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RESEARCH ARTICLE

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The role of 25(OH)D3 and circRNAs in early diagnosis of gestational diabetes mellitus

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Abstract

Objective: To explore the relationship between 25(OH)D3 and circular RNAs (circRNAs) in the early diagnosis of gestational diabetes mellitus (GDM) and to screen for biological markers for early prediction of GDM.

Methods: A cohort study was conducted using samples and data collected from pregnant women registered at the Li Huili hospital in China between April 2018 and January 2020. Four circRNAs (hsa_circ_0003218, hsa_circ_0002968, hsa_circ_0007430, and hsa_circ_0006260) were selected as potential biomarkers, and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to measure their concentration in the serum and to analyze their correlation with 25(OH)D3. The Pearson correlation test was used to assess the correlation between the 25(OH)D3, circRNAs, and various clinical variables. The area under the receiver operating characteristic (ROC) curve was used to assess the diagnostic value of circRNAs and 25(OH)D3 in the early stage of pregnancy.

Results: Weight, body mass index (BMI), triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and 25(OH)D3 were found to be risk factors for GDM. The level of 25(OH)D3 correlated significantly with HDL-C with a correlation coefficient of 0.298 ($p < 0.05$). The expression of hsa_circ_0003218 was significantly downregulated in the GDM group ($p < 0.05$). Hsa_circ_0002968, hsa_circ_0007430, and hsa_circ_0006260 did not show any differential expression between the two groups ($p > 0.05$). Furthermore, hsa_circ_0003218 level correlated significantly with 25(OH)D3 and the correlation coefficient was 0.357 ($p < 0.05$). The AUC of hsa_circ_0003218 combined with 25(OH)D3 was 0.789 ([0.700–0.877], $p < 0.001$), with sensitivity and specificity of 63.04% and 80.65%, respectively.

Conclusions: Hsa_circ_0003218 and 25(OH)D3 may jointly participate in the metabolic process of GDM. Thus, the combination of 25(OH)D3 and hsa_circ_0003218 represents a potential biomarker for the prediction of GDM in the early stages of pregnancy.

KEYWORDS
25(OH)D3, circRNAs, gestational diabetes mellitus, hsa_circ_0003218

1 | INTRODUCTION

Gestational diabetes mellitus (GDM) is a metabolic disorder that is generally diagnosed in the second or third trimester of pregnancy.[1] The incidence of GDM varies between countries and regions. GDM affects 1%–30% of pregnancies, with the highest prevalence in Africa, Asia, and India.[2] GDM is associated with serious adverse maternal outcomes including preeclampsia, type II diabetes mellitus, and long-term risk of developing metabolic syndrome.[3] Furthermore, GDM patients have abnormal fetal outcomes such as macrosomia, which is related to shoulder dystocia, infant respiratory distress syndrome, and neonatal hypoglycemia.[4,5] When diagnosed in the second trimester of gestation, GDM may affect the growth of the fetus.[6] Therefore, there is an urgent need to identify predictive biomarkers for the early diagnosis of GDM.

Epidemiological studies have shown that severe obesity, advanced maternal age, and family history of type II diabetes are associated with a high risk of GDM.[7–9] Recent reports suggest that vitamin D deficiency is also a risk factor for GDM.[10] Vitamin D is important during the rapid fetal developmental stages, particularly during bone calcification at the end of pregnancy.[11] Vitamin D is an essential vitamin for the human body and is mainly involved in the regulation of calcium and phosphorus metabolism, blood glucose metabolism, and immune functions in the form of 25(OH)D3.[7] A previous study indicated that vitamin D deficiency was associated with a higher risk of GDM.[12] Pregnant women with persistent vitamin D deficiency at 10–14 and 15–26 weeks of gestation had a 4.46-fold elevated risk for GDM compared with pregnant women with adequate vitamin D levels.[13] Vitamin D supplementation is reported to improve the metabolic profile, biomarkers of oxidative stress, and inflammation in patients without GDM.[14,15] However, the specific pathogenesis of 25(OH)D3 deficiency in GDM remains unclear.

CircRNAs comprise a recently identified class of small endogenous RNAs that have shown immense potential in clinical diagnosis and treatment. CircRNAs are categorized as noncoding and coding circRNAs,[16,17] and have emerged as important regulators of gene expression in some disease-related pathologies including neurological disorders, tumors, cardiovascular disorders, diabetes, and osteoarthritis.[18] CircRNAs occur as covalently closed continuous loops with covalently joined 3′- and 5′-ends formed by back-splicing events.[19] CircRNAs are characterized by their high abundance, stable structure, and high expression in specific tissues.[20] Dysregulated expression of circRNAs has been previously reported in GDM. However, little is known about the biological mechanism of circRNAs in GDM. The objective of this study was to explore the relationship between circRNAs and 25(OH)D3 in the early diagnosis of GDM and to identify potential biological markers for the early prediction of GDM.

2 | METHODS AND MATERIALS

This cohort study was conducted at the Li Huili Hospital (Ningbo, China) from April 2018 to January 2020. Plasma samples and data were collected from pregnant women with or without GDM at 12–14 weeks of gestation. Patients with metabolic syndrome, diabetes mellitus, hypertension, cardiovascular disease, thyroid disease, hematologic disease, and polycystic ovary syndrome prior to pregnancy, as well as infection, multiple pregnancy, and assisted reproductive technology-mediated pregnancy were excluded from the study. Our study was approved by the Ethics Committee of Ningbo Medical Center LiHuili Hospital (KYSB2020YJ051-01).

In this study, we evaluated the basic information and laboratory indicators from 45 pregnant women with GDM (GDM group) and 65 healthy pregnant women (control group). GDM was diagnosed according to the recommendations of the International Association of Diabetes and Pregnancy Study Group (IADPSG). Oral glucose tolerance test (OGTT) was performed with 75 g of glucose at 24–28 weeks of gestation. Fasting, 1 h, and 2 h plasma glucose levels were assessed, and the 75 g OTGG cut-off values were set at 5.1, 10, and 8.5 mmol/L, respectively. A diagnosis of GDM was confirmed when one or more values equaled or exceeded these thresholds.[21]

Total RNA was isolated from serum using the TRIzol® LS reagent (Invitrogen, Karlsruhe, Germany). Quantitative real-time PCR analysis (qRT-PCR) was performed on the LightCycler 480 II (Roche, Basel, Switzerland) using a LightCycler® 480 SYBR Green I Master Kit to compare the expression of circRNAs between the two groups.[15] Four circRNAs (hsa_circ_0003218, hsa_circ_0002968, hsa_circ_0007430, and hsa_circ_0006260) were selected as potential biomarkers, and their serum concentrations were measured by qRT-PCR. The following primer sequences were used in the qRT-PCR.

Hsa_circ_0003218 forward: 5′-GTTTAAAGATCCGTATCAGCAAGAC-3′, reverse: 5′-GATACGGATCTTTAAACGCACATAG-3′;

Hsa_circ_0002968 forward: 5′-CAGGAGCTCAAGGAATAGTATGGT-3′, reverse: 5′-CACGCTTGCTTCTGCTCATG-3′;

Hsa_circ_0007430 forward: 5′-GCGTCTCAGCATTTCCTATAAAAGA-3′,

reverse: 5′-CACATGCTAGATTGAGAGAATTCTGG-3′;

Hsa_circ_0006260 forward: 5′-TCCTTTGGTAATTTTGGGGTCAC-3′,

reverse: 5′-TGCCCTTTTTGCTAGCTGGT-3′.

Statistical analyses of the data were performed using the SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 for Windows (GraphPad Software, La Jolla, CA, USA). All values are expressed as a number (percentage) or the mean ± standard deviation. Comparisons between groups were performed using the independent sample *t* test. Pearson correlation test was used to analyze the association between two variables. The differential expression of the target gene between the control and GDM groups is expressed by the following formula: ΔCt = Ct circRNA-Ct β-Actin. The area under the curve (AUC) of 25(OH)D3 and circRNAs was calculated to evaluate the diagnostic value of 25(OH)D3 and circRNAs in GDM. Statistical significance was set at $p < 0.05$.

3 | RESULTS

The weight and BMI of the GDM group were significantly higher than those of the control group ($p < 0.05$, Table 1). As shown in Table 1, the

TABLE 1 Basic information and laboratory indicators of the pregnant women[a]

Characteristic	Control group ($n = 65$)	GDM group ($n = 40$)	p Value
Age, year	29.51 ± 3.56	30.69 ± 3.79	0.099
Weight, kg	53.82 ± 6.05	59.11 ± 9.47	0.001*
Height, cm	162.43 ± 4.39	161.20 ± 4.85	0.689
BMI, kg/m^2	20.39 ± 2.09	22.77 ± 3.71	0.000*
Fasting blood glucose, mmol/L	5.18 ± 0.59	5.03 ± 0.43	0.149
WBC,*10^9/L	8.02 ± 1.62	7.65 ± 1.84	0.456
RBC,*10^9/L	4.23 ± 0.40	4.30 ± 0.61	0.290
HB, g/L	126.12 ± 8.64	126.22 ± 9.40	0.955
PLT,*10^9/L	191.49 ± 40.15	200.78 ± 37.92	0.153
25(OH)D3, ng/ml	16.61 ± 6.29	14.14 ± 5.75	0.038*
Triglycerides, mmol/L	1.81 ± 0.80	2.32 ± 0.75	0.000*
Total cholesterol, mmol/L	5.00 ± 1.18	6.09 ± 0.97	0.000*
HDL-C, mmol/L	2.68 ± 1.61	2.02 ± 0.43	0.003*
LDL-C, mmol/L	3.55 ± 1.29	3.74 ± 0.71	0.307

Abbreviations: BMI, body mass index = weight (kg)/height^2 (cm^2); HB, hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

[a]Values are given as mean ± SD, unless otherwise indicated.

*p Value from independent samples t test or chi-squared test. $p < 0.05$ was considered statistically significant.

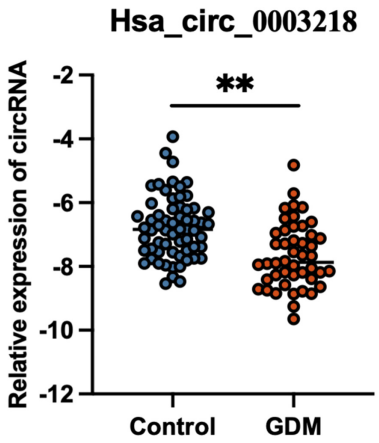

FIGURE 1 Expression of hsa_circ_0003218 between the control group ($n = 65$) and the GDM group ($n = 40$). The blue circles and red circles represent the healthy pregnant women and the GDM patients, respectively. $p < 0.05$ was considered statistically significant.

concentration of triglyceride and total cholesterol was significantly lower in the control group than that in the GDM group ($p < 0.05$). By contrast, the concentration of HDL-C and 25(OH)D3 was significantly higher in the control group than that in the GDM group ($p < 0.05$). There were no significant differences in age, height, or other laboratory indicators between the two groups ($p > 0.05$).

Figure 2A,B shows 25(OH)D3 level did not correlate with that of triglyceride and total cholesterol ($p > 0.05$). Figure 2C shows the level of 25(OH)D3 correlated significantly with HDL-C (correlation coefficient: 0.298; $p < 0.05$).

As shown in Figure 1, the expression of hsa_circ_0003218 was significantly lower in the GDM group than that in the control group ($p < 0.05$). However, there was no significant difference in the level of hsa_circ_0002968, hsa_circ_0007430 or hsa_circ_0006260 between the two groups ($p > 0.05$). Furthermore, as shown in Figure 2D, hsa_circ_0003218 level in the serum correlated significantly with that of 25(OH)D3 (correlation coefficient: 0.357; $p < 0.05$).

Next, we assessed the diagnostic value of 25(OH)D3 and hsa_circ_0003218 as biomarkers in the early diagnosis of GDM (Figure 3). Figure 3A shows the AUC of 25(OH)D3 was 0.736 ([0.641–0.831], $p < 0.001$, sensitivity of 63.04% and specificity of 74.19%), and Figure 3B shows the AUC of hsa_circ_0003218 was 0.743 ([0.647–0.839], $p < 0.001$, sensitivity of 54.35% and specificity of 87.10%). Figure 3C shows the AUC of hsa_circ_0003218 combined with 25(OH)D3 was 0.789 ([0.700–0.877], $p < 0.001$, sensitivity of 63.04% and specificity of 80.65%).

4 | DISCUSSION

We found that both weight and BMI were higher in the GDM group compared with that in the control group. The concentration of triglyceride and total cholesterol was higher in the GDM group than that in the healthy group, whereas the concentration of HDL-C and 25(OH)D3 was lower than in the GDM group than that in the control group. These data suggest that obesity, hyperlipidemia, and vitamin D deficiency may be high-risk factors for GDM. Furthermore, the correlation analysis of 25(OH)D3

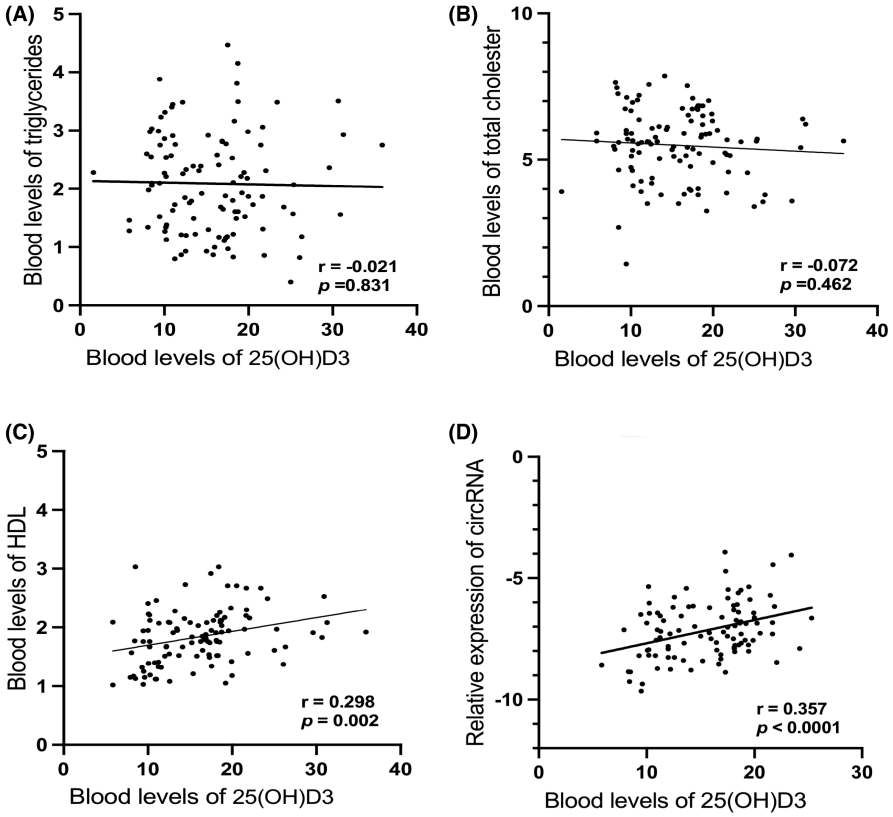

FIGURE 2 (A) Correlation analysis of the 25(OH)D3 level with triglycerides. (B) Correlation analysis of the 25(OH)D3 level with total cholesterol. (C) Correlation analysis of the 25(OH)D3 level with HDL-C. (D) Correlation analysis of the 25(OH)D3 level with hsa_circ_0003218. $p < 0.05$ was considered statistically significant.

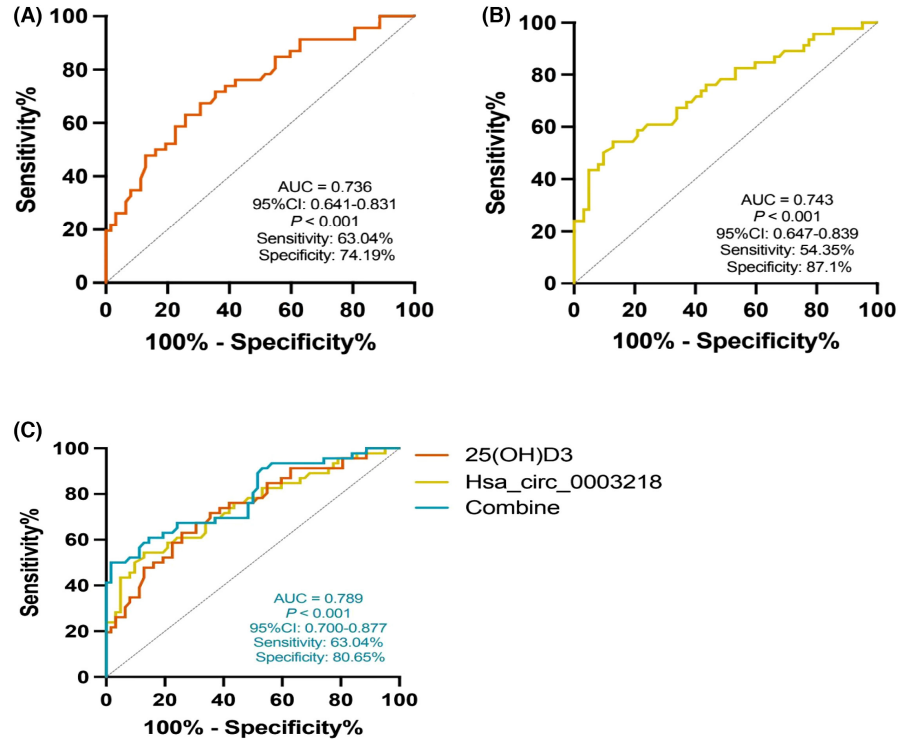

FIGURE 3 (A) Value of 25(OH)D3 as early diagnostic marker for GDM. (B) The value of hsa_circ_0003218 as early diagnostic marker for GDM. (C) The value of 25(OH)D3 and hsa_circ_0003218 as early diagnostic markers for GDM. AUC, area under the receiver operating characteristic curve; CI, confidence interval

level with serum lipid indexes showed that 25(OH)D3 correlated positively with HDL-C. This indicates that 25(OH)D3 may also be involved in the regulation of lipid metabolism during pregnancy. A light healthy diet, reasonable weight control, and appropriate vitamin D supplementation may reduce the incidence of GDM during pregnancy. According to a previous study, vitamin D deficiency appears to be associated with β-cell dysfunction and insulin resistance.[12] A 5 ng/ml increase in 25(OH)D3 concentration was associated with a 14% decrease in GDM risk.[22] Lower 25(OH)D3 concentration in overweight/obese pregnant women correlates

with a higher occurrence of GDM, which may be mediated by high molecular weight (HMW)-adiponectin.[23] Another study revealed that the development of hepatic steatosis in obesity and high levels of leptin and IL-6 impair 25(OH)D3 synthesis by affecting the vitamin D receptors (VDR)s.[24] In their randomized double-blinded clinical trial, Li et al. demonstrated that vitamin D supplementation significantly decreases the levels of total cholesterol and HDL-C, as well as the total cholesterol to HDL-C ratio.[25] However, the specific mechanism of vitamin D involvement in insulin resistance and lipid metabolism in GDM has not been clarified yet. Owing to the small sample size of our study, we could not examine whether the favorable effects of vitamin D supplementation are greater in women with baseline vitamin D deficiency than in those without the deficiency. Further studies need to be conducted with a larger sample size.

CircRNAs are involved in the regulation of glucose and lipid metabolism, such as triglyceride catabolism and lipid storage in GDM.[26,27] Therefore, we selected four circRNAs and compared their expression in the GDM and control groups to determine their potential to serve as stable and early predictive biomarkers of GDM. We found that hsa_circ_0003218 has potential diagnostic value in GDM. Furthermore, 25(OH)D3 was positively correlated with hsa_circ_0003218 ($p<0.05$). The AUC of hsa_circ_0003218 combined with 25(OH)D3 was 0.789 ([0.700–0.877], $p<0.001$) with a sensitivity of 63.04 and specificity of 80.65%. As circRNAs regulate gene expression at multiple levels, such as by sponging microRNAs or interacting with proteins,[28] we predict that the deregulation of circRNAs may lead to changes in the expression of target genes and possibly contribute to the development of GDM-related metabolic disorders. Further studies are needed to explore the potential association between the gene encoding the VDR and circRNAs in the metabolic process of GDM, so as to better understand the pathogenesis of GDM and identify novel strategies for early diagnosis and intervention.

Thus, this study demonstrates that 25(OH)D3 along with hsa_circ_0003218 may serve as biomarkers for early prediction of GDM owing to their joint involvement in the pathogenesis of GDM. This finding provides a potential strategy for the treatment of GDM by modulating the expression of 25(OH)D3 receptor and hsa_circ_0003218. However, further studies are needed to determine the specific biological mechanisms of circRNAs and the effects of vitamin D supplementation (in terms of dose and timing) on GDM risk.

AUTHOR CONTRIBUTIONS

Jinghui Zou involved in project management, manuscript writing, and data analysis; YanLiu, Jun Shen, and Aijiao Xue involved in data collection; Lulu Yan performed an experiment; Yisheng Zhang involved in project management and manuscript editing. All authors reviewed the article.

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data presented in this study can be found in online repositories.

The name of repositories and reference number can be found in the review.

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