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Role of Maternal Serum Vitamin B_{12} Concentration with Risk of Developing Gestational Diabetes Mellitus

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Abstract **Original Research Article**

Background: Gestational diabetes mellitus (GDM) is one of the most common complications in pregnant women. Vitamin B_{12} serves in synthesis of methionine from homocysteine. Low vitamin B_{12} inhibits DNA synthesis and elevates homocysteine. Elevated homocysteine level linked with insulin resistance that is associated with GDM. Various researchers suggested an association of serum vitamin B_{12} concentration with GDM. Estimation of serum vitamin B_{12} may be helpful in management of GDM. ***Objective:*** To observe the association of vitamin B_{12} concentration with risk of developing GDM. ***Methods:*** This cross-sectional study was carried out at the Department of Laboratory Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh between March 2021 and February 2022. A total of 86 women were enrolled; of them 43 were GDM women selected as cases and 43 were normal pregnant women taken as control group. Their demographic profile, relevant medical history with clinical examination findings were recorded. Serum vitamin B_{12} concentrations of the study population were estimated following standard procedure. ***Results:*** The mean serum vitamin B_{12} concentration was found significantly low in GDM women (158.7±91.8 pmol/L versus 264.5±100.9 pmol/L, p= 0.001). Pearson's correlation coefficient test between serum vitamin B_{12} concentration with fasting plasma glucose level, plasma glucose level 1 hours after 75 gm glucose and plasma glucose level 2 hours after 75 gm glucose showed a significantly negative correlation (r= -0.432, p= 0.004; r= -0.519, p<0.001 and r= -0.687, p<0.001). In multivariate logistic regression analysis, the odd ratio of vitamin B_{12} concentration with GDM was 1.997 (95% CI; p= 0.042). ***Conclusion:*** Serum vitamin B_{12} concentration is significantly low in GDM women, which is negatively correlated with plasma glucose levels among GDM women. Serum vitamin B_{12} concentration may be used as a predictive tool to identify risk of developing GDM.

Keywords: Correlation, Gestational Diabetes Mellitus (GDM). Pregnant Women, Serum Vitamin B_{12} Concentration.

1. INTRODUCTION

Gestational diabetes mellitus (GDM) is a common complication in pregnancy that is defined as any degree of glucose intolerance that first identified during pregnancy, regardless of whether the condition continues after pregnancy or not [1]. GDM is a serious pregnancy complication where women develop chronic hyperglycemia during gestation [2]. The prevalence of GDM in pregnancy is about 17% worldwide, which is 9.7% in Bangladesh [2-3]. The diagnosis of GDM include, when the fasting plasma glucose level is 5.1-6.9 mmol/L, after a 75 gm oral glucose load 1-hour plasma glucose level is ≥10.0 mmol/L, and 2-hour plasma glucose level is 8.5-11.0 mmol/L [4]. Pancreatic insulin release and chronic insulin resistance have roles in developing GDM. Insulin resistance is believed to be mediated by hormones released during pregnancy, such as placental lactogen, cortisol, progesterone, and estrogen [5]. Future metabolic dysfunction and diabetes have been linked to gestational diabetes mellitus (GDM) and reduced glucose tolerance during pregnancy [6, 7]. GDM is caused by a number of risk factors, including obesity, advanced maternal age, history of GDM, history of diabetes in first-degree relatives, and specific Asian and African ethnic women [7]. The health of both the mother and the fetus is negatively impacted by GDM in numerous ways. It increases the risk of perinatal morbidity and causes preeclampsia, polyhydramnios, and abortion during pregnancy [8].

Vitamin B_{12} is an essential water-soluble vitamin. It helps in maturation of erythrocytes and optimal nervous system function. Vitamin B_{12} is an important coenzyme during DNA synthesis [9]. Low level of maternal vitamin B_{12} will inhibit DNA synthesis by trapping folate as 5-methyltetrahydrofolate [10]. It causes reduction of mitochondrial DNA that is associated with insulin resistance [11]. Vitamin B_{12} may be involved in glucose intolerance due to its ability to regulate synthesis of homocysteine [12]. Low vitamin B_{12} levels lead to elevated levels of homocysteine [13]. Homocysteine at elevated concentration has been linked with insulin resistance [14-15]. Elevated homocysteine concentration impairs endothelial function in skeletal muscles, adipose tissue and liver, thus reducing insulin delivery to these insulin-sensitive tissues [16-17]. Elevated homocysteine concentration also plays a harmful role in pancreatic β-cell metabolism and insulin secretion [18]. It was reported that vitamin B_{12} insufficiency associated with higher risk of GDM [19]. Low vitamin B_{12} levels may be one of the primary mechanisms in driving the effect of high folate status on developing risk of GDM [20]. It's crucial to identify and treat women who are at higher risk of developing GDM in order to improve pregnancy outcomes. Monitoring vitamin B12 levels may help prevent complications from GDM in both the mother and the fetus. In this context, the aim of this study was to evaluate the association of vitamin B_{12} with risk of developing GDM.

2. METHODOLOGY

This cross-sectional study was conducted between March 2021 and February 2022 at the Department of Laboratory Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. A total of 86 women were enrolled following selection criteria; of them 43 were GDM women selected as case (Group I) and 43 were normal pregnant women taken as control (Group II). In this study diagnosed cases of GDM women from 28 week of gestation onward and apparently normal pregnant women from 28 week of gestation onward were included. The study excluded participants having a history of pre-eclampsia/chronic renal disease/liver disease/cardiovascular disease or diabetes mellitus, pregnant women with a history of prior GDM, pregnant women with mal-absorption syndrome and pregnant women taking any vitamin supplementations.

Study procedure

Prior to enrollment, each study participant provided written informed consent. Their demographic profile and relevant medical history were recorded. All study participants underwent a thorough clinical examination and pertinent investigations. Their body mass index (BMI), last menstrual cycle, anticipated delivery date, gestational age as determined by an ultrasound, and prenatal check-up findings were recorded. The gestational age of each group was calculated using the last menstruation date and verified by ultrasound. Serum vitamin B_{12} concentration of the study population was estimated following standard procedure. To ensure confidentiality, a different data collecting sheet was used for every participant.

Identification of GDM

The following criteria were used to diagnose GDM among the study population [4]:

- Plasma glucose level at fasting: 5.1 mmol/L - 6.9 mmol/L
- 1- hour after a 75 gm oral glucose load plasma glucose level ≥10.0 mmol/L and
- 2- hour after a 75 gm oral glucose load plasma glucose level 8.5-11.0 mmol/L

Collection and analysis of blood samples

Following an overnight (8–12 hours) fast, 5 ml of venous blood was drawn from each study participant's ante-cubital vein with all aseptic precautions. Then, 75 grams (gm) of glucose in 300 milliliters of water was given to each participant to drink. They were instructed to be sedentary for two hours and refrain from eating or drinking. After one and two hours of glucose consumption, three milliliters of venous blood were drawn for the second and third blood samples. Dry and clean test tubes were used to collect the blood samples. The test tubes were then sent to the Laboratory Medicine, BSMMU within 30 minutes of the blood samples collection. After being held upright for thirty minutes, every blood sample was centrifuged for five minutes at 3000 rpm in room temperature (22°C - 24°C). After that, the separated serum or plasma was taken in an eppendorf or a sample cup and labeled appropriately. Before analysis was completed, all separated serum and plasma were kept at -20°C. Blood glucose was measured by glucose-oxidase method in an automated biochemistry analyzer using the principle of photometric technique. Serum vitamin B_{12} concentration was measured by immunochemistry auto-analyzer using the Electrochemiluminescence Immunoassay.

Normal range of serum vitamin B_{12} concentration during pregnancy:

Serum vitamin B_{12} concentration during pregnancy is 160-490 pmol/L [19].

Statistical analysis of data

All collected data were verified and compiled accordingly. Both qualitative and quantitative data were presented as frequency with percentage, and mean with standard deviation (±SD) respectively. Statistical analysis was performed using window-based computer software Statistical Packages for Social Sciences (SPSS) version 26. Multivariate logistic regression analysis, Pearson's correlation coefficient test, the Unpaired t-test were used for the statistical analysis of the data. A p value less than 0.05 was regarded as statistically significant.

3. RESULTS

This study included total 86 pregnant women. Of them; 43 pregnant women with GDM from 28 week onward were assigned to group I, and another 43 normal pregnant women (without GDM) from 28 week onward were assigned to group II. The mean(±SD) age in group I was 32±6.02 years and that was 26±4.65 years in group II; which was ranged between 20-39 years. There were no patients (0.0%) in group I who were between the ages of 20 and 25 years, while group II had 22 patients (51.2%) who were in that age range. In groups I and II, there were 19 (44.2%) and 21 (40.8%) pregnant women who were between the ages of 26 and 30 years. Among those aged between 31 to 35 years; 19 (44.2%) and 0 (0.0%) were found in groups I and II respectively. There were 5 (11.6%) pregnant women in group I who were between the ages of 36 and 40 years, but none in group II (Figure- 1).

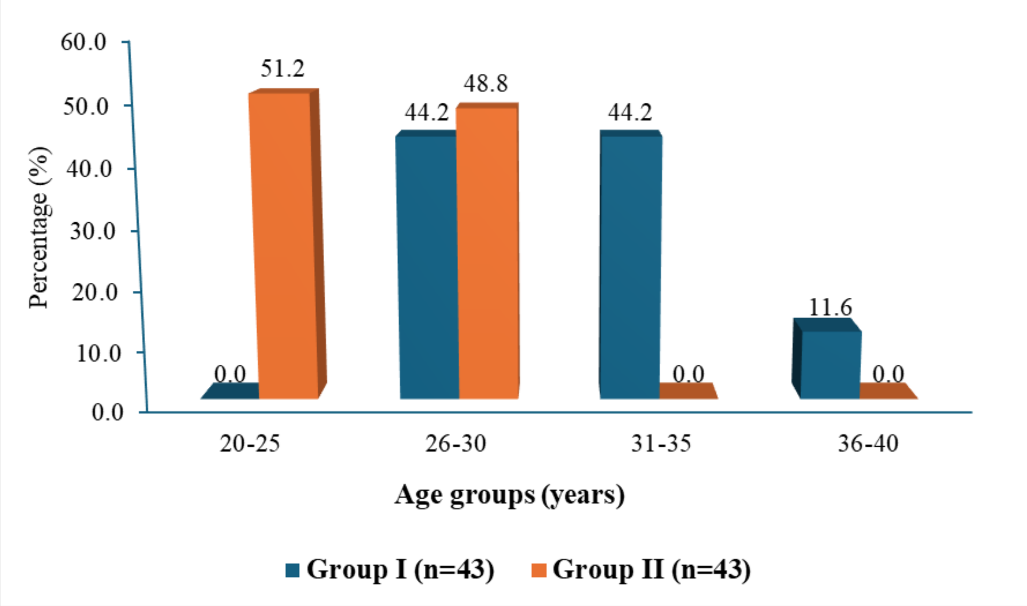

Figure- 1: Age distribution of the study subjects (N= 86)

The distribution of study subjects by gestational age as determined by ultrasonography (USG) showed that; the majority of the study participants [35, (81.4%)] in group I had between 28 to 36 weeks of gestation, while 33(76.7%) pregnant women in group II had the same gestational age. However, 10 (23.3%) participants in group II and 8 (18.6%) subjects in group I had ≥37 weeks of gestation, respectively. Gestational age was not significantly different between the groups (p= 0.398) (Table- 1).

Table- I: Distribution of the study population on gestational age determined by ultrasonography (N= 86)

Gestational age (weeks)	Group I (n=43)	Group II (n=43)	p value
	n (%)	n (%)	
28-36 weeks	35 (81.4)	33 (76.7)	
≥ 37 weeks	8 (18.6)	10 (23.3)	
Mean gestational age (weeks)	34.7±4.56	33.8±5.25	0.398^{ns}

Data were expressed as frequency, percentage and mean±SD,
Unpaired Student t-test was performed to compare between the groups, ns= Not significant

In this study 10 (23.3%) GDM women in group-I and 18 (41.9%) pregnant women in group-II were within normal body weight; but 24 (55.8%) study subjects in group-I and 22 (51.2%) study subjects in group-II were overweight; While, 9 (20.9%) subjects in group-I and 3 (6.9%) subjects in group-II were obese. The mean(±SD) body mass index (BMI) was 30.1±4.72 kg/m^2 in group I and that was 29.8±5.65 kg/m^2 in group II. The body mass index (BMI) was not significantly different among the groups (p= 0.790) (Table- 2).

Table- 2: Distribution of the study subjects on different body mass index (BMI) categories (N= 86)

Body mass index (BMI) categories	Group I (n=43) n (%)	Group II (n=43) n (%)	p value
Normal weight (19.0-24.99 kg/m^2)	10 (23.3)	18 (41.9)	
Overweight (25.0-29.9 kg/m^2)	24 (55.8)	22 (51.2)	
Obese (>30.0 kg/m^2)	9 (20.9)	3 (6.9)	
Mean±SD	30.1±4.72 kg/m^2	29.8±5.65 kg/m^2	0.790^{ns}
Range (minimum - maximum)	21.5 - 34.8 kg/m^2	19.4 -34.0 kg/m^2	

Data were expressed as frequency, percentage and mean±SD,
Unpaired Student t-test was performed to compare between the groups, ns= Not significant

It was observed that mean serum vitamin B_{12} concentration in group I was 158.7±91.8 pmol/L and that was 264.5±100.9 pmol/L in group II. The mean vitamin B_{12} concentration was significantly low in group I than group II (p= 0.001) (Table- 3).

Table- 3: Serum vitamin B_{12} concentration between two groups (N= 86)

Serum vitamin B_{12} concentration	Group I (n=43)	Group II (n=43)	p value
	n (%)	n (%)	
Normal (160-490 pmol/L)	25 (58.1)	34 (79.1)	
Low (<160 pmol/L)	18 (41.9)	9 (20.9)	
Mean±SD (pmol/L)	158.7±91.8	264.5±100.9	0.001^{s}

Data were expressed as frequency, percentage and mean±SD,
Unpaired Student t-test was performed to compare between the groups, s= significant

Using Pearson's correlation coefficient test, the relationship between serum vitamin B_{12} concentration and plasma glucose levels were examined. Serum vitamin B_{12} concentration had a significantly negative correlation with fasting plasma glucose level in women with GDM (r= -0.432, p= 0.004). In women with GDM, there was a significant negative correlation between serum vitamin B_{12} concentration and plasma glucose level one hour after 75 gm of glucose load (r= -0.519, p<0.001). Additionally, a significant negative correlation was observed between the serum vitamin B_{12} concentration in GDM women and their plasma glucose level two hours after 75 gm glucose load (r= -0.687, p<0.001) (Table 4).

Table- 4: Relationships between serum vitamin B_{12} concentration with fasting plasma glucose, 1-hour and 2-hours after 75 gm glucose levels in GDM women (n= 43)

Variables	r value	p value
Vitamin B_{12} versus fasting plasma glucose	-0.432	0.004
Vitamin B_{12} versus plasma glucose 1 hour after 75gm glucose	-0.519	<0.001
Vitamin B12 versus plasma glucose 2 hours after 75gm glucose	-0.687	<0.001

To evaluate the risk variables for developing GDM, a multivariate logistic regression analysis was performed. It was found that serum B_{12} concentration with age and body mass index (BMI) were the independent predictors for GDM. The findings indicated that older age was associated with almost three-fold increased risk of developing GDM (p= 0.021); body mass index (BMI) was associated with almost three-fold increased risk of developing GDM (p= 0.017), while serum B_{12} concentration was associated with a two-fold increased risk of developing GDM (p= 0.042) (Table 5).

Table- 5: Multivariate logistic regression analysis to assess the independent predictors of GDM (N= 86)

Variables	Odds Ratio (OR)	95% CI	p-value
Age (years)	2.786	1.188-5.546	0.021^{s}
BMI (kg/m^2)	2.567	1.182-5.573	0.017^{s}
Gestational age (weeks)	0.805	0.165-3.93	0.789^{ns}
Serum vitamin B_{12} (pmol/L)	1.997	1.990-1.005	0.042^{s}

s= Significant, ns= Not significant

4. DISCUSSION

Gestational diabetes mellitus (GDM) is a type of glucose intolerance with variable-intensity, that first appears or is identified during pregnancy [21]. Pregnancy-related glucose intolerance GDM can have detrimental effects on the mother and fetus [21-22]. When a pregnant woman cannot secrete enough insulin to offset the nutritional boost during pregnancy, along with the increased production of fat and anti-insulin hormones like the human placental hormones- prolactin, cortisol, and progesterone, they develop gestational diabetes [21]. Although the prevalence of GDM is around 18%, in several Asian nations it is over 20% [23]. Cobalamin, or vitamin B_{12}, is a water-soluble vitamin that is essential for cell metabolism and DNA methylation. Maternal body mass index (BMI), insulin resistance, and lipid profile are just a few of the health metrics that can be impacted by vitamin B_{12} deficiency, which is frequent during pregnancy [24-26]. It was reported that, vitamin B_{12} deficiency can result in insulin resistance and glucose intolerance, which can lead to GDM [27-29]. This study enrolled 86 pregnant women; of them 43 were pregnant women with GDM and another 43 were normal pregnant women (without GDM) to evaluate the association of vitamin B_{12} between GDM and normal pregnant women.

In this study, the mean age was 32±6.02 years in GDM women and that was 26±4.65 years in normal pregnant women. There was statistically significant difference between the groups ($p<0.001$). This result was comparable with similar previous studies [19, 30-31]. It was observed that majority (55.8%) of the GDM women were in 4th decade of life, while all normal pregnant women (without GDM) were in 3rd decade. This statement was an agreement of a related previous study [32]. This study showed that the mean gestational age was 34.7±4.56 weeks in GDM women and 33.8±5.25weeks in normal pregnant women. This finding was supported by a couple of related study [33-34]. Among the study population; mean body mass index (BMI) was 30.1±4.72 kg/m^2 in group I and that was 29.8±5.65kg/m^2 in group II. The body mass index (BMI) was not significantly different among the study groups ($p=0.256$). This finding was matched with a similar study [32].

The current study revealed that; 41.9% GDM women had low serum vitamin B_{12} concentration and only 20.9% normal pregnant women had low serum vitamin B_{12} concentration. The mean serum vitamin B_{12} concentration was significantly low in GDM women than normal pregnant women ($p<0.001$). In GDM group the mean serum vitamin B_{12} concentration was 158.7±91.8 pmol/L and in normal pregnant women that was 264.5±100.9 pmol/L. In this context, Sukumar N *et al.*, found that mean serum vitamin B_{12} concentration was lower in GDM women (169.0 pmol/L) than in normal pregnant women (195.6 pmol/L) which was statistically significant ($p<0.001$) [35]. Similarly, Ambreen AB *et al.*, reported that mean serum vitamin B_{12} concentration was 127.97±80.18 pmol/L in GDM women and 179.81±91.05 pmol/L in normal pregnant women which was statistically significant ($p<0.001$) [36]. The current study was supported by these previous studies [35-36].

In this study, Pearson's correlation coefficient test was done in order to assess the correlation between fasting plasma glucose level, plasma glucose level one hour after 75 gm of glucose load and plasma glucose level two hours after 75 gm of glucose load among GDM women. In women with GDM it was observed that; serum vitamin B_{12} concentration had a significantly negative correlation with these three glucose levels ($p<0.05$). Similar findings were observed in previous studies [37-38].

A multivariate logistic regression analysis was done to investigate the association of maternal vitamin B_{12} concentration with risk of developing GDM after adjusting for the effect of age, gestational age and BMI. Data analysis indicated that serum B_{12} concentration was associated with a two-fold increased risk of developing GDM [OR of 1.977 (95% CI-1.990-1.005; p=0.042). In accordance, Ambreen AB *et al.*, found that women with low serum vitamin B_{12} concentration had high odd ratio and increased risk of GDM (OR= 71%, 95% CI: 60.9-81.2%; p<0.0001) [36]. This present study showed older age had 3 times more risk for developing GDM [OR= 2.786 (95% CI:1.188-5.546; p= 0.021). Similarly, Lai JS *et al.*, found that older women had a significant higher risk for developing GDM (OR= 2.67, 95% CI: 2.13–3.34; p<0.001) [19]. In this current study BMI had OR of 2.567 (95% CI:1.182-5.573; p= 0.017) indicated a significant higher risk for developing GDM. Previous studies also documented that, increased BMI had significant risk of developing GDM [19, 36]. The current study findings were supported by these previous studies [19, 36].

The current study has some limitations. Firstly, it was a single center study. Secondly, the sample size was relatively small. Furthermore, patient age, BMI, gestational age and vitamin B_{12} concentration were the most common factors adjusted in multivariate analysis but other important factors were not taken into account.

5. CONCLUSION

This current study concluded that GDM women had low serum vitamin B_{12} concentration than normal pregnant women. The present study indicates a low serum vitamin B_{12} concentration increase the risk of developing GDM. Vitamin B_{12} concentration may be used as a predictive tool to identify risk of developing GDM. Follow up study is recommended for the better evaluation of vitamin B_{12} concentration in GDM women.

Conflicts of interest: Authors declare no conflict of interest.

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