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Original Article

Association of Serum Homocysteine with Gestational Diabetes Mellitus (GDM): A Case-Control Study

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Abstract:

Gestational diabetes mellitus (GDM) is associated with an increased risk of maternal and fetal mortality and morbidity. There is an increasing trend in the prevalence of GDM in Bangladesh. The role of homocysteine (Hcy) as an independent risk factor of GDM has not been extensively studied. The aim of this study was to evaluate the association of serum homocysteine levels in gestationaldiabetes mellitus. It was a case-control study among pregnant women attending the inpatient and outpatient departments of Obstetrics and Gynecology,BSMMU, Shahbag Dhaka, from April 2020 to March 2021. A total of 80 singleton pregnant women between 18-35 years of age were included in this study in their 24-40 weeks of gestation. Among them, 40 diagnosed women with GDM were considered as the cases and the rest of the 40 matched healthy pregnant women were selected as controls matching for age and gestational age.The overall average Hcy levels in the cases and controls were 5.48±1.3 µmol/L and 4.06±0.98 µmol/L, respectively. Both in the late second trimester and the third-trimester serum Hcy levels were significantly higher in the GDM cases than non-GDM healthy pregnant women ($p<0.05$). Considering Hcy level of 6.38 µmol/L as the cut-off value, GDM was 4.75 times more likely in pregnant women with elevated serum Hcy level (≥6.38) than those with < 6.38 µmol/L (OR=4.75; 95% CI = 0.941-23.985). There was a significant positive correlation of serum Hcy level with both fasting blood sugar level ($r = +0.600$, $p<0.001$) and 2 hrs after 75g glucose level ($r = +0.438$, $p<0.001$). Elevated level of serum homocysteine was found associated with GDM.

Key words: GDM, Homocysteine (Hcy) level.

Introduction:

Gestational diabetes mellitus (GDM) is exhibited as carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy[1]. It is the foremost common metabolic disorder throughout gestation that may cause several maternal and fetal complications during pregnancy and postnatal period. Women with GDM generally have few symptoms and it is most commonly recognized by screening during pregnancy[2]. It results in an increased maternal risk of preeclampsia, preterm labor, polyhydramnios, vaginal tearing, cesarean section and recurrent GDM and type-2 diabetes in later life. The major fetal and neonatal complications include perinatal mortality, macrosomia, obstetric trauma, hypoglycemia, hyperbilirubinemia, hypocalcemia and increased risk of obesity[3].

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Irrespective of variations in the population characteristics, screening methods and diagnostic criteria, an estimated 1-28% of pregnancies worldwide are impacted by the complications of GDM[4]. The prevalence of GDM in the U.S. was 7.6%,[5] while it reportedly affects one in every 23 pregnancies in the UK[6]. On the other hand, Bangladesh is the least developed country in Southeast Asia where maternal and child health is still underprivileged at some places with less access to healthcare services[7]. Here, the prevalence of GDM has been progressively increasing from 7.5% in 2012 to 18.1% in 2018[8].

In a normal pregnancy, there is an increase in insulin resistance (IR) which emerges in the second trimester and progresses over the late third trimester, is caused by increasing maternal weight and circulating hormonal factors produced by the mother and placenta, including human placental lactogen, progesterone, growth hormone, cortisol, and prolactin. To compensate for the peripheral IR during pregnancy, insulin secretion increases from a woman's pancreas. However, in women who develop GDM, there is diminished pancreatic β-cell reserve and the production of insulin fails to keep up with the increased insulin demand resulting in hyperglycemia. In addition, increased maternal adipose deposition, decreased exercise and increased calorie intake contribute to this state of relative glucose intolerance[9,10].

An increased level of oxidative stress is an important aspect of GDM that is involved in vascular damage and metabolic alterations[11]. Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of methionine. Elevated plasma Hcy concentration may induce excessive production of reactive oxygen species and impair the glutathione-related antioxidant defense system, leading to greater oxidative stress and lower antioxidant enzymatic activities[12]. Therefore, elevated maternal serum Hcy level could promote IR by inducing endoplasmic reticulum stress or up-regulating resistin production in adipose tissue,[13] which exaggerates β-cells function and results in GDM. Furthermore, during gestational diabetes, Hcy levels might increase, while serum Vit B12 and folic acid levels are known to decrease during GDM[14,15].

A large number of studies have focused on the relationship between Hcy and diabetes; however, the investigations between homocysteine and GDM were much fewer and their results were inconsistent. Likewise, in Bangladesh, there is a very limited number of studies on homocysteine levels in women with GDM. The present study aimed to evaluate the association of serum Hcy level with GDM. From the knowledge of the study, physicians might be able to assess the risk of developing GDM with the initiation of early effective management for prevention of the morbid effects and life-threatening consequences of GDM.

Materials and methods:

This case-control study was carried out on pregnant women attending the outpatient and inpatient departments of the Department of Obstetrics and Gynecology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka from April 2020 to March 2021 for prenatal care. A total of 40 cases and 40 controls who agreed to participate in the study were enrolled for this study upon fulfilling the selection criteria. Cases consisted of patients with GDM and controls comprised of healthy women without GDM matched for age between 18-35 years and gestational age 24-40 weeks. Data were collected from the patients on variables of interest using the semi-structured questionnaire designed for the interview, observation, clinical examination, hematological investigations and from hospital records of the patients. All the participants underwent standardized anthropometric measurements. With proper aseptic precautions, 3 ml of blood was collected from the ante-cubital vein using a sterile needle and syringe to estimate serum Hcy level. The blood sample was transferred into a clean, dry test tube and then taken for analysis to the laboratory of Department of Biochemistry and Molecular Biology, BSMMU where the total serum Hcy levels were determined by using an Alinity i Homocysteine Reagent Kit 09P28 in an automated analyzer (Atellica, Siemens, Germany). For this study purpose Hcy level 6.38 µmol/L was taken as cut off value,16 above which level it was considered a risk for developing GDM. All of the participants' oral glucose tolerance test (OGTT) reports were collected from their recent hospital records. GDM was diagnosed at the late second and third trimester of pregnancy if one or more of the following diagnostic criteria (WHO, 2013) were met - fasting plasma glucose: 5.1-6.9 mmol/l (92-125 mg/dl) and/or 2-hours plasma glucose following a 75 g oral glucose load: 8.5-11.0 mmol/l (153-199 mg/dl)1. Collected data were analyzed and compared using SPSS software v 26.0. A p-value less than 0.05 was considered statistically significant. The study was approved by the Institutional Review Board, BSMMU.

Results:

A total of 40 cases and 40 controls were enrolled in this study. The mean age was 28.7±3.45 years in the case group and 28.5±3.60 years in the control group. The majority of the patients were educated to the primary level, 42.5% in cases and 47.5% in the controls. Above four-fifth (82.5%) of the patients were homemakers among the cases compared to three-fourths (75%) of the control groups. Maximum participants (cases: 87.5%, and controls: 80.0%) had monthly family income between 15,000 to 30,000 BDT. These differences were not statistically significant between the case and control groups (Table I).

Table I: Distribution of patients according to socio-demographic characteristics of the study population.

Socio-demographic characteristics	Case (n = 40)		Control (n = 40)		p-value
	N	%	N	%	
Age (in years)					
Mean ± SD	28.7 ± 3.45		28.5 ± 3.60		[a]0.825[ns]
Range (min – max)	(18 – 35)		(18 – 35)		
Education					
Illiterate	10	25.0	7	17.5	[b]0.713[ns]
Primary	17	42.5	19	47.5	
Secondary & above	13	32.5	14	35.0	
Occupation					
Homemaker	33	82.5	30	75.0	[b]0.433[ns]
Student	4	10.0	8	20.0	
Service holder	3	7.5	2	5.0	
Monthly household income (in taka)					
15,000 to 30,000 BDT	35	87.5	32	80.0	[b]0.363[ns]
> 30,000 BDT	5	12.5	8	20.0	

ns = not significant

[a]p value reached from unpaired t-test

[b]p value reached from chi-square test

Majority of the respondents were on their third trimester of pregnancy in both groups (cases: 72.5% and controls: 75.0%). Total 80.0% of patients in the control group were multigravida, compared to 70% in the case group. In this study, gestational age in the cases was 31.4±4.20 weeks and in the control group 32.4±4.80 weeks. Majority of the of patients' BMI belonged to 25-29.9 kg/m2 (overweight) in both groups, which were 82.5% in case and 75.0% in control groups. These differences were not statistically significant (p >0.05) between the two groups (Table II).

Table II: Distribution of patients according to obstetric and anthropometric parameters of the study population

Obstetrical and anthropmetric parameters	Case (n = 40)		Control (n = 40)		p value
	N	%	N	%	
Gravida					
Primi	12	30.0	8	20.0	[a]0.302[ns]
Multi	28	70.0	32	80.0	
Trimester of pregnancy					
Second trimester	11	27.5	10	25.0	[a]0.799[ns]
Third trimester	29	72.5	30	75.0	
Gestational age (in weeks)					
Mean ± SD	31.4 ± 4.20		32.4 ± 4.80		[b]0.301[ns]
Range (min – max)	(24 – 38)		(24 – 39)		
Body mass index (kg/m2)					
Normal (18.5 – 24.9)	5	12.5	10	25.0	
Overweight (25.0 – 29.9)	33	82.5	30	75.0	
Obese (≥ 30)	2	5.0	0	0.0	
Mean ± SD	27.6 ± 1.76		27.0 ± 1.98		[b]0.144[ns]
Range (min – max)	(23.9 – 32.5)		(22.7 – 29.8)		

ns = not significant

[a]p value reached from chi-square test

[b]p value reached from unpaired t-test

There was no significant difference in distribution of the study subjects according to their previous history of obstetrical complications (p>0.05), though in cases preterm delivery and congenital birth defects were observed more, 28.6% and 3.6% respectively, than the controls (Table III).

Table III: Distribution of study population according previous obstetrical complications (in multigravid respondents)

Previous obstetrical complications	Case (n = 28)		Control (n = 32)		p value
	N	%	N	%	
History of preterm delivery					
Yes	8	28.6	3	9.4	[a]0.094[ns]
No	20	71.4	29	90.6	
History of congenital defect of baby					
Yes	1	3.6	0	0.0	[a]0.467[ns]
No	27	96.4	32	100.0	

ns = not significant

[a]p value reached from chi-square test

Mean Hcy was found 5.48±1.30 µmol/L in cases and 4.06±0.98 µmol/L in control group. The difference between two groups was statistically significant ($p < 0.001$) (Figure 1).

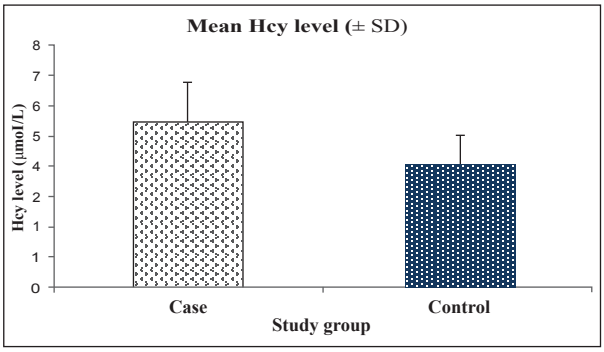


Figure I: Distribution of maternal serum Hcy level in the study population (cases = 5.48 ± 1.30 µmol/L and controls = 4.06 ± 0.98 µmol/L, p<0.001)

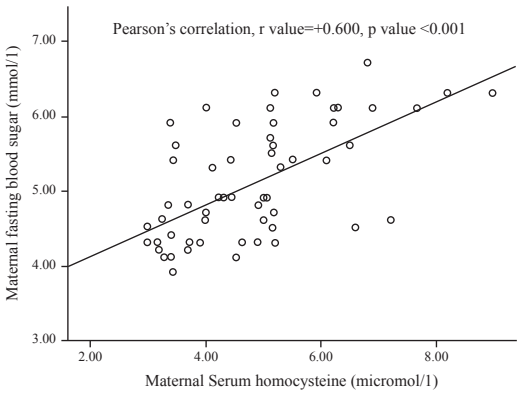
There was a significant difference in regards to raised Hcy level in between case and control groups (p=0.043), and the respondents with Hcy level ≥6.38 µmol/L had 4.75 times more chance to develop GDM compared to that of the respondent with homocysteine level < 6.38 µ mol/L (OR=4.750; 95% CI = 0.941-23.985) (Table V).

Table V: Odds ratios (OR) and 95% confidence intervals (CI) for GDM according serum homocysteine level in pregnancy (n=80)

	Groups			
Serum Hcy (µmol/L)	Case n(%) (n=40)	Control n(%) (n=40)	p-value	OR (95% CI)
≥ 6.38	8 (80.0)	2 (20.0)	[a]0.043*	4.75 (.941-23.985)
< 6.38	32 (45.7)	38 (54.3)		

*significant
[a]p value reached from chi-square test

Figure 2: observed a significant positive correlation of maternal serum Hcy level with both fasting blood sugar and 2 hours after 75g of glucose blood sugar (r=+0.600, p<0.001 and r=0.438, p<0.001, respectively).



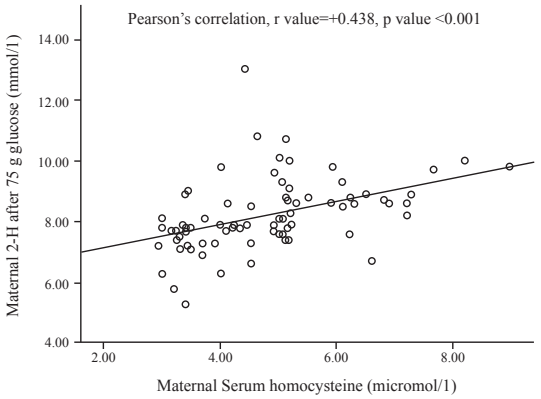


Figure 2: Scatterplot diagram showing the correlation between maternal FBS and 2HA 75g glucose blood sugar with serum homocysteine level

Discussion:

An elevated plasma Hcy level may increase the overall risk of GDM. This case-control study was carried out to evaluate the association of maternal serum Hcy level with GDM, and therefore measure serum Hcy level of normal healthy pregnant and women with GDM in their 24 to 40 weeks of gestation.

Based on the results of this study, the mean serum total Hcy concentration was significantly (p<0.001) higher among women with GDM (5.48±1.30 µmol/L) than that of normal controls (4.06±0.98 µmol/L) and both in the second and third trimester of pregnancy serum Hcy levels were found significantly higher than the normal healthy pregnant women. This finding is similar to that reported by Seghieri and associate, where serum Hcy was significantly higher in the group with GDM compared with nondiabetic women[17].

In this study, the majority of the respondents were between 24-29 years of age groups, 42.5% in the cases and 47.5% in the controls. The majority of respondents (45.0%) were educated up to the primary level, while 21.2% were found illiterate. Above three-quarters of the patients were homemakers (78.8%), and the monthly family income was between 15000-30000 Taka (83.8%). No statistical significance was observed regarding the study population's socio-demographic characteristics. These findings were supported by Cheung and Byth, who enumerated that socially disadvantaged GDM women are less likely to seek perinatal care[18].

In the present study majority of the respondents belonged to their third trimester of pregnancy, 72.5% and 75.0% in case and control groups, respectively,

while three-quarter of the study subjects (75.0%) were multigravida. Gestational age was taken as matching criteria, with the mean (±SD) duration of the respondents' gestational age was 31.4±4.20 weeks among the cases and 32.4±4.80 weeks in the control group. The overall average body mass index (BMI) in this study subjects were found almost similar 27.6±1.76 kg/m^2 among the case group and 27.0±1.98 kg/m^2 in the control group. According to Carpenter,[19] gravidas with GDM generally demonstrate higher degrees of post-pregnancy insulin resistance, β-cell dysfunction, higher BMI, central obesity, and exaggerated hyperlipidaemia, suggesting that GDM is a transient manifestation of long standing metabolic dysfunction.

In the current study, previous obstetrical complications and the number of babies with congenital defects were very low and did not show any statistical significance (p>0.05), though history of preterm delivery was seen in 28.6% of the cases and history of congenital defect of baby in 3.6%. Among the study subjects, 27.5% of the cases and 12.5% of the controls had a positive family history of GDM (p=0.094). According to Steegers-Theunissen and associates, there was a significant relation between tHcy concentration and stillbirth, neural tube defects, and clubfoot with plasma Hcy[20].

The respondents with Hcy level ≥6.38 μmol/L had 4.75 times more chance to develop GDM compared to that of the respondent with homocysteine level < 6.38 μmol/L (OR=4.750; 95% CI = 0.941-23.985). Moreover, the correlation between maternal serum Hcy level and fasting and 2 hours after 75g of glucose blood sugar showed significant positive correlations. This backs our hypothesis that elevated serum Hcy level is associated with GDM.

These findings co-relate with reports by Sharmila, who revealed there is significant association between homocysteine with GDM and hyperhomocysteinemia was found in 56.66% of patients with GDM[21]. Movahed and associates, in their study observed, in GDM, serum Hcy level was significantly higher (P<0.001), and folic acid was significantly lower (P<0.001) than normal pregnancy group[22]. They concluded that folic acid has a role in the regulation of serum Hcy level and blood sugar. Deng et al. also found women with GDM had a higher plasma Hcy level than normal glucose tolerant (NGT) women (6.61 ± 1.32 vs. 6.17 ± 1.29 μmol/L, P = 0.001). The GDM risk was 1.79 (OR=1.79, 95% CI 1.18–2.72, P=0.006) times higher in women whose plasma tHcy level was ≥7.29 μmol/L compared to women with plasma tHcy level < 5.75 μmol/L[23].

Though the current study was conducted in a selected tertiary level hospital with small sample size, therefore, all the findings of this study had given rise to concerns about higher level of serum homocysteine among the GDM patients.

Conclusion:

In this study, findings conveyed that elevated maternal Hcy level was associated with GDM. And it is recommended that screening for hyperhomocysteinemia might be considered a part of the routine antenatal check-up. Further multi-centric study with a larger sample size could be done to determine the validity of the findings of the present study.

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