Y | 26 (25.24) | 3 (21.43) | | |
| N | 77 (74.76) | 11 (78.57) | | |
| Stein-Leventhal syndrome [n (%)] | | | 5.704 | 0.017 |
| Y | 39 (37.86) | 10 (71.43) | | |
| N | 64 (62.14) | 4 (28.57) | | |
| Gravidity (time) | | | 0.070 | 0.792 |
| ≥2 | 48 (46.60) | 6 (42.86) | | |
| <2 | 55 (53.40) | 8 (57.14) | | |
| Parity (time) | | | 8.172 | 0.003 |
| ≥2 | 45 (43.69) | 12 (85.71) | | |
| <2 | 58 (56.31) | 2 (14.29) | | |

Table 7. Values

| Factor | Value |
|--------------------------|--------------|
| Age | >30=0, ≤30=1 |
| BMI | <25=0, ≥25=1 |
| Stein-Leventhal syndrome | Y=0, N=1 |
| Parity | ≥2=0, <2=1 |

lead to higher risks of long-process delivery, C-section, shoulder dystocia and delivery trauma, as well as anoxia and intrauterine death of the fetus. In this study, more stable control of blood sugar and better efficacy against DGM were observed in the SG, which were also supported by the lower incidence of adverse outcome of newborns in the same group.

microRNAs (MiRNAs) are a type of molecular modulator extensively recently found in human tissues and body liquids, and they play a vital role in the development of diabetes and its complications. miR-16 is one of them [20, 21]. During the study, serum miR-16 was tested in both groups before and after treatment. The results revealed a lower value in the SG as compared with the CG. According to related reports, diabetes may generate destructive impacts on blood vessels and lead to microvascular complications, such as retinopathy (which can lead to blindness), nephropathy (which can lead to end-stage nephropathy or renal failure), and painful neuropathy (which can lead to amputation). miR-16 level is associated with vascular complications of diabetes, in particular, proliferative retinopathy. In this study, the miR-16 level was reduced, proving that the combination of Metformin and insulin lispro can reduce the incidence of diabetes complications [22, 23]. The value of miR-16 in the prediction of GDM was also explored. Referring to the ROC analysis results, the serum miR-16's specificity to the prediction of GDM was 67.27% with certain predictive value. It is speculated that the value of miR-16 in the prediction of the incidence of diabetic complications is better, but it still needs to be further explored.

During the study, the hazards affecting GDM treatment effects were also analyzed by Logistic regression, and were found to be age, BMI, Stein-Leventhal syndrome and parity on an independent basis. According to the report by Lo DeSisto, et al. [24, 25], advanced age, pregestational obesity, Stein-Leventhal syndrome, and parity were independent hazards of

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Table 8. Logistic multifactor analysis

| | B | S.E. | Wals | Sig. | Exp (B) | EXP (B) 95% C.I. | |
|--------------------------|--------|-------|-------|-------|---------|------------------|-------------|
| | | | | | | Lower limit | Upper limit |
| Age | 0.968 | 0.684 | 2.003 | 0.017 | 5.634 | 0.689 | 10.07 |
| BMI | -0.451 | 0.624 | 0.565 | 0.035 | 0.837 | 0.197 | 2.063 |
| Stein-Leventhal syndrome | -0.037 | 0.587 | 0.004 | 0.006 | 1.463 | 0.305 | 3.046 |
| Parity | -0.342 | 0.586 | 0.341 | 0.040 | 0.971 | 0.225 | 2.242 |

GDM, which were consistent with the results obtained in this study, indicating that those relevant hazards shall be paid attention to during the treatment of GDM. Obesity shall be controlled, and delivery at an advanced age shall be avoided, in order to achieve more outstanding treatment effects of GDM.

However, the mechanism of miR-16 in GDM was not explored in this study, and some limitations caused our failure to follow up with patients for the statistics of incidences of type 2 diabetes. Therefore, in the subsequent studies, those would be the directions to provide better guides for use in clinic.

In conclusion, the combination of Metformin and insulin lispro in the treatment of gestational diabetes is more effective and safe with lower incidence of adverse outcome of newborns and reduced serum miR-16, deserving popularization.

Disclosure of conflict of interest

None.

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