Original Article
Efficacy of metformin in respective combination with insulin aspart and biosynthetic human insulin in the treatment of gestational diabetes mellitus and their effects on pregnancy outcomes

Rongzu Tu[1], Zhai'e Lu[2]

Departments of [1]Internal Medicine, [2]Obstetric, Ningbo Women and Children's Hospital, Ningbo, Zhejiang Province, China

Abstract: Objective: To explore the efficacy of metformin in respective combination with insulin aspart and biosynthetic human insulin in the treatment of gestational diabetes mellitus (GDM) and their effects on pregnancy outcomes. Methods: A total of 145 patients with GDM were selected as the subjects for a prospective, non-randomized, concurrent controlled study. Among them, patients received metformin combined with biosynthetic human insulin therapy was assigned into group A (78 patients), and the rest treated with metformin combined with insulin aspart were enrolled in group B (67 patients). The blood glucose index, superoxide dismutase (SOD) and malondialdehyde (MDA) levels, complications, neonatal adverse events were compared between the two groups, and the pregnancy outcomes were performed with factorial analysis. Results: After treatment, the FBG, 2 h PG and HbA1c in group B declined by 4.54±0.63 mmol/L, 6.44±0.79 mmol/L, 2.22±0.44%), respectively, which was significantly greater than those of group A (2.84±0.45 mmol/L, 3.65±0.53 mmol/L, 1.34±0.33%; P<0.05). In terms of SOD, its ascending range in group B (52.54±8.89 U/mL) was markedly greater than that of group A (31.24±6.24 U/mL) while the MDA in group B dropped by 18.34±3.78 mmol/L, which was remarkably greater than that in group A (8.68±2.34 mmol/L; P<0.05). The incidence of gestational hypertension and cesarean section in group B was significantly lower than that in group A (P<0.05), so was the case with the incidence of hypoglycemia and jaundice (P<0.05). What's more, it was found that pre-pregnancy BMI, dietary preference, exercise habits and family history of diabetes mellitus were independent factors affecting pregnancy outcomes in GDM patients. Conclusion: Metformin combined with insulin aspart can more effectively control blood glucose and improve pregnancy outcomes than metformin combined with biosynthetic human insulin.

Keywords: Metformin, insulin aspart, biosynthetic human insulin, gestational diabetes, pregnancy outcome

Introduction

Gestational diabetes mellitus (GDM), defined as the first occurrence of carbohydrate intolerance in pregnancy, affects up to 14% of the gestational population worldwide and goes in parallel with the increase of people with diabetes. It is estimated that there were 451 million people (aged 18-99) with diabetes worldwide in 2017, and the figure is expected to increase to 693 million by 2045 [1, 2]. Untimely treatment will lead to an increased risk of complications such as pre-eclampsia and preterm birth, as well as excessive fetal growth and problems during pregnancy and delivery like birth tears,

increased caesarean delivery and higher risk of perinatal neonatal disease [3]. Therefore, how to safely and effectively treat GDM is of great significance to maternal and fetal health.

When comes to the treatment of GDM, most patients can achieve normal blood glucose through nutritional treatment, but still up to 30% of patients need insulin and other hypoglycemic drugs [4]. At present, insulin is acknowledged to be the first-line clinical treatment for GDM [5]. However, there are many kinds of insulin, whose injection time, onset time and action time varies enormously from each other, exerting significant different hypoglycemic

effect. Therefore, the selection of the very insulin most suitable to treat GDM patients has always been one of the focuses of clinical research [6]. Among them, insulin aspart is an ultra-short-acting insulin analogue, which can quickly take effect within 10-15 min after subcutaneous injection. By binding to insulin receptors on muscle cells and fat, it can increase glucose intake, inhibit the release of glucose into the blood by the liver, and thereby reduce blood glucose in the body [7, 8]. Biosynthetic human insulin is a creation of bioengineering technology. After the purification of insulin, which is fermented by non-pathogenic microorganisms such as Escherichia coli and yeast, its amino acid sequence and biological activity are highly consistent with the insulin secreted by human body [8]. While metformin is a second-generation hypoglycemic agent, which can not only reduce human basal plasma insulin level and inhibit liver glucose release, but also lower insulin resistance, which could be served as an insulin sensitizer [9]. Studies in recent years have proved that metformin combined with insulin can effectively treat diabetes mellitus, and the former is recommended as an adjuvant for insulin in some clinical guidelines [10-12].

However, there are few reports on the comparison of metformin combined with insulin aspart and biosynthetic human insulin in the treatment of GDM. Therefore, this study aims to find a more safe and effective treatment scheme for GDM patients by comparing the effects of these two therapies on GDM and pregnancy outcomes.

Materials and methods

General information

From March 2015 to June 2018, 145 patients with GDM admitted to Ningbo Women and Children's Hospital were selected as the subjects for a prospective, non-randomized, concurrent and controlled study, in which 78 patients treated with metformin combined with biosynthetic human insulin were included into group A, and the remaining 67 patients receiving metformin combined with insulin aspart were assigned into group B. Inclusion criteria: Patients met the GDM diagnostic criteria, aged 22-45 years, with gestational age of 24-36 weeks, who could not effectively control blood glucose after lifestyle intervention (including

diet and exercise recommendations) and met the usual criteria for starting insulin therapy in the hospital were included [13]. In contrast, the exclusion criteria were as follows: Patients with drug contraindication in this study; patients with severe cardiac, hepatic or renal insufficiency; patients with incomplete clinical data; non-monogamous patients; abnormal fetuses; patients with communication barrier; multipara with family history or previous history of GDM; smokers or alcoholics. This study was approved by the medical ethics committee of our hospital, and all the study subjects and their families understood the process and purpose of the study and signed the informed consent.

Treatment

Subjects were treated with reference to the Standards of Medical Care in Diabetes published in 2013 [14]. Upon admission, all the patients were treated immediately with health education, diet adjustment and exercise instruction. Group A was given a daily subcutaneous injection of 0.8-1 U/(kg·d) biosynthetic human insulin (Novo Nordisk Company) before bedtime, followed by metformin (Sino-American Shanghai Squibb Pharmaceutical Co., Ltd.) at a dose of 0.5 g/time, 2 times a day. While group B received subcutaneous injection with insulin asparagus at an initial dose of 0.2-0.3 IU/(kg·d) before dinner every day (Novo Nordisk Company, China), and metformin was given at the same usage and dosage as group A. The changes of patients in the two groups were observed until the birth of the newborn.

Observation indicators

The main results of this study were the control of blood glucose and pregnancy outcomes, and the secondary results were the occurrence of complications and the changes of serum SOD and MDA levels.

COBAS INTEGRA 800 automatic biochemical analyzer (manufactured by Roche Switzerland) was employed to detect fasting blood glucose (FBG), 2 h postprandial blood glucose (2 h PG) and glycosylated hemoglobin (HbA1c) before and after treatment.

After continuous treatment for 1 month in both groups, 3 mL fasting venous blood was extracted in the morning [15]. Serum was collected

after centrifugation and stored in the refrigerator at -20°C for later use. Then the serum levels of SOD and MDA were measured in strict accordance with the corresponding kit instructions of SOD ELISA and MDA ELISA (Kamiya, USA, KT-50849, KT-53246).

Complications such as gestational hypertension, polyhydramnios (amniotic fluid volume >2000 mL) or oligohydramnios (amniotic fluid volume <300 mL), premature delivery (<37 weeks of pregnancy), cesarean section, postpartum hemorrhage (blood loss of >500 mL within 24 h after delivery) were recorded [16]. In addition, the occurrence of adverse pregnancy outcomes such as macrosomia (postnatal weight ≥4000 g), pathological jaundice, hypoglycemia (intravenous glucose injection needed), respiratory distress (respiratory distress syndrome or respiratory support needed) were recorded after delivery [17].

Statistical methods

Statistical analysis was performed using SPSS 21.0 (EASYBIO Company), and the data was plotted by GraphPad Prism 7. The counting data were expressed by case number/percentage (n (%)), and a chi-square test was adopted for inter-group comparison. While the measurement data were expressed as mean ± standard deviation ($\overline{x}$ ± sd). An independent t-test was employed for comparison of measurement data between the two groups, and a paired t-test was applied for intra-group comparison before and after treatment. With adverse pregnancy outcome as the dependent variable and pre-pregnancy body mass index (BMI), dietary preference, exercise habit and family history of diabetes as the independent variables, multivariate logistic regression was used to analyze the factors affecting adverse pregnancy outcomes. P<0.05 indicated a statistically significant difference.

Results

Comparison of general information between the two groups of patients and newborns

As to the patients, no significant differences were observed in general information represented by age, pregnancy and pre-pregnancy BMI, education level, dietary preference, place of residence, exercise habits, family history of

diabetes, or pregnancy and parity (P>0.05). While the newborns in both groups were successfully delivered without death, and there was no significant difference in gestational age and body weight between the two groups (P>0.05; **Table 1**).

Comparison of blood glucose levels before and after treatment of patients in the two groups

Before treatment, the levels of FBG, 2 h PG and HbA1c in group A were 8.56±1.82 mmol/L, 12.14±2.45 mmol/L, (7.85±1.31% respectively, and those in group B were 8.71±1.79 mmol/L, 11.89±2.65 mmol/L, 7.68±1.22%. It was obvious that there were no significant differences as regard to FBG, 2 h PG and HbA1c levels between the two groups (P>0.05) before treatment. While after it, the levels of FBG, 2 h PG and HbA1c in group A were 5.55±1.11 mmol/L, 8.55±0.89 mmol/L and 6.41±0.91%, respectively, which were significantly lower than those before treatment (P<0.05). And the corresponding level of FBG, 2 h PG and HbA1c in group B after treatment was 4.27±0.98 mmol/L, 5.67±0.78 mmol/L and 5.32±0.76%, which were also markedly lower than that before treatment (P<0.05). The above data revealed that after treatment, the decrease of FBG, 2 h PG and HbA1c in group B, which was 4.54± 0.63 mmol/L, 6.44±0.79 mmol/L and 2.22± 0.44% respectively, was significantly greater than that in group A (2.84±0.45 mmol/L, 3.65±0.53 mmol/L, 1.34±0.33%; P<0.05). See **Figure 1**.

Comparison of SOD and MDA levels before and after treatment of patients in the two groups

Before treatment, the levels of SOD and MDA in group A were 175.52±25.54 U/mL and 66.64±8.46 mmol/L respectively, and those in group B were 178.46±23.55 U/mL and 67.89±9.78 mmol/L respectively, which indicated that the SOD and MDA levels did not identify any significant difference between the two groups (P>0.05). While after treatment, the SOD and MDA levels in group A were 209.46±28.55 U/mL, 57.62±5.18 mmol/L, respectively, suggesting that the SOD level of group A significantly increased by 31.24±6.24 U/mL, while MDA level markedly reduced by 8.68±2.34 mmol/L (P<0.05). As to group B, the levels of SOD and MDA after treatment were 228.82±24.32 U/mL and 48.62±4.67 mmol/L,

Table 1. Comparison of general information ($\bar{x} \pm$ sd, n (%))

Group	Group A (n=78)	Group B (n=67)	χ^2/t	P
Age (year)	29.34±4.98	30.23±5.24	1.047	0.297
Pregnancy week (week)	28.69±3.45	29.65±3.78	1.598	0.112
BMI before pregnancy (kg/m^2)	25.24±2.67	25.76±2.89	1.125	0.262
Degree of education (n, %)			0.490	0.484
Below senior high school	43 (55.13)	45 (67.16)		
Senior high school or above	35 (44.87)	22 (32.84)		
Food preference (n, %)			0.170	0.680
Light diet	56 (71.79)	46 (68.66)		
Heavy diet	22 (28.21)	21 (31.34)		
Place of residence (n, %)			2.484	0.115
City	34 (43.59)	38 (56.72)		
Counryside	44 (56.41)	29 (43.28)		
Exercise (n, %)			0.189	0.664
Yes	23 (29.49)	22 (32.84)		
No	55 (70.51)	45 (67.16)		
Family history of diabetes mellitus (n, %)			1.233	0.267
Yes	7 (8.97)	10 (14.93)		
No	71 (91.03)	57 (85.07)		
Gravidity (n, %)			1.516	0.469
1 time	30 (38.46)	22 (32.84)		
2 times	26 (33.33)	29 (43.28)		
At least 3 times	22 (28.21)	16 (23.88)		
Parity (n, %)			0.627	0.429
Primipara	47 (60.26)	41 (53.73)		
Multipara	31 (39.74)	26 (46.27)		
Newborn's birth weight (kg)	3.61±0.53	3.48±0.61	1.373	0.172
Gestational age at delivery of newborns (week)	38.84±1.67	39.33±1.56	1.816	0.715

Note: BMI, body mass index.

respectively, suggesting that its SOD level significantly increased by 52.54±8.89 U/mL, while MDA level markedly declined by 18.34±3.78 mmol/L than those before treatment (P<0.05). The above data demonstrated that, after treatment, the increase of SOD in group B, which was 52.54±8.89 U/mL, was significantly greater than that in group A (31.24±6.24 U/mL), and the decrease in MDA in group B (18.34±3.78 mmol/L) was significantly greater than group A (8.68±2.34 mmol/L; P<0.05). See **Figure 2**.

Comparison of complications after treatment of patients in the two groups

There was no significant difference in the occurrence of polyhydramnios or oligohydramnios, premature delivery or postpartum hemorrhage between the two groups after treatment

(P>0.05), but the occurrence of gestational hypertension and cesarean section in group B was significantly lower than that in group A (P<0.05). See **Table 2**.

Comparison of adverse conditions between the two groups of newborns

No significant difference was observed in the occurrence of respiratory distress and macrosomia between the two groups (P>0.05), whereas, the occurrence of hypoglycemia and jaundice in group B was significantly lower than that in group A (P<0.05). See **Table 3**.

Univariate analysis of pregnancy outcomes in GDM patients

According to pregnancy outcomes, the patients were divided into a good outcome group (n=84)
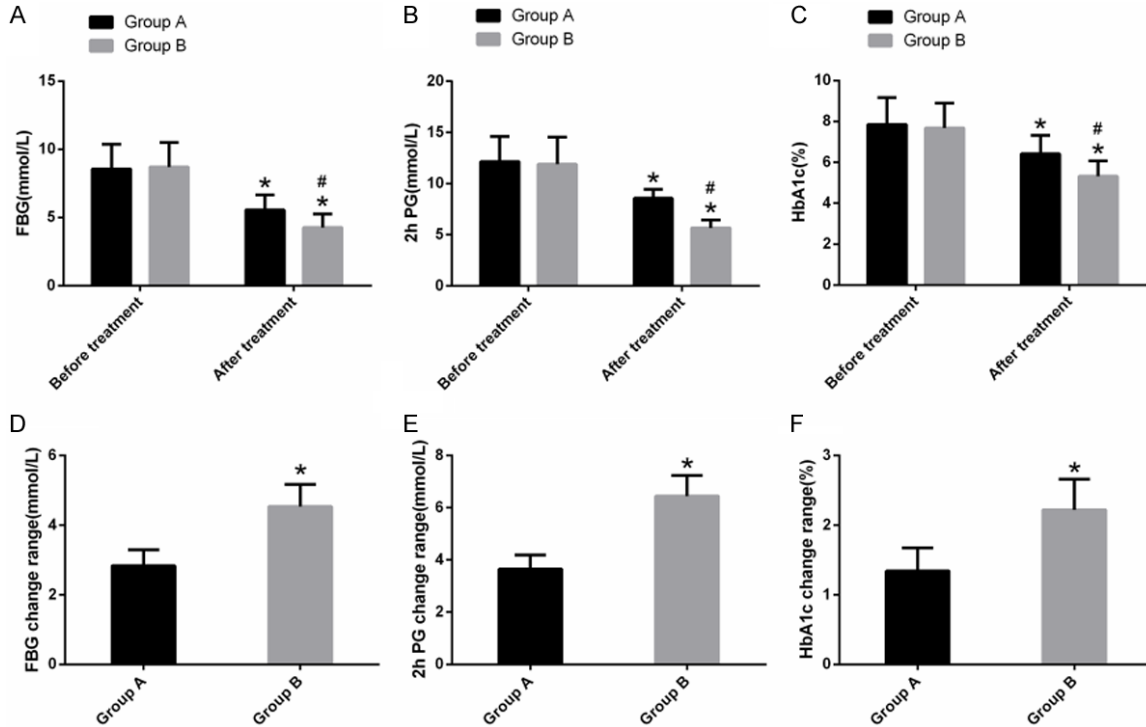
Figure 1. Comparison of blood glucose before and after treatment. A. Comparison of FBG; B. Comparison of 2 h PG; C. Comparison of HbA1c; D. Comparison of FBG change range; E. Comparison of 2h PG change range; F. Comparison of HbA1c change range. Compared to before treatment within group, *P<0.05; compared with group A, #P<0.05. 2 h PG, 2 h postprandial blood glucose; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin.
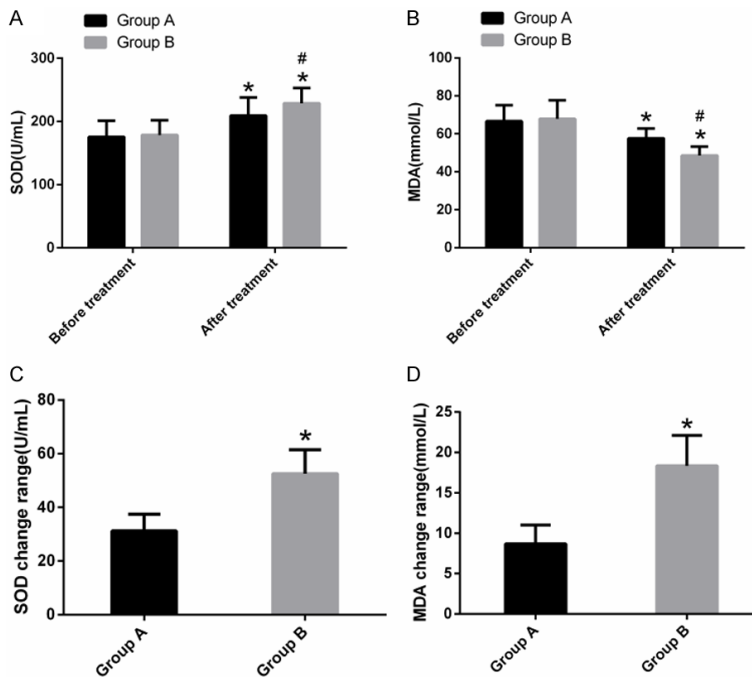

Figure 2. Comparison of SOD and MDA levels before and after treatment. A. SOD level; B. MDA level; C. SOD level change range; D. MDA level change range. Compared to before treatment within group, *P<0.05; compared with group A, #P<0.05. SOD, superoxide dismutase; MDA, malondialdehyde.

and a poor outcome group (n=61), and the general and clinicopathological factors of the two groups were analyzed by univariate analysis. The results indicated that age, irrespective of gestational age, place of residence, education level, drinking history, pregnancy times and parity (P>0.05), pre-pregnancy BMI, dietary preference, exercise habits and family history of diabetes may be the factors affecting the pregnancy outcome of GDM patients (P<0.05). See **Table 4**.

Multivariate analysis of pregnancy outcomes in GDM patients

With the adverse pregnancy outcome as the dependent variable, and pre-pregnancy BMI, dietary preference, exer-

Table 2. Comparison of complication after treatment (n, %)

Group	Group A (n=78)	Group B (n=67)	χ^2	P
Gestational hypertension	12 (15.38)	3 (4.48)	4.623	0.032
Polyhydramnios or oligoamnios	8 (10.26)	2 (2.99)	2.968	0107
Premature delivery	5 (6.41)	1 (1.49)	1.132	0.287
cesarean delivery	10 (12.82)	2 (2.99)	4.593	0.032
Postpartum hemorrhage	14 (17.95)	5 (7.46)	3.480	0.062

Table 3. Comparison of adverse reaction in newborns (n, %)

Group	Group A (n=78)	Group B (n=67)	χ^2	P
Hypoglycemia	26 (33.33)	11 (16.42)	5.426	0.020
Icterus	20 (25.64)	7 (10.45)	5.490	0.019
Respiratory distress	12 (6.41)	6 (1.49)	1.370	0.242
Fetal macrosomia	6 (7.69)	3 (4.48)	0.207	0.649

Note: BMI, body mass index.

cise habits and family history of diabetes as independent variables, multivariate logistic regression was employed to analyze the factors affecting adverse pregnancy outcome, which revealed that pre-pregnancy BMI, dietary preference, exercise habits and family history of diabetes were independent factors influencing pregnancy outcomes in GDM patients. See **Table 5**.

Discussion

Insulin aspart and biosynthetic human insulin are commonly used insulin in the treatment of gestational diabetes, both of which can effectively control patients' blood glucose, however, with some certain limitations. For instance, diabetic patients often develop insulin resistance during treatment, resulting in poor hypoglycemic effect. And oral anti-diabetic drugs provide a solution to this problem, as stated in some studies, they can reduce insulin antibody level and restore plasma glucose level [18]. Among them, metformin can reduce insulin resistance and lower blood glucose levels by inhibiting gluconeogenesis and hepatic glucose production, while improving peripheral tissue insulin sensitivity, and play a protective role on heart and pancreatic β cells [19]. Studies have demonstrated that in patients with type 2 diabetes treated with insulin intensification, the combination of insulin and metformin can better control blood glucose and reduce insulin demand and less weight gain compared with insulin monotherapy [20]. Still, other studies showed that metformin combined with insulin is more effective in reducing HbA1c (inter-group difference: -0.42%, P<0.001), weight gain (inter-group difference: -2.6 kg, P<0.001), and insulin use (1.04 IU/kg vs. 1.36 IU/kg; P< 0.001) in patients with type 2 diabetes than placebo plus insulin [10]. The results of present study showed that after treatment, the levels of FBG, 2 h PG, and HbA1c were dropped significantly in both groups, with those of group B being significantly lower than group A, indicating that metformin combined with insulin aspart can cooperate with each other to quickly and effectively control blood glucose in SDM patients from multiple aspects.

Studies have found that GDM can cause the imbalance of oxidation and antioxidant in the body, leading to oxidative damage in the disc vessels, and thus resulting in adverse pregnancy outcomes in GDM patients. Therefore, restoring the oxidation and antioxidant balance in GDM is the key to improving adverse pregnancy outcomes [21-23]. SOD is an indispensable enzyme in the process of scavenging free radicals, which can protect myocardial cells from damage, and its level reflects the changes of antioxidant capacity after myocardial injury to a certain extent [24]. While MDA is the product of lipid peroxidation in the body, which can reflex the free radical attack after myocardial injury [25]. The current study showed that, both groups presented increased SOD and decreased MDA levels after 5 days of treatment, and the SOD and MDA levels in group B were better than those in group A, indicating that metformin combined with insulin aspart could interact to correct oxidation and antioxidant balance in GDM patients. Subsequently, we observed that the occurrence of gestational hypertension and cesarean section in group B was significantly lower than that in group A, so was the case with the occurrence of neonatal hypoglycemia and jaundice, which may be due to the fact that metformin combined with insulin aspart could not only more effectively control the blood glucose, but also correct the balance of oxidation and antioxidant activity in

Table 4. Univariate analysis of pregnancy outcome in GDM patients (n)

Group	Good outcome group	Adverse outcome group	χ^2	P
Age (year)			0.873	0.350
>35	41	25		
≤35	43	36		
Pregnancy week (week)			2.535	0.060
>28	27	29		
≤28	57	32		
BMI before pregnancy (kg/m^2)			28.483	<0.001
>25	51	10		
≤25	33	51		
Degree of education (n, %)			0.114	0.736
Below senior high school	50	38		
Senior high school or above	34	23		
Food preference (n, %)			13.323	<0.001
Light diet	69	33		
Heavy diet	15	28		
Place of residence (n, %)			1.558	0.212
City	38	34		
Counryside	46	27		
Exercise (n, %)			8.317	0.004
Yes	34	11		
No	50	50		
Drinking history			2.474	0.116
Yes	31	15		
No	53	46		
Family history of diabetes mellitus (n, %)			16.842	<0.001
Yes	2	15		
No	82	46		
Gravidity (n, %)			2.180	0.336
1 time	34	18		
2 times	32	24		
At least 3 times	19	19		
Parity (n, %)			0.465	0.495
Primipara	49	39		
Multipara	35	22		

Note: GDM, gestational diabetes mellitus; BMI, body mass index.

investigated. Further logistic multivariate regression analysis revealed that pre-pregnancy BMI, dietary preference, exercise habits, and family history of diabetes may be the factors influencing the pregnancy outcomes of GDM patients. Though at present, the exact mechanism behind GDM has not been clarified, it has already confirmed that GDM patients can significantly reduce the risk of maternal and infant complications after treatment [26]. Therefore, strict GDM screening should be carried out for pregnant women with obesity and family history of diabetes in clinical practice in order to detect abnormal glucose metabolism in pregnancy earlier, so that intervention treatment can be given as soon as possible to improve pregnancy outcomes.

However, there are some shortcomings in this study. First, the small sample size leads to some limitations and one-sidedness of the results. Second, the optimal combination dose between the two drugs has not been explored. Last but not the least, we have not followed up the patients and newborns for a long time, so the long-term impact of the treatment remains a subject of investigation. Nevertheless, these deficiencies will be continuously improved in our follow-up research.

GDM patients. Another possibility was that insulin aspart enhanced the protective ability of metformin on heart and pancreatic β cells, thereby improving pregnancy outcomes. However, the specific mechanism remains to be confirmed by further basic experiments, so as to provide a theoretical basis for the adjuvant use of antioxidant therapy in the future.

At the end of the study, the factors affecting the pregnancy outcomes of GDM patients were

In conclusion, compared with metformin combined with biosynthetic human insulin, metformin combined with insulin aspart is more effective in controlling blood glucose. Besides, it can better reduce the risk of developing hyperten-

Table 5. Multivariate analysis of pregnancy outcome in GDM patients

Group	B	S.E.	Wals	P	OR	95% CI
Exercise	1.093	0.511	4.667	0.031	2.967	1.103-8.021
Food preference	1.066	0.455	5.345	0.022	2.848	1.154-6.945
BMI before pregnancy	1.989	0.602	9.927	0.002	6.688	2.045-21.81
Family history of diabetes mellitus	1.426	0.466	5.071	0.009	2.791	1.145-6.844

Note: GDM, gestational diabetes mellitus; BMI, body mass index; OR, odds ratio; CI confidence interval.

sive disorder complicating pregnancy, lower cesarean section rate, and improve pregnancy outcomes of GDM patients.

Disclosure of conflict of interest

None.

Address correspondence to: Zhai'e Lu, Department of Obstetric, Ningbo Women and Children's Hospital, No. 339 Liuting Street, Haishu District, Ningbo 315000, Zhejiang Province, China. Tel: +86-0574-87651722; Fax: +86-0574-87651722; E-mail: luzaie2lze5@163.com

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