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ORIGINAL ARTICLE

Role of First-Trimester Serum C1q/TNF-Related Protein 9 in Gestational Diabetes Mellitus

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SUMMARY

Background: **The current study aims to explore the relationship between gestational diabetes mellitus (GDM) and C1q/TNF-related protein 9 (CTRP9) level in early pregnancy.**
Methods: **Clinical data of 63 GDM patients and 70 normal pregnant women were included in the present study. Binary logistic regression analysis was used to explore the risk factors for GDM. To determine the value of CTPR9 for predicting GDM, the area under the receiver operating characteristic curve (AUC-ROC) was analyzed. Pearson's correlation assay was performed to explore the relationship between serum CTRP9 and body mass index (BMI) or oral glucose tolerance test (OGTT).**
Results: **Our data showed that the age, median maternal pre-pregnancy BMI, and fasting blood glucose during pregnancy of GDM group were significantly higher than those of the control group. ELISA showed the level of first-trimester serum CTRP9 was significantly decreased in GDM patients compared with that of healthy controls. Multiple logistic regression analysis showed that first-trimester serum CTRP9 and BMI were risk factors of GDM. The AUC-ROC showed that the diagnostic efficiency of CTRP9 + BMI was much higher than that of BMI alone. Moreover, first-trimester serum CTRP9 was found to be negatively correlated with BMI or OGTT in GDM patients.**
Conclusions: **Serum CTRP9 was an independent risk factor for the progression of GDM in pregnant women. Combined use of first-trimester serum CTRP9 and maternal pre-pregnancy BMI may be able to more accurately predict the occurrence of GDM.**
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KEY WORDS

CTRP9, gestational diabetes mellitus, first-trimester, risk factor

INTRODUCTION

Gestational diabetes mellitus (GDM), defined as glucose intolerance initially diagnosed during pregnancy, is becoming a great threat to maternal and neonatal health [1,2]. It is reported that the incidence of pre-eclampsia, macrosomia, and cesarean section are significantly increased for women with GDM [3]. Furthermore, the offspring are more likely to develop diabetes in youth [4, 5]. Therefore, early detection and prevention are of great importance for the management of GDM.

C1q/TNF- related protein 9 (CTRP9), a member of CTRP super family, is the closest paralog of adiponectin (APN) [6]. Increasing evidence has suggested that CTRP9 plays a key role in maintaining energy homeostasis via altering insulin sensitivity [6-8]. For instance, upregulation of CTRP9 dramatically decreased weight gain induced by a high-fat diet [6]. Lower serum CTRP9 is shown to be inversely correlated with age, fasting glucose, and homeostasis model assessment for insulin resistance [7]. In addition, CTRP9 knockout is shown to exaggerate lipotoxicity in high-fat, diet-induced, cardiac hypertrophy [8]. However, CTRP9 has not been fully investigated in women with GDM, especially those in the first-trimester.

In the present study, we explored the level of serum CTRP9 in women at the early stages of pregnancy and then explored the feasibility of first-trimester serum CTRP9 as a biomarker to monitor the subjects susceptible to GDM.

MATERIALS AND METHODS

Patient samples

Participants were recruited in the first trimester (10 weeks) from March 2017 to March 2018 at Qingdao Eighth People's Hospital. GDM was defined at 24 - 28 gestational weeks, using established criteria from the International Association of Diabetes and Pregnancy Study Groups (IADPSG) based on the results of a standard 2-hour, 75-g oral glucose tolerance test (OGTT) [9]. Exclusion criteria: 1. Less than 18 years old and more than 45 years old; 2. Combined with hypertension, cardiovascular, endocrine and kidney diseases; 3. Pre-pregnancy diabetes; 4. Abnormal chromosome number of fetus. Pregnant women were diagnosed with GDM when any glucose values exceeded the standard cutoff levels: fasting were dmmol/L, 1 hour sting wmmol/L, 2 hours ting wmmol/L [9]. Controls with normal blood glucose throughout the pregnancy were recruited from the same period at Qingdao Eighth People's Hospital. Written informed consent was obtained from all the eligible participants and the study protocol was approved by the Medical Ethical Committee of Qingdao Eighth People's Hospital. Details of all the participants were shown in Table 1.

Enzyme-linked immunosorbent assay (ELISA)

The serum levels of CTRP9 were determined using an Aviscera Biosciences ELISA kit (Aviscera Biosciences, Inc., Santa Clara, CA, USA; Cat. No.: SK00081-02) according to the instruction.

Statistical analysis

All results were expressed as mean ± standard error of mean (M ± S.E.M). Kolmogorov-Smirnov test was conducted to ensure the normal distribution of the data and our data met the null hypothesis of the normal distribution. Categorical variables were analyzed for between-group differences using the χ^2 test. Conditional logistic regression models were used to compute the odds ratio (OR) and 95% confidence interval (CI) between CTRP9 levels and risk of GDM. Comparisons between groups were made using a 2-tailed Student's *t*-test. For comparisons of multiple groups, analysis of variance (ANOVA) was used. Receiver operating characteristic (ROC) analysis was performed to explore the diagnostic value of first-trimester serum CTRP9. p-value < 0.05 was considered to be statistically significant.

RESULTS

Comparison of the general data between GDM group and controls

According to our data, maternal age, median maternal pre-pregnancy BMI and fasting blood glucose during pregnancy of the GDM group were significantly higher than those of the control group (Table 1). The median gestational age at delivery was significantly lower in the GDM group than that of controls. There was no significant difference in terms of maternal family history of diabetes, maternal family history of hypertension, parity, and gender of newborn between the two groups (Table 1).

Reduced first trimester serum CTRP9 in GDM patients

We then determined the level of serum CTRP9 in GDM patients and healthy controls in the first trimester (10 weeks). ELISA showed that the level of serum CTRP9 was 104.82 ± 27.85 pg/mL in GDM patients. In contrast, the level of serum CTRP9 was 213.56 ± 34.18 pg/mL in healthy controls (Figure 1). Our data showed that the first trimester serum CTRP9 level was significantly lower in GDM patients than that of controls.

First trimester serum CTRP9 was an independent risk factor of GDM

Based on the above data, we further analyzed the potential risk factors using multivariate logistic regression analysis. As shown in Table 2, maternal age, median maternal pre-pregnancy BMI, and first trimester serum CTRP9 were independent risk factors of GDM.

Combination of first trimester serum CTRP9 and BMI demonstrated better performance in the prediction of GDM

Subsequently, the diagnostic value of first trimester serum CTRP9 and BMI in predicting the risk of GDM was analyzed using the AUC-ROC. The AUCs of CTRP9, BMI, and CTRP9 + BMI in GDM group were 0.776 (95% CI = 0.699 - 0.853, p < 0.001), 0.671 (95% CI = 0.580 - 0.761, p < 0.001), and 0.821 (95% CI = 0.751 - 0.892, p < 0.001), respectively (Figure 2). Obviously, the AUC of CTRP9 + BMI was the highest, followed by CTRP9 and BMI.

Table 1. Comparison of the general data between GDM group and controls.

Variable	GDM group (n = 63)	Controls (n = 70)	p
Maternal age (years)	31.54 ± 3.67	29.28 ± 3.94	< 0.001
Median maternal pre-pregnancy BMI (kg/m^2)	21.06 ± 1.52	20.18 ± 1.32	< 0.001
Median gestational age at delivery (weeks)	38.62 ± 1.01	39.45 ± 1.21	< 0.001
Fasting blood glucose during pregnancy	6.99 ± 1.20	4.86 ± 0.81	< 0.001
Maternal family history of diabetes, n	5	3	> 0.05
Maternal family history of hypertension, n	8	6	> 0.05
Parity			> 0.05
Nulliparous, n	35	40	
Parous, n	28	30	
Gender of newborn			> 0.05
Male, n	31	35	
Female, n	32	35	

Table 2. Logistic regression analysis of independent risk factors among GDM patients.

Variable	OR	95% CI	p
Maternal age	1.781	1.037 - 3.059	0.041
Maternal pre-pregnancy BMI	2.537	1.342 - 4.797	0.025
Fasting blood glucose during pregnancy	1.752	0.896 - 3.069	0.056
Gestational age at delivery	0.972	0.720 - 1.312	0.064
Parity	1.251	1.078 - 1.452	0.053
CTRP9	0.701	0.507 - 0.972	0.043

Negative correlation between first trimester serum CTRP9 and OGTT

We then analyzed the correlation between first trimester serum CTRP9 and BMI or OGTT. Pearson's correlation assay showed a negative correlation between serum CTRP9 and BMI ($r = -0.216$, $p < 0.05$) and OGTT ($r = -0.315$, $p < 0.05$), respectively (Figure 3A and B). These data further indicated that reduction of CTRP was a risk factor for GDM.

DISCUSSION

GDM is a common complication of pregnancy and is very harmful to the health of mothers and children [10, 11]. For GDM diagnosis, glucose testing is usually carried out between 24 - 28 weeks of gestation [12-14]. However, insulin resistance appears at that time since the rapidly growing fetus needs to preserve nutrients [15]. Therefore, detection of first trimester biomarkers may shed light on the early management of GDM and improve the targeted intervention [15,16].

Recently, the role of CTRP9 in glucose metabolism has been widely reported [17,18]. For instance, loss of CTRP9 increases food intake and reduces insulin resistance in mice [17]. Decreased serum CTRP9 has been shown in type 2 diabetes patients [18]. However, no studies have investigated the relationship between first trimester serum CTRP9 and GDM development. The purpose of this study is to explore the relationship between GDM and CTRP9 level in early pregnancy to provide a clinical basis for early prediction of GDM occurrence and early improvement of perinatal outcome. Studies have shown that BMI before pregnancy, age of pregnant women, GDM history, macrosomia history, and multiple pregnancy history are closely related to the development of GDM [19,20]. In line with previous findings, the age, BMI before pregnancy, and fasting blood glucose during pregnancy in the GDM group were significantly higher than those in the control group.

The relationship of CTRP9 with metabolism has been

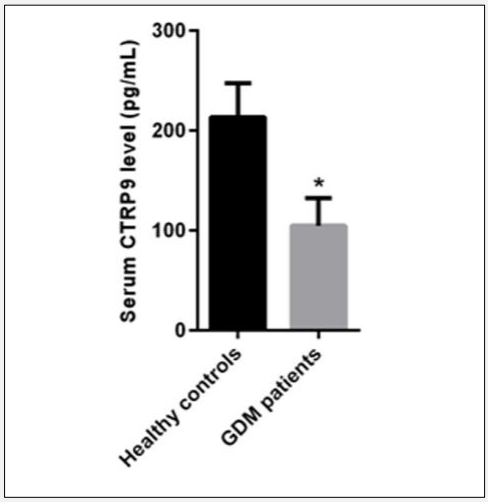

Figure 1. ELISA showed the level of first trimester serum CTRP9 level was significantly decreased in GDM patients compared with that of healthy controls.

*** - p < 0.01 vs. controls.**

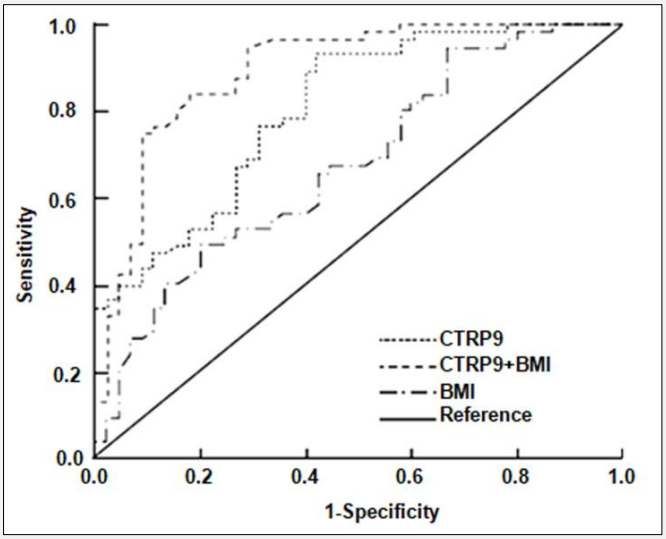

Figure 2. ROC analysis was carried out to analyze the diagnostic efficiency of CTRP9 and BMI in GDM patients.

well studied especially for GDM [19,21]. Several studies found that CTRP9 was reduced in the circulating system of type 2 diabetes patients [18,22]. In diet-induced obese mice, CTRP9 protects perivascular adipose tissue via AMPK-eNOS signaling [23]. Additionally, CTRP9 is found to be a novel metabolic regulator that

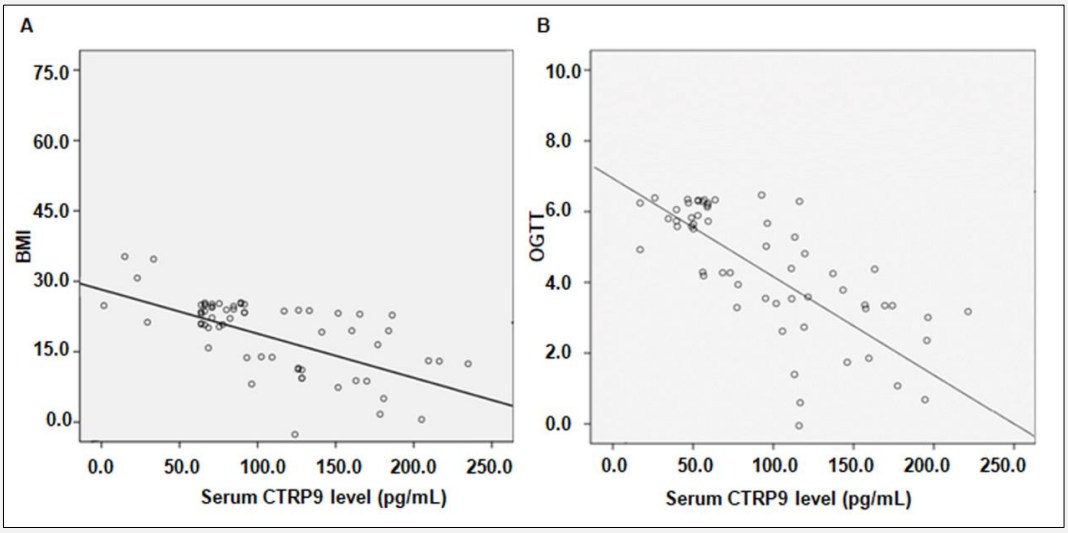

Figure 3. Pearson's correlation assay was performed to evaluate the correlation between serum CTRP9 and BMI or OGTT.

A negative correlation was found between first trimester serum CTRP9 and BMI (A) and OGTT (B).

links adipose tissue to lipid metabolism in skeletal muscle and liver [6]. These findings indicate that CTRP9 may be a novel component of the metabolic network that is involved in systemic energy balance. Here, we evaluated the level of first trimester serum CTRP9 in GDM women. For the first time, we showed novel data that compared GDM patients with normoglycemic patients. Serum CTRP9 level was significantly decreased in GDM patients, suggesting a possible role of CTRP9 in GDM screening in early pregnancy.

Additionally, multiple logistic regression analysis showed that CTRP9 was the risk factor of GDM. The AUC-ROC showed that the diagnostic efficiency of CTRP9 + BMI was much higher than that of BMI alone, suggesting that this combination improved the value for predicting GDM. We propose that adding first trimester serum CTRP9 to pre-pregnancy screening may be a predictor of GDM. However, the sample size of this study is relatively small, so it can only be preliminarily inferred that CTRP9 has value in predicting the occurrence of GDM. Multi-center and large sample clinical research are necessary in future studies.

CONCLUSION

The low level of first trimester serum CTRP9 is an independent risk factor for the occurrence of GDM. Furthermore, the combination of first trimester serum CTRP9 and maternal pre-pregnancy BMI may be able to accurately predict the occurrence of GDM.

Declaration of Interest:
We declare no conflicts of interest.

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