Original Article

Frequency of Vitamin B_{12} Deficiency in Gestational Diabetes Mellitus Patients Reporting at a Tertiary Care Hospital

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ABSTRACT

Objective: To determine the frequency of Vitamin B_{12} deficiency in Gestational Diabetes mellitus (GDM) patients reporting at a tertiary care hospital.

Study Design: Observational Study

Place and Duration of Study: This study was conducted at the Department of Biochemistry, Gynecology & Obstetrics Liaquat University Hospital Jamshoro from Jan 2018 to December 2018.

Materials and Methods: A sample of 291 pregnant women grouped as; 216 diagnosed GDM cases and 75 controls was selected according to criteria. Fasting blood glucose ≥100 mg/dl was taken as GDM. Venous blood samples were centrifuged to separate sera used for the estimation of blood glucose (hexokinase method) and Vitamin B12 (ECLIA assay method). Statistical SPSS software 22.0 (IBM, Inc USA) analyzed data using Student t-test and Chi-square test at 95% CI ($P \leq 0.05$).

Results: Age (mean±SD) of control and cases was 37.9± 9.51 and 36.3±9.42 years respectively (P=0.81). Vitamin B_{12} of control and cases was 215.6±43.7 and 155.1±80.7 ng/mL (P=0.0001). Vitamin B12 deficiency was noted in 70.39% of GDM cases compared to 51.6% in controls (P=0.0001). Vitamin B12 shows negative correlation with FBG (r= - 0.176, P=0.031) and RBG (r= - 0.230, P=0.0001).

Conclusion: The present study reports 70.39% frequency of vitamin B12 deficiency in gestational diabetes mellitus compared to 51.6% in controls.

Key Words: Vitamin B_{12}, Gestational Diabetes mellitus, Blood glucose

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a disorder of glucose metabolism characterized by hyperglycemia and glycosuria first time observed during pregnancy. GDM is defined as hyperglycemia due to glucose intolerance during pregnancy.[1] It`s true prevalence is lacking in developing countries because of lack of registries. However, prevalence of 1- 14% is suggested for the GDM. Frequency and prevalence of GDM varies across the World.

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Approximately 7% of women who conceive develop GMD and burden is estimated as >200,000 cases per year.[2] GDM women are prone to fetal malformation & macrosomia, pre-eclampsia, eclampsia. Cesarean section ratio is increased in GDM women.[3,4] One of the risk factor for GDM is the obesity and its prevalence is rising in the urban society.[5] Insulin resistance is present in obese women and role for GDM during pregnancy. Pregnancy is a state of insulin resistance due to altered hormones. Insulin resistance occurs at 24–28 weeks of gestation and progresses till last trimester.[6] GDM women show a change in blood glucose, lipids and insulin levels. This accounts as primary metabolic defect that manifests as GDM in its severe form.[7] Primary metabolic defect of GDM is worsened by malnutrition and vitamin deficiencies. B complex vitamins are necessary for glucose metabolism.[8] A previous study[9] reported high frequency of vitamin B12 deficiency in pregnant women and was associated with insulin resistance, hyperglycemia, hyperlipidemia, adiposity and glycosuria compared to those with normal B12 levels. Vitamin B_{12} is essentially required for the nuclear maturation of cell through nucleic acid biosynthesis by methylation. Methylation is required for the DNA synthesis, protein and phospholipids synthesis and neurotransmitters. Myelination of brain

needs methylation reaction. Blood cells show megaloblastic changes and anemia. Defective myelination, polyhydramnios, birth defects, defective brain development, respiratory distress syndrome and still births are common in pregnant women with vitamin B_{12} deficiency.[8,9] However, the research on the epigenetic effect correlating with hypo vitamin B_{12} during pregnancy is scarce.[8-10] Search of national literature for Pakistan shows lack of sufficient data on the topic of frequency and prevalence of vitamin B12 deficiency in the women suffering from gestational diabetes mellitus. Therefore; the present study was conducted in gestational diabetes mellitus women presenting at our tertiary care hospital to evaluate the frequency of vitamin B_{12} deficiency.

MATERIALS AND METHODS

The present case control observation study was conducted at the Department of Biochemistry, Gynecology & Obstetrics Liaquat University Hospital Jamshoro. A sample of 291 (n= 291) diagnosed cases of pregnant women was selected through non-probability purposive technique. The study covered duration of Jan 2018 to December 2018. Pregnant women fulfilling the criteria of GDM were segregated as cases (n=216). Normal healthy pregnant with normoglycemia were termed as controls (n=75). Inclusion criteria followed were; voluntary pregnant women, diagnosed Gestational Diabetes mellitus, age 20 – 40 years, pregnancy of 2nd and 3rd trimester, singleton fetus and healthy women were selected as cases. Known cases of DM, polycystic ovarian syndrome (PCOS), post-partum female, and those suffering from major systemic disease were excluded. Patients were interviewed for the purpose of study, protocol, harms and benefits. Only volunteers were entered in the study protocol. Volunteer pregnant women – both cases and control were asked to sign the consent form. Biodata, physical examination findings and laboratory findings were saved in a proforma. Data was confidential locked in lockers. Institutional ethical approval was taken in prior from the ethical review committee of institute. Research ethical standards were in accordance to the "Helsinki`s Declaration" for patient handling. Volunteers were asked to comply the study protocol. Venous blood samples were taken from peripheral vein. Fasting and random blood samples were collected for glucose and vitamin B12 estimation. Five ml blood was collected in vacutainer and NaF tubes. Samples were centrifuged for 15 minutes (at x3000 rpm) to separate sera. Samples were stored and preserved in refrigerators. Blood Glucose was estimated by hexokinase method. Vitamin B12 was analyzed by ELISA commercial kit (Abcam, USA) (competitive immuno- assay, Neoplate) as per instructions of the manufacturer. Quantity was measured at 450nm absorbance. Biochemical estimation of variables was performed on Cobas chemistry analyzer. Vitamin B12 deficiency and normal levels were taken as cited previously.[11] Study variable data was calculated on SPSS 21.0 version (for Windows release). Continuous data age, FBG, RBG and vitamin B12 were calculated by Student`s t-test and data was presented as mean+/- SD. 95% confidence interval was considered of statistical significance ($P \leq 0.05$). Vitamin B12 categories of insufficiency and deficiency were calculated by Chi- square test. Correlation co-efficient (r-value) and P-value were analyzed by Pearson`s analysis. Statistical significance of results was taken at 95% CI ($P \leq 0.05$).

RESULTS

Age (mean±SD) of control and cases was 37.9± 9.51 and 36.3±9.42 years respectively (P=0.81). Fasting and random blood glucose reveals significant difference ($P<0.05$).

Table No.1: Age and Biochemical findings in control and cases (n=291)

	Control	Cases	t-value	P-value
Age	37.9±9.51	36.3±9.52	1.33	0.81
FBG (mg/dl)	80.5±10.5	148.1±55.6	10.3	0.0001
RBG (mg/dl)	141.0±21.33	241.1±70.5	12.05	0.0001
Vitamin B_{12} (ng/mL)	215.6±43.7	155.1±80.7	1.136	0.0001

Table No.2: Vitamin B_{12} (ng/mL) in control and cases (n=291)

	Control		Cases		X^2-value	P-value
	No.	%	No.	%		
Normal levels	37	49.3	64	29.6	87.00	0.0001
Borderline deficiency	27	36.0	36	16.6		
Deficiency	09	12.0	30	13.8		
Severe deficiency	02	2.6	86	39.81		
Total	75	100	216	100		

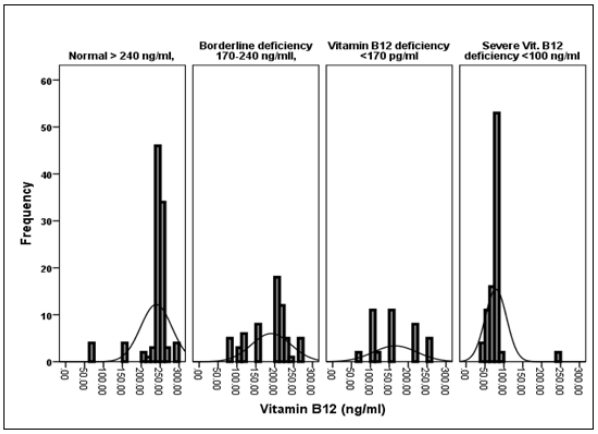
Table No.3: Vitamin B_{12} level (ng/mL) in cases (n=216)

	Mean	SD	95% CI for Mean		Min.	Max.
			L. Bound	U. Bound		
Normal levels	239.56	41.36	231.39	247.73	70.00	298.00
Borderline deficiency	190.74	52.424	177.54	203.94	87.00	270.00
Deficiency	165.43	57.813	146.69	184.17	67.00	261.00
Severe deficiency	78.71	28.416	72.69	84.73	40.00	245.00
Total	170.41	78.536	161.35	179.48	40.00	298.00

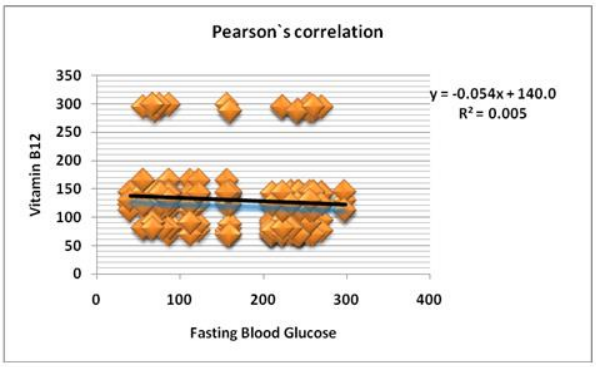
Table No.4: Pearson`s analysis of Vitamin B_{12} †

Fasting blood glucose	r-value*	- 0.176
	P-value**	0.031
Random blood glucose	r-value*	- 0.230
	P-value**	0.0001

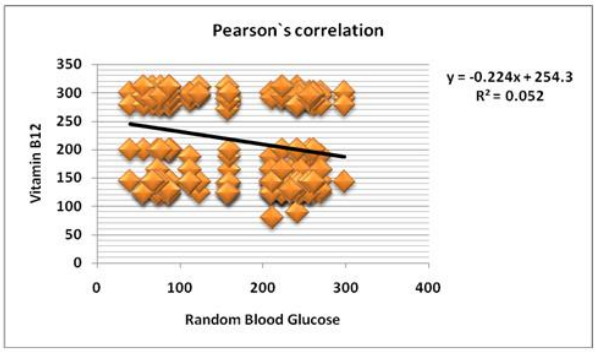
†. Correlation calculated at 0.05 level
*. r-value - Correlation co-efficient
**. P-value - Statistical significance



Graph No.1: Bar graph showing vitamin B12 distribution in cases



Graph No.2: Scatter plot shows negative correlation of vitamin B_{12} and Fasting Blood Glucose (r= - 0.176, P=0.0001) in cases



Graph No.3: Scatter plot shows negative correlation of vitamin B_{12} and Fasting Blood Glucose (r= - 0.230, P=0.0001) in cases

Vitamin B_{12} of control and cases was 215.6±43.7 and 155.1±80.7 ng/mL (P=0.0001) (table 1). Table 2 shows the frequency of vitamin B12 levels of normal, borderline deficiency, deficiency and severe deficiency. Normal vitamin B_{12} level was noted in 49.3% and 29.6% of control and cases. Vitamin B12 deficiency was noted in 70.39% of GDM cases compared to 51.6% in controls (P=0.0001) (table 2). Table 3 shows the vitamin B_{12} (mean±SD) in normal, borderline deficiency, deficiency and severe deficiency in control and cases (Graph 1). Pearson`s correlation analysis of vitamin B12 and blood glucose is shown in table 4. Vitamin B12 shows negative correlation with FBG (r= - 0.176, P=0.031) and RBG (r= - 0.230, P=0.0001). Negative correlation proved statistically significant (P<0.05) (Graph 2 and 3).

DISCUSSION

The present study is first observational research probing into the vitamin B12 status of pregnant women suffering from GDM. The present study shows 70.39% vitamin B12 deficiency in GDM cases compared to 51.6% in controls (P=0.0001). This shows the gravity of Vitamin B12 deficiency in pregnant women suffering from gestation diabetes mellitus. Our observation is in agreement with previous studies cited as.[12,13] Vitamin B12 deficiency in normal pregnant women has been reported by a previous study.[14] While vitamin B12 deficiency in pregnant women with GDM is in agreement with another previous study.[15] Increased insulin resistance and adiposity in pregnant women with vitamin B12 deficiency was reported.[15] Above study, further added that the risk of GDM is twice more frequent pregnant women with cobalamin deficiency compared to cobalamin non-deficient pregnant women. The finding is in keeping with present and previous study.[14] A previous study[16] found frequency of 67% vitamin B12 deficiency in pregnancy compared to 39% in controls. Our findings of 70.39% vitamin B12 deficiency in GDM cases and 51.6% in controls are relatively higher compared to above study. Inconsistent frequency may be due to different social class, dietary habits, sample size, etc. However, vitamin B12 deficiency is a consistent finding. Vitamin B12 deficiency is prevalent in developing countries[17,18] due to dietary deficiency, this is the most probable reason of high frequency noted in the present study. Vitamin B12 deficiency has also been reported in the British pregnant women[19] who are taking balanced diet without nutritional deficiency. Other important observation of present study is the negative association/correlation of vitamin B12 with blood glucose levels. Vitamin B12 shows negative association with the fasting (r= - 0.176, P=0.031) and random (r= - 0.230, P=0.0001) blood glucose levels. Negative correlation proved statistically significant (P<0.05) (Graph 2 and 3). The observation of negative association of vitamin B12 with fasting blood glucose (r= -0.09; p=0.006) is in line with a previous study.[19] Another previous study[20] from UK reported the low vitamin B12 levels in pregnant women

suffering from GDM, and further added 2.59 higher odds ratio of developing GDM in vitamin B12 deficient women with pregnancy. The findings are consistent with observation of vitamin B12 deficiency in present study. A previous study[16] reported negative correlation of vitamin B12 levels with gestational age (β=-0.57) (p=0.0021), gravidity (β=-0.28) (p=0.01) and fasting blood glucose (r=-0.29) (p=0.004). Negative association of vitamin B12 and fasting blood glucose is consistent with present study. Some of previous studies[21-23] reported vitamin B12 deficiency is exaggerated by metformin therapy that was exclusion in the present study. From the evidence based findings of high frequency of vitamin B12 deficiency in light of published literature, it is advised for strict monitoring of vitamin B12 for betterment of pregnancy outcome. Limitations of present study are; small sample size, nutritional status and dietary habits are not clear. Cause effect association of vitamin B12 and GDM are questionable because of cross sectional study design. However, the prospective study and potential confounding factor – smoking exclusion add to the strength of the study results.

CONCLUSION

The present study reports 70.39% frequency of vitamin B12 deficiency in gestational diabetes mellitus compared to 51.6% in controls. Vitamin B_{12} shows negative correlation with fasting and random blood glucose. Vitamin B_{12} deficiency may cause serious maternal and fetal outcome that should be screened and managed properly for better fetal and maternal outcome.

Author's Contribution:

Concept & Design of Study:	Shehmeen Khan Khanzada
Drafting:	Sabreena Abbas Khokhar, Fouzia Shaikh
Data Analysis:	Muhammad Akbar, Akram Munir
Revisiting Critically:	Shehmeen Khan Khanzada, Sabreena Abbas Khokhar
Final Approval of version:	Shehmeen Khan Khanzada

Conflict of Interest: The study has no conflict of interest to declare by any author.

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