

Association of Elevated Serum Homocysteine Level in Women with Gestational Diabetes Mellitus

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Abstract

Background: In gestational diabetes mellitus (GDM), risk prediction is mostly based on maternal history and clinical risk factors and may not optimally identify high risk pregnancies. Therefore, universal screening is widely recommended. Homocysteine levels during pregnancy in women with GDM have been studied; however, it remains unclear whether hyperhomocysteinemia is a useful predictor of GDM. **Objective:** To determine the association of serum homocysteine level in women with gestational diabetes mellitus. **Methods:** Case control study was conducted in the Department of Obstetrics and Gynaecology, Institute of Child and Mother Health (ICMH), Dhaka. Pregnant women in their 24 weeks to 40 weeks of gestation attended for antenatal care diagnosed as GDM was selected as cases. Non-diabetic pregnant women matching with cases by age and gestational age were selected as control in this study. GDM was diagnosed by oral glucose tolerance test (OGTT). The serum homocysteine level of these patients was measured. Descriptive and inferential analysis was carried out using SPSS version 22.0. P-value less than 0.05 will be considered as statistically significant. **Results:** Among the cases fasting blood sugar (6.13 ± 0.66) and controls (4.60 ± 0.57). Similarly, 2 hours after 75 gm. glucose blood sugar among cases (8.46 ± 0.88) and controls (6.32 ± 0.96). Both results were statistically significant $p=0.001$. Majority of patients were at third trimester of pregnancy (67.0%). 37.5% were primigravida and 2.5% were multigravida. Mean gestational age of cases (30.25 ± 2.74) and controls (30.02 ± 3.03). Among the cases 62.1% had history of GDM where 37.9% don't have history of GDM. Among control group only 7.7% had history of GDM. This result statistically significant $p=0.001$. 6.9% cases had history of macrocosmic child which was not statistically significant $p=0.49$. There was no history of still birth or other congenital defect. Mean serum homocysteine level among cases (6.50 ± 1.72) and controls (5.20 ± 1.87) which was statistically significant $p=0.001$. Positive correlation observed between maternal serum homocysteine level and maternal blood sugar ($r=0.209$, $p=0.005$) in this study. Respondents with high homocysteine level have 3.94 times more chance to develop GDM (OR=3.94; 95% CI = 1.59-9.77). **Conclusion:** Finding from the present study suggests that maternal elevated serum homocysteine level in pregnancy is significantly associated with Gestational Diabetes Mellitus.

Keywords: Serum Homocysteine Level, Gestational, Diabetes Mellitus.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of varying severity with onset or first recognition during pregnancy [1]. It is a serious pregnancy complication, in which women without previously diagnosed diabetes develop chronic

hyperglycemia during gestation. In most cases, this hyperglycemia is the result of impaired glucose tolerance due to pancreatic beta cell dysfunction on a background of chronic insulin resistance. It is the most frequent metabolic disorder occurring during pregnancy and its prevalence may range from 1 to 16% of all

pregnancies [2]. According to the most recent (2017) International Diabetes Federation (IDF) estimates, GDM affects approximately 14% of pregnancies worldwide, representing approximately 18 million births annually. During healthy pregnancy, the mother's body undergoes a series of physiological changes in order to support the demands of the growing fetus. During early gestation, insulin sensitivity increases, promoting the uptake of glucose into adipose stores in preparation for the energy demands of later pregnancy. However, as pregnancy progresses, a surge of local and placental hormones, including estrogen, progesterone, leptin, cortisol, placental lactogen, and placental growth hormone together promote a state of insulin resistance [3]. As a result, blood glucose is slightly elevated, and this glucose is readily transported across the placenta to fuel the growth of the fetus. This mild state of insulin resistance also promotes endogenous glucose production and the breakdown of fat stores, resulting in a further increase in blood glucose and free fatty acid concentrations [4, 5]. International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria be used in the diagnosis of GDM which is 24-28 weeks OGTT by 75 gm of glucose fasting plasma glucose (5.1-6.9) mmol/L (≥ 92 mg/dl), or 2 hours after 75 gm of glucose load blood glucose level (8.5-11.0) mmol/L [6]. Epidemiological studies of risk factors for GDM are limited and are typically afflicted by confounding factors. In addition, inconsistencies in diagnostic criteria for GDM and measurements of risk factors make it difficult to compare findings across studies. Despite these concerns, several risk factors for GDM emerge consistently. These include overweight/obesity, excessive gestational weight gain, westernized diet, ethnicity, genetic polymorphisms, advanced maternal age, intrauterine environment (low or high birth weight), family and personal history of GDM, and other diseases of insulin resistance, such as polycystic ovarian syndrome (PCOS). Reactive oxygen species (ROS) are described as free radical and non-radical derivatives of oxygen, and include superoxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2). A hyperglycemic environment is associated with oxidative stress, and GDM women have been reported to overproduce free radicals and have impaired free-radical scavenging mechanisms. ROS inhibit insulin-stimulated glucose uptake by interfering with both IRS-1 and GLUT4. ROS also slow glycogen synthesis in the liver and muscle. Pro-inflammatory cytokines, such as TNF, may also contribute to oxidative stress by increasing the expression and the activation of ROS precursors, like NADPH oxidase 4 (NOX4). Universal screening for GDM is common in most developed nations, using a variety of tests including an oral glucose tolerance test (OGTT) at 24–28 weeks gestation for diagnosis. Serum homocysteine has previously been found to be reduced in all trimesters of pregnancy (due to either a physiological response to the pregnancy, an increase in estrogen, hemodilution from an increased plasma volume, or

increased demand for methionine by both the mother and the fetus) compared to non-pregnant controls [7]. Previous studies showed that hyperhomocysteinemia is associated with adverse pregnancy outcomes or early pregnancy losses. Recent data about plasma homocysteine levels and glucose tolerance in both diabetic and non-diabetic pregnant women has also demonstrated an association between insulin resistance and hyperhomocysteinemia [8]. Additionally, it has been demonstrated that elevated plasma levels of homocysteine increase the risk of macro vascular disease and atherothrombosis, especially in individuals with Type 2 diabetes or impaired glucose tolerance and common carotid artery intima-media thickness in women with gestational diabetes mellitus (GDM) compared with unaffected women (Steegers-Theunissen [9]. Early identification of women at high risk of GDM may also facilitate early streamlined antenatal care with enhanced continuity, targeted lifestyle interventions to reduce excessive gestational weight gain and potentially reduce GDM and T2DM. It may also allow timely screening and prompt GDM management, with improved patient experiences and clinical outcomes. With rising GDM prevalence, opportunities for potential prevention of GDM and its complications provide rationale for early pregnancy GDM risk screening [10].

MATERIALS AND METHODS

Study Design: This study was a case control study.

Place of Study: This study was carried out in the Department of Obstetrics and Gynecology of Institute of Child and Mother Health (ICMH), Matuail, Dhaka, Bangladesh.

Period of Study: September 2018 to August 2019.

Study Population: The study population was included the Pregnant women attending the antenatal clinic in their 24 weeks to 40 weeks of pregnancy in the Department of Obstetrics and Gynecology of ICMH.

Case: GDM patient gestational age within 24 weeks to 40 weeks attending in ICMH.

Controls: Healthy pregnant woman gestational age within 24 weeks to 40 weeks attending in ICMH.

Sampling Method: Purposive sampling was done according to the availability of the patients who was fulfill the inclusion criteria.

Sample Size Determination:

Using the following formula

$$n = \left(\frac{r+1}{r} \right) \frac{(Z_{\alpha} + Z_{\beta})^2 (\sigma^2)}{(\mu_1 - \mu_2)^2}$$

Considering 10% non-response rate and missing value calculated.

Sample size, n = 44 cases, 44 controls.

Inclusion Criteria:

Case:

- Pregnant women with GDM attended to antenatal care in ICMH.
- Age of all patients was within 18 to 35 years.
- Gestational age was within 24 to 40 weeks.
- Pregnant women who had given consent to participate in the study.

Control:

- Healthy Pregnant women attended to antenatal care in ICMH.
- Age of all patients was within 18 to 35 years.
- Gestational age was within 24 to 40 weeks.
- Pregnant women who had given consent to participate in the study.

Exclusion Criteria:

- Known case of diabetic patient.
- Patient on drug/ medication likely to alter glucose metabolism e.g. Sulfonylureas, protease inhibitors, stavudine, steroids and thiazide diuretics, beta agonist.
- Pregnancy with any other medical or obstetrical disorder.
- Patients with multiple pregnancies.
- Pregnant women with smoking or tobacco chewing habit.

Operational Definitions:

- **Gestational Diabetes Mellitus:** Gestational Diabetes Mellitus is diagnosed if one or more of the following criteria are met [11, 6]
 - ☐ Fasting plasma glucose 5.1-6.9 mmol/l
 - ☐ 2 hours plasma glucose 8.5-11mmol/l following 75 gm oral glucose load [11].
- **Homocysteine Level:** Normal level of serum homocysteine is 4-15 µmol/L.
Cut-off value of serum homocysteine is 6.38 µmol/L. >6.38 and ≤ 6.38 normal [8].

Gestational diabetes mellitus was diagnosed at any time in pregnancy if one or more of the following criteria are met	
Fasting plasma glucose	5.1-6.9 mmol/L (92-125 mg/dL)
2-h plasma glucose following a 75 g oral glucose load	8.5-11.0 mmol/L (153-199 mg/dL)

Measurement of Serum Homocysteine

Homocysteine assay is a one-step immunoassay for the quantitative determination of total L-homocysteine in human serum or plasma using CMIA technology, with flexible assay protocols, referred to as Chemiflex. Bound or dimerized homocysteine (oxidized form) is reduced by dithiothreitol (DTT) to free homocysteine, which is then converted to S-adenosyl homocysteine (SAH) by the action of the recombinant enzyme S-adenosyl homocysteine hydrolase (rSAHHase) in the presence of excess adenosine. The SAH then competes with acridinium-labeled S-adenosyl cysteine for particle-bound monoclonal antibody. Following a wash stage

Study Procedure

The study population was pregnant women attending in the department of Obstetrics and Gynecology, ICMH fulfilling the inclusion and exclusion criteria. All participants were in their 24 weeks to 40 weeks of pregnancy, primi or multigravida and with a single fetus. The purpose and procedure of the study was discussed with the patients. Informed written consent was taken from those who agree to participate in the study. Ethical committee clearance was obtained from the institution. Thorough clinical examination was done in all the subjects. Blood was taken from the ante cubital vein using a sterile needle and syringe. As the serum concentration of Hcy increases artificially due to time and temperature dependent release from RBCs the blood samples were centrifuged immediately in a cooling centrifuge technique. For each and every subject separate data collection sheet was prepared. Data was collected from the patients on variables of interest using the structured questionnaire design by interview, observation, clinical examination, biochemical investigations of the patients.

Data Collection

Case and control were selected purposively according to the availability of the respondent. Detailed Obstetric and medical history and clinical information were obtained by preformed structured questionnaire.

Blood Collection

Maternal blood samples were drawn from the antecubital vein (in an arm without intravenous infusion ongoing). 5 milliliters blood was drawn with proper aseptic precautions. The blood sample was transferred into a clean, dry test tube and taken to the laboratory.

Measurement of Blood Glucose Level (OGTT): After arrival in the laboratory, venous blood for the clients withdrawn from cubital vein [11].

and magnetic separation, pre-trigger and trigger solutions are added to the reaction mixture and the resulting chemiluminescence is measured as relative light units (RLUs). An indirect relationship exists between the amount of homocysteine in the sample and the RLUs detected by the ARCHITECT i System Optics. Most laboratories normal homocysteine levels in the blood between 4 to 15micromoles/liter (µmol/L). Any measurement above 15 is considered high. Any measurement below 12 is considered low. Optimal homocysteine levels are below 10 to 12. Hyperhomocysteinemia was classified into moderate, intermediate, and severe types based on the level of homocysteine and are:

Moderate (15 to 30 µmol/L)
 Intermediate (30 to 100 µmol/L)
 Severe (greater than 100 µmol/L)
 Cut off value of serum homocysteine 6.38 (µmol/L), >6.38 raised and ≤ 6.38 normal [8].

Data Analysis

Statistical analyses were carried out by using Windows based Statistical Package for Social Sciences (SPSS- 22). The descriptive statistics of the study was presented in tables, figures or suitable graphs, frequency, percentage, mean ± SD as per the requirement of qualitative and quantitative variables. Chi square test was done to observe the association between case and control. Unpaired t test was done to

see the difference of homocysteine level between case and control. Pearson correlation coefficient analysis was used to observe the association between maternal serum homocysteine level and GDM. Odds ratio and 95% confidence interval was also estimated for the outcome. The p value <0.05 was considered as statistically significant.

RESULTS

This study carried out to assess the association of serum homocysteine level in women with gestational diabetes mellitus. A total of 44 cases and 44 controls were selected for the study. Findings of the study were presented by graphs and tables.

Table 1: Socio-demographic characteristics of the respondents.

Socio-demographic characteristics	Case n=44 (%)	Control n=44 (%)	Total n=88 (%)	p value
Age				
<20 years	0 (0.0)	3 (6.8)	3(3.4)	0.10 ^b
20-30 years	33 (75.0)	35 (79.5)	68(77.3)	
30 years & above	11(25.0)	6 (13.6)	17(19.3)	
Educational status				
Primary or below	21 (47.7)	25 (56.8)	46(52.3)	0.31 ^a
SSC	14 (31.8)	15 (34.1)	29(33.0)	
HSC and above	9 (20.5)	4 (9.1)	13(14.8)	
Occupation				
Housewife	40 (90.9)	39 (88.6)	79(89.8)	0.75 ^b
Student	2 (4.5)	4 (9.1)	6(6.8)	
Service	2 (4.5)	1 (2.3)	3(3.4)	
Monthly family income (Taka)				
15000-30000 taka	36(81.8)	44 (100.0)	80(90.9)	0.006 ^b
More than 30000 taka	8 (18.2)	0 (0.0)	8(9.1)	

a= Pearson Chi-Square test, b= Fisher's Exact Test

Socio-demographic characteristics of the patients are shown in table-1. Majority of the respondents were between 20-30 years (77.3%) The proportions of patient of different educational status were primary or below (52.3%). Most of the patients

were housewife (89.8%) and monthly family income of majority 15000-30000 Taka (90.9%). Statistically significant result observed between case and control in monthly family income (p=0.006).

Table 2: Blood pressure of the respondents (case=44, control=44)

Blood pressure	Case Mean ± SD	Control Mean ± SD	p value
Systolic blood pressure	107.27 ± 9.73	106.82 ± 9.34	0.82 ^c
Diastolic blood pressure	71.36 ± 7.34	71.14 ± 6.89	0.88 ^c

c=unpaired t test

Mean systolic blood pressure of case (107.27 ± 9.73) and control (106.82 ± 9.34) were equal. Similarly, diastolic blood pressure also equal. There was no

statistical difference observed between case and control group (Table-2).

Table 3: Obstetrical characteristics of the respondents (case=44, control=44)

Obstetrical characteristics	Case n=44 (%)	Control n=44 (%)	Total n=88 (%)	p value
Trimester of pregnancy				
Second trimester	10 (22.7)	19 (43.2)	29(33.0)	0.04 ^a
Third trimester	34(77.3)	25 (58.6)	59(67.0)	

Gravida				
Primi	15 (34.1)	18 (40.9)	33(37.5)	0.62 ^a
Multi	29 (65.9)	26 (59.1)	55(62.5)	
Gestational age (mean ± SD)				
Duration in weeks	30.25 ± 2.74	30.02 ± 3.03		0.714 ^c

a= Pearson Chi-Square, c=unpaired t test

The obstetrical conditions of the patients are shown in table-3. Majority of patients were at third trimester of pregnancy (67.0%). 37.5% were primigravida and 62.5% were multigravida. Mean

gestational age of case (30.25 ± 2.74) and control (30.02 ± 3.03). There was no statistically significant result is observed in obstetrical characteristics of case control group.

Table 4: Complication of current pregnancy of the respondents (case=44, control=44)

Complication of current pregnancy	Case n = 44 (%)	Control n = 44 (%)	p value
Polyhydramnios			
Present	3 (6.8)	0 (0.0)	0.12 ^b
Absent	41 (93.2)	44 (100.0)	
Preterm labour			
Present	0 (0.0)	0 (0.0)	
Absent	44 (100.0)	44 (100.0)	
PROM			
Present	0 (0.0)	0 (0.0)	
Absent	44 (100.0)	44 (100.0)	

b= Fisher's Exact Test

The Complication of current pregnancy of the respondents is shown in table-4. Majority of respondents don't have any complication. Only 6.8%

cases were suffering from polyhydramnios which was not statistically significant p=0.12.

Table 5: History of previous obstetrical complications of the respondents.

H/O Complication	Case n = 29 (%)	Control n = 26 (%)	p value
History of GDM			
Present	18 (62.1)	2 (7.7)	0.001 ^a
Absent	11 (37.9)	24 (92.3)	
History of macrocosmic baby			
Present	2 (6.9)	0 (0.0)	0.49 ^b
Absent	27 (93.1)	26 (100.0)	
History of still birth			
Present	0 (0.0)	0 (0.0)	
Absent	29 (100.0)	26 (100.0)	
History of IUD			
Present	0 (0.0)	0 (0.0)	
Absent	29 (100.0)	26 (100.0)	
History of repeated pregnancy loss			
Present	0 (0.0)	0 (0.0)	
Absent	29 (100.0)	26 (100.0)	
History of congenital defect of baby			
Present	0 (0.0)	0 (0.0)	
Absent	29 (100.0)	26 (100.0)	

a= Pearson Chi-Square, b= Fisher's Exact Test

The history of previous pregnancy obstetrical complications of the respondents is shown in table-5. Among the case 62.1% had history of GDM where 37.9% don't have history of GDM. Among control

group only 7.7% had history of GDM. This result statistically significant p = 0.001. 6.9% cases had history of macrocosmic child which was not statistically significant p=0.49.

Table 6: Association of serum homocysteine level and gestational diabetes mellitus.

Serum homocysteine level	Case (n=44) Mean ± SD	Control (n=44) Mean ± SD	p value
Serum homocysteine level	6.50 ± 1.72	5.20 ± 1.87	0.001 ^c

c=unpaired t test

Mean serum homocysteine level among cases (6.50 ± 1.72) and controls (5.20 ± 1.87) which was statistically significant p=0.001 (Table-6).

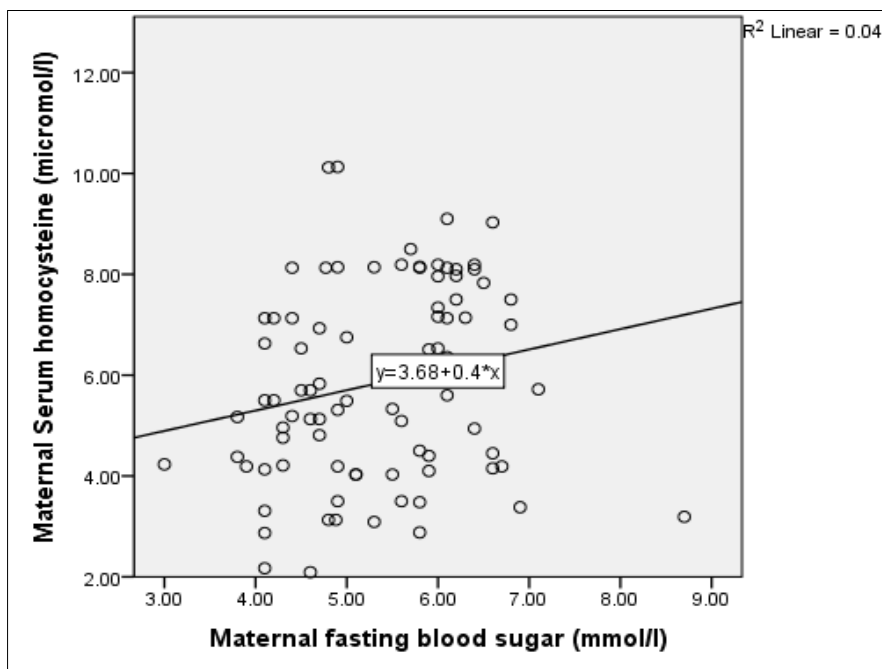


Figure I: Correlation between FBS and S. homocysteine (r=0.209, p<0.05)

Correlation between maternal serum homocysteine level and fasting blood sugar is shown in

figure-I where positive correlation observed (r=0.209, p<0.05).

Table 7: Odds ratios (OR) and 95% confidence intervals (CI) for GDM according to serum homocysteine concentrations in pregnancy.

Serum homocysteine (µmol/L)	Case n (%)	Control n (%)	p- value	OR (95% CI)
>6.38	25 (56.8)	11 (25.0)	0.005	3.94 (1.59-9.77)
≤ 6.38	19 (43.2)	33 (75.0)		

Here, serum homocysteine level 6.38µmol/L is considered as cut off value. High level of serum homocysteine was observed in case group (56.8%) compared with control (25.0%) group which was statistically significant (P= 0.005). All respondents of control group had low level of serum homocysteine (75.0%) compare to case group (43.2%). Respondents with high homocysteine level have 3.94 times more chance to develop GDM (OR=3.94; 95% CI = 1.59-9.77) (Table-7).

DISCUSSION

A recent meta-analysis show homocysteine levels were significantly elevated in women with GDM compared with those without GDM (weighted mean difference 0.77, 95% confidence interval 0.44–1.10). This evidence more consistent during the second

trimester measurement of homocysteine (weighted mean difference 0.95, 95% confidence interval 0.67–1.23) and for women aged older than 30 years (weighted mean difference 0.90, 95% confidence interval 0.63–1.17[12]. According to other study Serum homocysteine significantly higher in the group with GDM compared with non-diabetic women and significantly related to serum albumin as well as to serum uric acid. No relationship observed between sHcy and serum vitamin B12, serum triglycerides, or high-density lipoprotein (HDL) cholesterol, mean blood pressure. GDM remained significantly associated with higher sHcy concentrations also after adjusting for age, serum folate, albumin, uric acid, and vitamin B12 (p =.006) [13]. Fasting plasma homocysteine levels correlated significantly with insulin secretion in response to OGTT even after adjustment for body mass index (p< .05) in hypertensive patients but not in

normotensive individuals. However, fasting plasma homocysteine concentrations showed no correlations with steady-state plasma glucose concentration, a measurement of insulin sensitivity, during an insulin suppression test in groups of hypertensives ($n = 42$) and normotensive ($n = 37$) subjects. When the steady-state plasma glucose concentrations were divided into three tertiles, fasting plasma homocysteine concentrations showed no difference across these three groups in either hypertensive patients or normotensive subjects [14]. According to Guven *et al.*, [15] mean serum homocysteine concentration of women in gestational diabetes, glucose intolerants and normal controls at 24–28 weeks of gestation 9.0 ± 3.1 , 8.1 ± 2.5 and 7.4 ± 1.6 $\mu\text{mol/L}$, respectively. The only statistically difference in homocysteine levels observed between women with gestational diabetes and normal controls ($p < 0.01$) [15]. In this study majority of patients were at third trimester of pregnancy (67.0%). 37.5% were primigravida and 62.5% were multigravida. Mean gestational age of case (30.25 ± 2.74) and control (30.02 ± 3.03). There was no statistically significant result is observed in obstetrical characteristics of case control group. In this study among the case 62.1% had history of GDM where 37.9% don't have history of GDM. Among control group only 7.7% had history of GDM. This result statistically significant $p=0.001$. 6.9% cases had history of macrosomic child which was not statistically significant $p=0.49$. There was no history of still birth or other congenital defect. According to Steegers-Theunissen *et al.*, [8] there also a significant relation between tHcy concentration and stillbirth, neural tube defects and clubfoot with plasma tHcy. Placental abruption had no relation with tHcy [8]. This result is not similar to my study. According to this study Mean serum homocysteine level among cases (6.50 ± 1.72) and controls (5.20 ± 1.87) which was statistically significant $p=0.001$. Study of Turkish women found that patients with gestational diabetes and women with abnormal screening test results (>135 mg/dL) but normal OGTT results have higher homocysteine levels than normal pregnant women [16]. Study on 170 women, 18 (10.6%) converted to diabetes during the 4-year follow-up period. Mean age, BMI, fasting insulin, and total cholesterol at baseline (6 weeks' postpartum test) were similar in the three study groups (i.e., normal, IGT, and diabetes). Fasting glucose levels, insulin-to-glucose ratios, and homocysteine levels were significantly higher in the diabetic group ($p < 0.05$). Higher glucose at the time of the diagnosis of GDM and higher homocysteine levels at baseline were independently associated with the onset of postpartum diabetes. These relationships were independent of age, BMI, and family history of diabetes [8] which is similar to this study. Positive correlation observed between maternal serum homocysteine level and maternal blood sugar ($r=0.209$, $p=0.051$) in this study. According to Alatab *et al.*, [17] a trend of elevation of homocysteine is presented in women with GDM, that is more prominent in women with impaired GTT, and shows a

significant correlation with history of GDM similar to this study. Here serum homocysteine level $6.38 \mu\text{mol/L}$ is considered as cut off value. High level of serum homocysteine was observed in case (56.8%) compared with control (25.0%) group which was statistically significant ($p=0.005$). All respondents of control group had low level of serum homocysteine (75.0%) compare to case (43.2%). Respondents with high homocysteine level have 3.94 times more chance to develop GDM ($\text{OR}=3.94$; 95% CI = 1.59-9.77) which is similar to other study conducted by (Cho N 2005) [8] According to this study respondents with high homocysteine level have 3.94 times more chance to develop GDM which is similar to other study.

CONCLUSION

In this study, finding conveyed that maternal elevated serum homocysteine level in pregnancy is significantly associated with Gestational Diabetes Mellitus.

Limitations of the Study

This study had some limitations as well;

- Study was conducted in a single hospital. So, the study population might not represent the whole community,
- The sample was taken purposively. So, there may be chance of bias which can influence the results,
- Sample size was small,
- Limited resources and facilities.

Therefore, the study findings cannot be generalized to the entire population.

RECOMMENDATIONS

To make more conclusive results the following recommendations are proposed for further studies;

- Similar type of study can be done with large sample size.
- Other co factors related to GDM should be evaluated.

Further national multicenter prospective studies can be done.

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