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Original Article

Association of Maternal Serum Homocysteine Level with Gestational Diabetes Mellitus (GDM)

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ABSTRACT

Background: Gestational diabetes mellitus (GDM) is a common metabolic disorder of pregnancy associated with adverse maternal and neonatal outcomes. Elevated maternal serum homocysteine has been associated with insulin resistance and GDM, although findings have been inconsistent. The relationship is of particular interest during mid-pregnancy, when physiological insulin resistance increases and GDM commonly becomes clinically apparent, with implications for pregnancy outcomes. **Aim of the study:** To determine the association between serum homocysteine level and gestational diabetes mellitus among pregnant women. **Methods & Materials:** This hospital-based case-control study was conducted at Bangladesh Medical University, Dhaka, from August 2024 to July 2025. A total of 64 pregnant women aged 18–35 years with a gestational age of 24–28 weeks were enrolled, including 32 women with GDM and 32 non-GDM controls. GDM was diagnosed using a 75-g oral glucose tolerance test according to WHO criteria. Serum homocysteine was measured using a chemiluminescent microparticle immunoassay. Levels of 4–15 µmol/L were considered normal, and >15 µmol/L were considered increased. Data were analyzed using SPSS version 26.0, with $p<0.05$ considered statistically significant. **Results:** Mean serum homocysteine was significantly higher in women with GDM than in controls (10.3±6.2 vs. 7.2±3.3 µmol/L; p=0.016). Increased homocysteine (>15 µmol/L) was found in 56.3% of GDM participants compared with 9.4% of controls. Elevated homocysteine was significantly associated with GDM (aOR 12.5; 95% CI: 3.1–49.3; p=0.001). **Conclusion:** Women with GDM had significantly higher serum homocysteine levels and a greater frequency of increased homocysteine than non-GDM women. Serum homocysteine could have potential as an adjunctive marker of metabolic risk in mid-pregnancy, but larger longitudinal studies are needed.

Keywords: *Gestational diabetes mellitus; Homocysteine; Pregnancy; Insulin resistance; Metabolic risk*

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as diabetes diagnosed in the second and third trimester of pregnancy that was not clearly overt diabetes prior to gestation [1]. Worldwide incidence of GDM is gradually increasing every year varying from 3% to 14% [2]. GDM is the most frequent metabolic disorder occurring during pregnancy and its prevalence may range from 1-16% of all pregnancies [3]. Southeast Asia had the highest prevalence of GDM at 24.2%. Alarmingly, the prevalence of GDM in Bangladesh has been progressively increasing from 7.5% in 2012 to 18.1% in 2018 [4]. Homocysteine is a sulphur amino acid and a normal intermediate in methionine metabolism. Metabolism of homocysteine occurs by two main pathways: remethylation to methionine or transulfuration to cystathionine and then to cystine. Any problem in either pathway may cause accumulation of homocysteine within the body. When excess homocysteine is produced in the body and not readily converted into methionine or cysteine, it is excreted out of the tightly regulated cell environment into the blood. It is the role of the liver and kidney to remove excess homocysteine from the blood [5]. During early pregnancy, glucose tolerance is normal or slightly improved with normal peripheral sensitivity to insulin and hepatic basal glucose production. As gestational age progresses maternal insulin sensitivity declines creating an insulin resistant state to meet the energy demands of both the mother and the rapidly growing fetus [6]. It may become worse during the rest of the pregnancy and be worst in the late third trimester. Therefore, GDM commonly develops in the late second trimester and disappears post-delivery [7]. GDM during pregnancy is associated with preeclampsia, fetal macrosomia, needs emergency caesarean section, birth trauma and neonatal hypoglycemia. During normal pregnancy mother's body undergo physiological changes in order to support the demand of growing fetus. One of the important metabolic adaptations in order to maintain glucose homeostasis in pregnant women and compensate through hypertrophy and hyperplasia of pancreatic beta cell, as well as increased glucose stimulated insulin secretion [8]. The risk factors for developing gestational diabetes mellitus are pre-pregnancy BMI >30 Kg/m2, previous history of gestational diabetes mellitus, macrosomic baby, family history of diabetes [9]. It exerts detrimental effects on a number of cell lineages including endothelial cells through production of reactive oxygen species. Both acute and chronic exposure to homocysteine shows detrimental effects on B-cell metabolism and insulin secretion [10]. Elevated homocysteine level has been reported as a major risk factor for vascular complications of diabetes, as well as being associated with clinical conditions of insulin resistance [11]. Higher maternal serum homocysteine levels were a risk factor for developing GDM and a positive correlation with insulin resistance [12], while few others could not establish significant correlation [13]. The potential role of elevated homocysteine in insulin resistance, endothelial dysfunction, and oxidative stress, investigating its association with GDM may provide an opportunity for earlier identification of women at increased metabolic risk and

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guide timely preventive interventions. The study aimed to determine the association of high level of serum homocysteine level in women with gestational diabetes mellitus.

METHODS & MATERIALS

The study was a hospital-based case-control study conducted in the Outpatient Department of Fetomaternal Medicine and the Department of General Obstetrics and Gynecology, Bangladesh Medical University (BMU), Dhaka, from August 2024 to July 2025. A total of 64 participants were included, comprising 32 GDM cases and 32 controls.

Inclusion Criteria

- Singleton/ Healthy pregnant women with gestational age within 24 to 28 weeks.
- Pregnant women diagnosed as GDM.
- Age within 18 to 35 years.
- Pregnant women who had given consent to participate in the study.

Exclusion Criteria

- Known case of diabetes mellitus.
- Patient on drug/ medication likely to alter glucose metabolism
- Any other medical disorder such as thyroid disease, kidney disease, liver disease, inborn error of homocysteine metabolism, psoriasis and nutrient deficiency
- Multiple pregnancies.
- Pregnant women with smoking or tobacco chewing habit.

Ethical Considerations

Ethical approval was obtained from the Institutional Review Board of BMU. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants after explaining the study purpose and procedures. Participant confidentiality and anonymity were maintained throughout data collection, laboratory processing, and analysis.

Data Collection

Participants were selected by purposive (non-random) sampling according to predefined eligibility criteria. The sample size was calculated using a two-sample mean comparison based on previously reported homocysteine levels, with a 5% significance level and 80% statistical power, yielding 29 participants per group. 10% non-response or missing-data rate was allowed. Sociodemographic and obstetric information, including age, education, monthly family income, gestational age, gravida, and body mass index (BMI), was collected through face-to-face interviews using a prestructured questionnaire, supplemented by clinical examination and relevant investigations. GDM was diagnosed using a 75-g oral glucose tolerance test according to WHO criteria.

Blood Collection and Laboratory Assessment

Following aseptic precautions, 3 mL of venous blood was collected from each participant for serum homocysteine estimation. Samples were allowed to clot for 30 minutes at room temperature and centrifuged at 1000×g for 20 minutes. Serum was analyzed immediately or stored at ≤−20°C until analysis. Total serum homocysteine was measured using a chemiluminescent microparticle immunoassay (CMIA). A serum homocysteine concentration of 4–15 µmol/L was considered normal, whereas >15 µmol/L was considered elevated.

Statistical Analysis

Data were analyzed using SPSS version 26.0. Continuous variables were expressed as mean±SD and compared using the independent-samples t-test. Categorical variables were presented as frequencies and percentages and compared using the χ^2 or Fisher's exact test. Correlation analysis was performed where appropriate. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Among women with GDM, 46.9% were aged 18–25 years, 34.4% were aged 26–34 years, and 18.8% were aged 35–40 years, compared with 62.5%, 37.5%, and 0.0%, respectively, among controls. The mean age was 27±6.2 years in the GDM group and 24.8±3.9 years in controls (p=0.104). Educational qualification, religion, occupation, and monthly income showed no significant between-group differences (p=0.342, 0.497, 0.281, and 0.486, respectively). Primary, SSC, HSC, and other educational levels accounted for 12.5%, 40.6%, 40.6%, and 6.3% among cases, compared with 6.3%, 25.0%, 62.5%, and 6.3% among controls. Most participants were Muslim (96.9% and 90.6%), while Hindu participants accounted for 3.1% and 6.3% and Christian participants for 0.0% and 3.1%. Housewives constituted 71.9% of cases and 75.0% of controls, with no significant difference in occupational distribution. Monthly income of 10,000–25,000 was reported in 53.1% of cases and 59.4% of controls, while income >25,000 was reported in 46.9% and 37.5%, respectively. The mean BMI was 25.6±3.1 kg/m² among cases and 24.8±2.4 kg/m² among controls (p=0.267) (*Table I*).

Table I
Distribution of the participants according to sociodemographic characteristics (*n*=64).

Variables	Case (GDM) (n=32)		Control (Non-GDM) (n=32)		p-value
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
Age					
18-25	15	46.9	20	62.5	0.034a
26-34	11	34.4	12	37.5	
35-40	6	18.8	0	0.0	
MeanSD	276.2		24.83.9		0.104c
Educational qualification					
Primary	4	12.5	2	6.3	0.342a
SSC	13	40.6	8	25.0	
HSC	13	40.6	20	62.5	
Others	2	6.3	2	6.3	
Religion					
Islam	31	96.9	29	90.6	0.497a
Hindu	1	3.1	2	6.3	
Christian	0	0.0	1	3.1	
Occupation					
Housewife	23	71.9	24	75.0	0.281a
Student	2	6.3	3	9.4	
Service holder	7	21.9	3	9.4	
Others	0	0.0	2	6.3	
Monthly income					
<10000	0	0.0	1	3.1	0.486a
10000-25000	17	53.1	19	59.4	
>25000	15	46.9	12	37.5	
BMI					
MeanSD	25.63.1		24.82.4		0.267c

a= chi square test; c= independent sample t-test.

Table II shows that mean gestational age was comparable between groups (25.5±1.39 vs 25.3±1.4 weeks; p=0.140). Primigravida women constituted 43.8% of the GDM group and 59.4% of controls, whereas multigravida women comprised 56.3% and 40.6%, respectively (p=0.211). Previous pregnancy complications were reported in 6.3% of cases and 9.4% of controls (p=1.0). Previous adverse perinatal outcomes were present in 21.9% of cases and 18.8% of controls (p=0.756). A first-degree family history of diabetes was reported in 21.9% of cases and 28.1% of controls (p=0.564).

Table II
Distribution of the participants according to pregnancy related variables (*n*=64).

Variables	Case (GDM) (n=32)		Control (Non-GDM) (n=32)		p-value
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)	
Gestational age (in weeks)					
Mean	25.51.39		25.31.4		0.140c
Gravida					
Primigravida	14	43.8	19	59.4	0.211a
Multigravida	18	56.3	13	40.6	
Complications of previous pregnancy (preeclamsia and previous H/O GDM)					
Yes	2	6.3	3	9.4	1.0b
No	30	93.8	29	90.6	
Perinatal Outcome (Previous macrosomia, stillbirth, IUD or congenital anomaly)					
Yes	7	21.9	6	18.8	0.756a
No	25	78.1	26	81.3	
F/H od DM in 1st degree relative					
Yes	7	21.9	9	28.1	0.564a
No	25	78.1	23	71.9	

a= chi square test; b= fisher's exact test; c= independent sample t-test.

Serum homocysteine levels were significantly higher among women with GDM, with a mean of 10.3±6.2 µmol/L compared with 7.2±3.3 µmol/L in controls (p=0.016). The corresponding median (IQR) values were 10 (4.26–16.6) and 6.25 (4.8–7.8) µmol/L, respectively (*Table III*)

Table III
Mean of homocysteine level between case and control group (*n*=64).

Homocysteine	Groups		p-value
	Case (GDM) (n=32)	Control (Non-GDM) (n=32)	
MeanSD	10.36.2	7.23.3	0.016c
Median (IQR)	10 (4.26-16.6)	6.25 (4.8-7.8)	

Group 1 showed a higher median homocysteine level (≈10) with greater variability than Group 2 (≈6.2), which had a narrower distribution and several high outliers (Figure 1). The box plot showed higher serum homocysteine levels in women with GDM than in controls. The median level was 10.0 µmol/L in the GDM group compared with 6.25 µmol/L in controls, with greater variability in the GDM group (IQR: 4.26–16.6 vs. 4.8–7.8 µmol/L) (*Figure 1*).

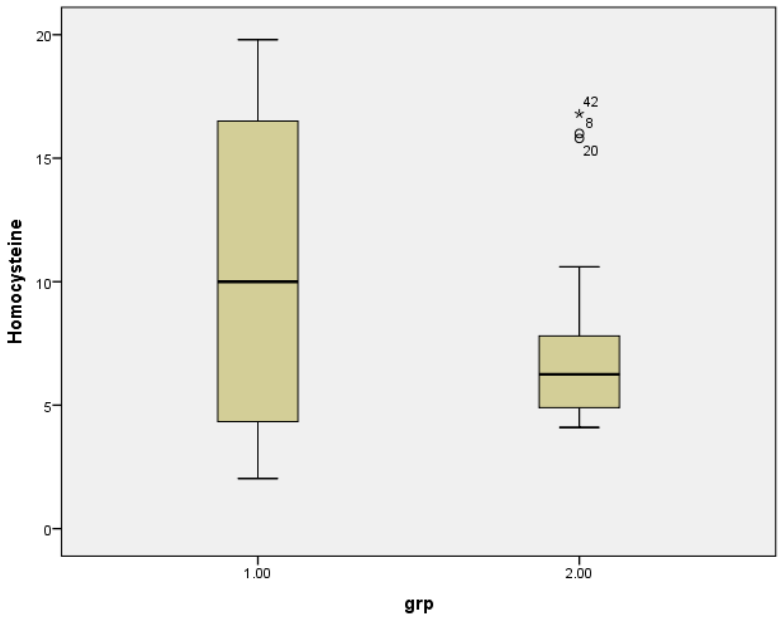


Figure 1 Box plot showing distribution of homocysteine level between case and control group.

Table IV presents that increased homocysteine (>15 µmol/L) was observed in 56.3% of GDM participants compared with 9.4% of controls, whereas normal levels (4–15 µmol/L) were observed in 43.8% and 90.6%, respectively. Elevated homocysteine was significantly associated with GDM (aOR 12.5, 95% CI 3.1–49.3; p=0.001).

Table IV
Association between levels of homocysteine in case and control group (*n*=64).

Homocysteine	Case (GDM) (n=32)		Control (Non-GDM) (n=32)		aOR	95% CI	p-value
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)			
Normal (4-15 µmol/L)	14	43.8	29	90.6	12.5	3.1-49.3	0.001b
Increased (>15 µmol/L)	18	56.3	3	9.4			

DISCUSSION

Gestational diabetes mellitus (GDM) is glucose intolerance first recognized during pregnancy and is characterized by abnormal maternal glycemic regulation. It is associated with maternal metabolic dysfunction and adverse pregnancy outcomes. The sociodemographic profile showed that age was the only variable with a statistically significant difference between the GDM and control groups (p=0.034). The largest proportion in both groups were aged 18–25 years, but women aged 35–40 years occurred only among GDM participants. Mean age was also higher in the GDM group (27.0±6.2 vs 24.8±3.9 years), although this difference was not significant (p=0.104). This pattern is consistent with established evidence that GDM frequency increases with maternal age. A large analysis demonstrated progressively higher GDM odds with advancing age, particularly from 30 years onward [14]. Educational qualification, religion, occupation, and monthly income were comparable between groups (all p>0.05), suggesting limited sociodemographic differentiation. BMI was slightly higher among GDM participants (25.6±3.1 vs 24.8±2.4 kg/m²), but the difference was not significant (p=0.267). This may reflect the multifactorial nature of GDM, in which adiposity interacts with maternal age and pregnancy-related insulin resistance rather than acting alone [15]. The categorical age result may therefore be more informative than the mean-age comparison. Pregnancy-related characteristics were broadly similar between groups. Mean gestational age was 25.5±1.39 weeks among GDM participants and 25.3±1.4 weeks among controls (p=0.140), indicating comparable timing of assessment. Multigravidity was more frequent among GDM participants (56.3% vs 40.6%), but the difference was not significant (p=0.211). Previous pregnancy complications were uncommon and showed no significant difference (p=1.0), while previous macrosomia, stillbirth, intrauterine death, or congenital anomaly were also comparable (21.9% vs 18.8%; p=0.756). First-degree family history of diabetes was reported in 21.9% of GDM participants and 28.1% of controls (p=0.564). Although these characteristics are clinically relevant during GDM assessment, their lack of statistical difference suggests that they did not explain the principal biochemical finding. Similar gestational ages also strengthen interpretation of the homocysteine comparison because maternal homocysteine varies during pregnancy [16,17]. The absence of significant differences should not be interpreted as evidence that these factors are clinically unimportant, particularly because the sample size may have limited statistical power for less frequent exposures. Serum homocysteine demonstrated the clearest biochemical difference. Mean homocysteine was higher in GDM participants than controls (10.3±6.2 vs 7.2±3.3 µmol/L; p=0.016), with median

values of 10.0 µmol/L (IQR 4.26–16.6) and 6.25 µmol/L (IQR 4.8–7.8), respectively. The box plot similarly showed a higher central tendency and greater dispersion among GDM participants. These findings agree with earlier clinical observations of higher homocysteine in GDM [18]. They are also consistent with a meta-analysis reporting significantly higher homocysteine among women with GDM, particularly during the second trimester [19]. A later systematic review reached a similar conclusion [20]. Possible mechanisms include oxidative stress, endothelial dysfunction, inflammatory signaling, and impaired β-cell function, all of which may contribute to abnormal glucose regulation [19,16]. However, these mechanisms remain explanatory rather than causal because the present case-control design cannot establish temporal direction. Gong et al. similarly found that the difference was particularly evident when homocysteine was measured in the second trimester, which is relevant because the present participants were assessed at approximately 25 weeks of gestation [19]. The categorical analysis further demonstrated a strong association. Increased homocysteine (>15 µmol/L) occurred in 56.3% of GDM participants compared with 9.4% of controls, while normal concentrations were present in 43.8% and 90.6%, respectively. The difference was highly significant (p=0.001), with an adjusted odds ratio of 12.5 (95% CI 3.1–49.3). This effect size is greater than estimates reported in several previous studies, where the strength of association has varied [20, 21]. A recent Mendelian randomization study also identified a positive association between genetically predicted homocysteine and GDM (OR 1.28, 95% CI 1.09–1.51), supporting a potentially relevant biological relationship [22]. Nevertheless, findings are not uniform. Radzicka et al. reported no significant difference in homocysteine between GDM and control groups, while other studies have described variable relationships involving homocysteine, folate, vitamin B12, and glycemia [16,17]. Differences in gestational age, ethnicity, nutritional status, assay methods, diagnostic criteria, and metabolic adjustment may contribute to this heterogeneity. In the present study, folate and vitamin B12 were not measured, limiting assessment of important determinants of homocysteine metabolism [17]. The relatively wide confidence interval also indicates uncertainty around the magnitude of the observed association and argues against interpreting the estimate as a definitive population-level effect. Overall, elevated maternal serum homocysteine was the principal biochemical feature distinguishing women with GDM from controls, whereas most sociodemographic and pregnancy-related variables were not significantly different. The consistent elevation in mean and median levels and the strong categorical association suggest that homocysteine may have value as an adjunctive marker for closer metabolic assessment. However, the wide confidence interval around the odds ratio indicates imprecision, and the case-control design cannot determine whether elevated homocysteine precedes GDM or accompanies it. Future prospective studies should evaluate homocysteine alongside folate, vitamin B12, BMI, renal function, insulin resistance, and glycemic indices. Current diabetes-in-pregnancy guidance continues to emphasize established glucose-based screening rather than homocysteine testing [23]. Thus, these findings support further investigation of homocysteine as a potential adjunctive biomarker, not as a standalone diagnostic test. Such evaluation could clarify whether homocysteine adds information beyond maternal characteristics and glucose testing.

LIMITATIONS

This study was limited by its small, single-center sample from BMU, Dhaka, which may restrict generalizability. Potential confounders, including vitamin B12, folate, renal function, and dietary intake, were not adjusted for. The short follow-up period also precluded assessment of long-term maternal and neonatal outcomes and postpartum glucose tolerance.

CONCLUSION & RECOMMENDATIONS

Elevated maternal serum homocysteine was significantly associated with GDM, suggesting its potential role as a marker of metabolic risk during mid-pregnancy. Homocysteine assessment may support early identification of women at increased risk of GDM and facilitate timely intervention. Routine testing may be considered in high-risk women, particularly those with previous GDM or elevated BMI. Larger multicentre longitudinal studies incorporating vitamin B12, folate, dietary factors, and complementary biomarkers such as SHBG and hs-CRP are warranted to clarify causality and improve early risk-prediction models.

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CONFLICT OF INTEREST

None declared

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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