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Pre-gestational diabetes mellitus, gestational diabetes mellitus, and its association with the MTHFR C677T polymorphism

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Abstract

Gestational diabetes mellitus (GDM) is a common condition during pregnancy and is associated with an increased risk of pre-eclampsia. The methylenetetrahydrofolate reductase (MTHFR) gene plays a crucial role in folate metabolism and has been implicated in GDM. To investigate the relationship between the MTHFR C677T gene polymorphism and the conditions of GDM and gestational prediabetes in pregnant women. A case-control study was conducted in 114 pregnant women with GDM and 96 pregnant women without GDM, from the first trimester to the prenatal examination at Can Tho Obstetrics Hospital. The pregnant women underwent a 1-hour (G1) and 2-hour (G2) oral glucose tolerance test (OGTT) and genetic polymorphism analysis based on real-time PCR technique. In pregnant women with GDM, weight, concentrations of G0, G1, G2, and folic acid were higher than those in the non-GDM group, with $P < .05$. When analyzing the subgroup without gestational diabetes, we found that the rate of prediabetes was 16.6% (16/96 pregnant women). In this group, blood glucose levels at 1 hour and 2 hours during the OGTT were higher compared to the normal glucose group ($P < .05$). A 2-hour post-OGTT glucose level of 7.78 mmol/L had a sensitivity of 93.8%, a specificity of 100%, and an area under the curve of 0.987 for diagnosing gestational prediabetes ($P < .001$). However, there were no statistically significant differences in the CC, CT, and TT polymorphisms of the MTHFR C677T gene among pregnant women with or without pre-gestational and GDM. Both fasting blood glucose and 2-hour glucose concentrations during the OGTT, as well as folic acid concentrations, were higher in both the pre-gestational and GDM groups compared to the non-gestational diabetes cohort. However, the analysis of MTHFR C677T polymorphisms revealed no statistically significant differences among the groups, highlighting the necessity for more extensive investigations to gain deeper insights into this relationship.

Abbreviations: GDM = gestational diabetes mellitus, IFG = impaired fasting glucose, IGT = impaired glucose tolerance, MTHFR = methylenetetrahydrofolate reductase, OGTT = oral glucose tolerance test.

Keywords: gestational diabetes mellitus, MTHFR C677T, pre-gestational diabetes mellitus

1. Introduction

Gestational diabetes mellitus (GDM) is a prevalent condition during pregnancy.[1] The prevalence of GDM in Vietnam is approximately 22.8% according to the standards of the World Health Organization.[2] GDM is glucose intolerance and insulin resistance with onset or first recognition during pregnancy.[3] This condition has numerous adverse impacts on both mothers and their offspring.[4] For mothers, GDM is associated with an increased risk of pre-eclampsia, cesarean delivery, difficult labor, and the development of type 2 diabetes mellitus in the postpartum period. Furthermore, offspring born to mothers with GDM are at an increased risk of developing obesity, impaired glucose tolerance (IGT), and type 2 diabetes mellitus during childhood or early adulthood.[4,5] Overweight, obesity, and IGT are significant risk factors for GDM, perpetuating a cycle of obesity and diabetes across generations.[1]

The inheritance of the specific genetic variant methylenetetrahydrofolate reductase (MTHFR) C677T (rs1801133) in the gene encoding the MTHFR enzyme is considered a potent determinant significantly altering the concentration of certain substances in pregnant women during gestation, including red blood cell folate concentration. The MTHFR gene plays a crucial role in folate metabolism, facilitating the conversion of 5', 10'-MTHFR, a methyl donor, to regenerate methylated homocysteine into methionine.[6–8] The prevalence of the MTHFR 677TT genotype varies across ethnic groups and regions, with a frequency of approximately 15% in Japanese populations.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Individuals with the TT genotype have significantly higher tHcy levels and lower serum folate levels than those with the CT and CC genotypes,[9] Some studies suggest that the TT genotype is a polymorphism that affects folate regulation.[10,11] In Vietnam, studies show that the frequency of the 677CC, 677CT, and 677TT genotypes in Vietnamese women with recurrent pregnancy loss is 65.4%, 30.8%, and 3.8%, respectively. However, there have been no studies indicating the relationship or potential risk of the MTHFR C677T gene polymorphism concerning GDM.[12] Meanwhile, pre-GDM is a concerning issue in many countries,[13] the estimated prevalence of IGT and impaired fasting glucose (IFG) in individuals aged 20 to 79 in Vietnam, after age-adjusted comparative prevalence, was 8.9% and 2.2%, respectively in 2021, and is projected to be 9.5% and 2.4% in 2045.[14] Therefore, we conducted this study to investigate the association of the MTHFR C677T polymorphism with pregestational and GDM in pregnant women during the first trimester in the Vietnamese population.

2. Methods

2.1. Study design and study population

Pregnant women in their first trimester who had their first prenatal visit after becoming pregnant and were found to have hyperglycemia at Can Tho Obstetrics Hospital from June 2023 to January 2024 were included in the study. Participants were divided into 2 groups: those with GDM and those without. We divided our subjects into 2 groups: the group of pregnant women with GDM and the group of pregnant women without GDM. We further analyzed the non-GDM subgroup, dividing it into 2 branches: pre-GDM versus non-pre-GDM, to investigate certain markers that may predict early pre-GDM versus GDM.

2.1.1. Including criteria.

2.1.1.1. Case group selection criteria. Pregnant women were diagnosed with GDM using the 75 g oral glucose tolerance test (OGTT) were included. The test was conducted in the morning after an 8-hour fasting period. Blood glucose levels were measured at fasting (prior to glucose ingestion), and at 1- and 2-hour intervals post-ingestion. The diagnosis of GDM was confirmed when two out of 3 blood samples meet the following criteria: Fasting blood glucose ≥ 5.1 mmol/L (92 mg/dL) and/or 1-hour blood glucose ≥ 10 mmol/L (180 mg/dL) and/or 2-hour blood glucose ≥ 8.5 mmol/L (153 mg/dL). According to the Carpenter/Coustan diagnostic criteria[15]:

1. Fasting blood glucose: ≥95 mg/dL (5.3 mmol/L)
2. At 1 hour: ≥180 mg/dL (10.0 mmol/L)
3. At 2 hours: ≥155 mg/dL (8.6 mmol/L)
4. At 3 hours: ≥140 mg/dL (7.8 mmol/L)

2.1.1.2. Control group selection criteria. Pregnant women in their first trimester, without GDM, are in good general health, and have equivalent characteristics to the case group.

2.1.2. Excluding criteria.

1 Pregnant women with impaired cognitive function or unstable mental status.
2 preexisting diagnoses of diabetes mellitus before pregnancy.
3 History of autoimmune diseases (such as systemic lupus erythematosus) or current use of corticosteroids, have hyperthyroidism or hypothyroidism.

2.2. Variables

MTHFR C677T polymorphism characteristics: there are 3 genotypes: CC, CT, and TT. Specifically, CC represents the genotype without the alanine mutation (wild-type homozygote), CT represents the genotype with 1 alanine mutation (heterozygous mutant), and TT represents the genotype with 2 alanine mutations (homozygous mutant). Three pre-gestational diabetes conditions were considered, as defined by WHO 2006 criteria[13]: IFG by G0 = 6.1 to 6.9 mmol/l (110–125 mg/dL), IGT defined by G2 = 7.8 to 11.1 mmol/l (140–200 mg/dL), or combined IFG and IGT. Normal glucose tolerance was defined by G0 < 6.1 mmol/l (110 mg/dL) and G2 < 7.8 mmol/l (140 mg/dL).

2.3. Data collection and measurements

All study participants were given 400 µg/d folic acid and multivitamins from the time of pregnancy after meeting the inclusion and exclusion criteria. They were instructed to fast for at least 8 hours before blood collection for laboratory testing. Two venous blood samples were drawn from each participant into separate tubes containing anticoagulant. The tubes were labeled with the corresponding participant's identification number, which matched the number in the patient list and medical records. DNA testing and genetic analysis were performed using blood collected in EDTA anticoagulant tubes. The blood samples from the second tube were transported to the Molecular Biology Laboratory of Can Tho University of Medicine and Pharmacy for MTHFR C677T polymorphism analysis. The blood samples were stored in a deep freezer (−80°C) until further analysis. The MTHFR C677T polymorphism analysis was conducted as follows: Venous blood samples from study participants were anticoagulated with EDTA. DNA was extracted from venous blood using the Quick-DNA™ Miniprep Kit protocol. DNA concentration and purity were measured by absorbance at A260/A280 using a spectrophotometer. DNA purity was assessed by measuring the A260/A280 ratio, with a value between 1.8 and 2.0 indicating pure DNA. The frequency of alleles and genotypes of MTHFR C677T was determined using the GeneProof MTHFR C677T Real-time PCR Kit, following the provided instructions.

2.4. Data analysis

The collected data was processed using medical statistical methods. SPSS 22 software was used for data entry and analysis. Category variables were described using frequencies and percentages. Continuous variables were assessed for normal distribution. For normally distributed variables, the mean and standard deviation were calculated. For non-normally distributed variables, median, maximum value, minimum value, and interquartile range were used. The Student *t* test was used to compare the mean values of normally distributed variables, while the Kruskall–Wallis test was used for non-normally distributed variables. Statistical significance was considered at $P < .05$.

2.5. Ethics approval

This study had been approved by the Ethics Committee in Biomedical Research of Can Tho University of Medicine and Pharmacy (23. 155.HV/PCT-HĐĐĐ, 03/20/2023).

3. Results

3.1. MTHFR C677T polymorphism and characteristics between GDM and pre-GDM groups

Table 1 demonstrates that gestational age, weight, glucose concentrations after 0-, 1, and 2 hours during the OGTT, and folic acid levels were significantly higher in the GDM group compared to the non-GDM group ($P < .05$). However, there was no significant difference in MTHFR C677T polymorphism between the group with and without GDM.

Table 2 demonstrates that glucose concentrations after 1 and 2 hours during the OGTT were significantly higher in the

pre-GDM group compared to the non-pre-GDM group ($P < .05$). However, there was no significant difference in MTHFR C677T polymorphism between the group with and without pre-GDM.

3.2. Predictive value of certain indicators for pre-GDM

Table 3 and Figure 1 demonstrate that G2 exhibits high sensitivity and specificity in predicting pre-GDM among women during the first trimester (with a sensitivity of 93.8%, specificity of 100%, a threshold of 7.78, and an area under the curve of 0.987).

4. Discussion

4.1. Principal findings

Our study revealed that in first-trimester pregnant women, the GDM group exhibited higher values in terms of weight, glucose concentrations at 0, 1, and 2 hours after the glucose tolerance test, and folic acid levels compared to the non-GDM group, with $P < .05$. Additionally, 1- and 2-hour post-OGTT glucose concentrations were significantly higher ($P < .05$) in the pre-GDM group compared to the normal glycemic control group. However, there were no statistically significant differences in the distribution of MTHFR C677T polymorphisms (CC, CT, TT) between the groups with or without pre-gestational and GDM (Fig. 1).

Table 1

Comparison of MTHFR C677T polymorphism characteristics between gestational diabetes mellitus group and the non-gestational diabetes mellitus group.

Characteristics	Gestational diabetes mellitus (n = 114)	Non-gestational diabetes mellitus (n = 96)	*P*
Maternal age (yr)	32.40 ± 5.53	31.69 ± 11.72	.562
Weight (kg)	56.02 ± 7.94	53.64 ± 6.68	.021
Height (m)	1.94 ± 0.35	1.56 ± 0.05	.353
BMI (kg/m^2)	22.31 ± 3.10	21.91 ± 2.80	.335
Family history	21 (58.3)	15 (41.7)	.592
MTHFR C677T polymorphism			
CC	74 (51.7)	69 (48.3)	.559
CT	37 (59.7)	25 (40.3)	
TT	3 (60)	2 (40)	
G0 (mmol/L)	4.96 ± 1.08	4.32 ± 0.57	<.001
G1 (mmol/L)	11.20 ± 1.99	7.85 ± 7.54	<.001
G2 (mmol/L)	9.50 ± 1.80	6.43 ± 1.41	<.001
Acid folic (ng/mL)	19.19 ± 8.70	14.76 ± 6.75	<.001

Table 2

Comparison of MTHFR C677T polymorphism characteristics between pre-gestational diabetes mellitus group and the non-pre-gestational diabetes mellitus group.

Characteristics	Pre-gestational diabetes mellitus (n = 16)	Non-pre-gestational diabetes mellitus (n = 80)	*P*
Maternal age (yr)	31 (24–35.5)	30 (27–34)	.716
Weight (kg)	54 (50–65)	52 (49–56)	.261
Height (m)	1.57 (1.56–1.60)	1.56 (1.52–1.60)	.205
BMI (kg/m^2)	21.64 (19.49–26.25)	21.48 (20.14–22.68)	.488
Family history	2 (13.3)	13 (86.7)	.706
MTHFR C677T polymorphism			
CC	11 (15.9)	58 (84.1)	.731
CT	5 (20.0)	20 (80.0)	
TT	0 (0)	2 (100)	
G0 (mmol/L)	4.29 (4.17–4.61)	4.20 (3.9–4.49)	.109
G1 (mmol/L)	7.7 (6.96–8.34)	6.9 (6.1–7.98)	.05
G2 (mmol/L)	8.2 (8.07–8.40)	6.2 (5.62–6.95)	<.001
Acid folic (ng/mL)	15.65 (13.60–19.53)	13.7 (9.34–17.58)	.191

4.2. Strengths and weaknesses of the study

Our study aimed to compare the MTHFR C677T polymorphism between the GDM group and the non-GDM group. The initial hypothesis was that there would be a significant difference between the 2 groups. Contrary to our initial hypothesis, our findings indicated no significant difference in the distribution of MTHFR C677T polymorphisms between these 2 groups. However, due to the limited sample size, larger case-control studies are warranted to further investigate the role of MTHFR C677T polymorphism in GDM patients, a high-risk group for subsequent diabetes development and adverse pregnancy outcomes.

4.3. Possible explanations and comparison with other studies

In the first trimester, pregnant women in the GDM group exhibited higher fasting blood glucose levels, as well as glucose levels at 1-hour and 2-hours during the OGTT, compared to the non-GDM group. This pattern was also observed when analyzing the pre-GDM group and the normal glycemic control group. These findings are consistent with previous studies.[7,16,17] Pregnancy is a state that predisposes women to develop glucose regulation disorders due to increased insulin resistance. GDM may occur when this physiological insulin resistance is exacerbated and accompanied by a relative insulin deficiency.[15] Pregnancy is considered a predisposing factor for GDM due to the reduced insulin sensitivity of tissues, leading to increased insulin requirements in women with preexisting diabetes. Changes in glucose metabolism and the rapid recovery of insulin action occur during the postpartum period. Insulin resistance is attributed to placental hormone secretion, such as lactogen, estrogen, and progesterone, which stimulate insulin secretion while antagonizing its action.[5,13,15] At the molecular level, insulin resistance is characterized by impaired insulin signaling, leading to insufficient transport across the cell membrane facilitated by glucose transporter 4 (GLUT4) - the primary transporter responsible for transporting glucose into cells for energy utilization. Compared to normal pregnancies, insulin-stimulated glucose uptake is diminished by 54% in GDM. Decreased tyrosine or increased phosphorylation of serine/threonine residues of insulin receptors also diminish insulin signaling.[18] We observed the CC genotype, which does not carry the mutation allele, to be the most prevalent polymorphic genotype, followed by the CT heterozygous mutation genotype, and lastly, the TT homozygous mutation genotype in both the GDM group and the non-GDM group. However, there was no statistically significant difference between the groups, consistent with previous studies.[19] Nevertheless, the folic acid concentration was higher in the GDM/pre-GDM group compared to the control group.[19,20] MTHFR is involved in the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a pathway that facilitates the removal of the methyl group

Table 3

The value of G1, G2 in predicting pre-gestational and gestational diabetes mellitus.

Characteristics	Sensitivity	Specificity	Threshold	AUC
G1 (mmol/L)	81.3	51.25	6.92	0.652
G2 (mmol/L)	93.8	100	7.78	0.987

AUC = area under the curve.

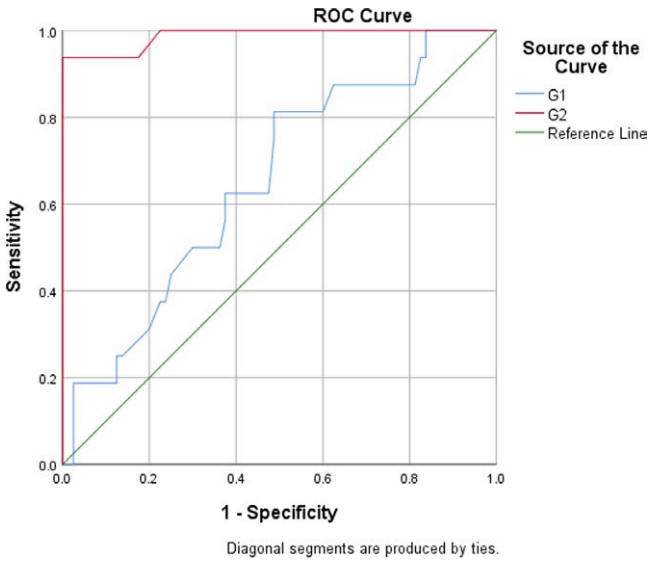

Figure 1. ROC curve of G1, G2 in predicting pre-gestational diabetes mellitus. ROC = receiver operating curve.

from Hcy to form Methionine. This enzyme also influences the methyl group of nucleic acids, hormones, neurotransmitters, as well as the synthesis of purines and pyrimidines.[6,19] Based on our study, the CC and CT genotypes have the highest prevalence, while the rare TT genotype, which has a lesser impact on folate regulation, suggests that changes in serum folic acid levels may not be significantly associated with the MTHFR genotype (see Tables 1 and 2).

5. Conclusions

In the first trimester, pregnant women with GDM exhibit higher weight, glucose levels at 0, 1, and 2 hours post-OGTT, and folic acid concentration compared to those without GDM, with $P < .05$. Additionally, the glucose concentration at 2 hours post-OGTT demonstrated high sensitivity and specificity for prediabetes diagnosis. However, the polymorphisms CC, CT, TT of the MTHFR C677T gene do not exhibit statistically significant differences between the group with and without prediabetes and GDM.

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