Original Article

Hypovitaminosis B_{12} in Pregnant Women Suffering from Gestational Diabetes Mellitus

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ABSTRACT

Objective: To determine the frequency of vitamin B12 deficiency (hypovitaminosis B_{12}) in Pregnant Women suffering from Gestational Diabetes mellitus,

Study Design: Observational / case control study

Place and Duration of study: This study was conducted at the Department of Pathology and Gynecology/Obstetrics, Indus Medical College, Tando Muhammad Khan from Jan 2018 to September 2019.

Materials and Methods: A sample of 200 pregnant women was divided into control normal pregnant women and GDM diagnosed cases. Subjects were selected by non – probability convenient sampling fulfilling criteria. Blood samples were collected by venesection and centrifuged to get sera. Hematological findings, blood glucose, HbA1c and vitamin B_{12} were estimated. Hypovitaminosis B12 was defined as <300 pg/ml. Continuous variable were analyzed by Student`s t-test and presented as mean +/- SD. Categorical data was cross tabulated by Chi – square test and presented as frequency and % at 95% CI ($P \leq 0.05$).

Results: Vitamin B12 in control was 316.2±116.9 pg/ml and cases 270.5±131.1 pg/ml (P=0.023). Hypovitaminosis B12 (<300 pg/mL) was noted in 39% of control compared to 61% in GDM cases (P=0.0001). Vitamin B12 shows significant negative correlation with age (r= - 0.262, P=0.0001), gestational age (r= - 0.238, P=0.001), gravida (r= - 0.277, P=0.0001), FBG (r= - 0.284, P=0.0001) and RBG (r= - 0.229, P=0.0001). Vitamin B12 shows non – significant correlation with HbA1c (r= - 0.014, P=0.083).

Conclusion: We found hypovitaminosis B_{12} in 61% of GDM cases. Vitamin B12 shows significant negative correlation with age, gestational age, gravida, fasting and random blood glucose.

Key Words: Vitamin B_{12}, Glycemic control, Gestational Diabetes, Sindh

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance occurring first time in gestation that may disappear after parturition.[1] Approximately 7–14% of pregnancies are complicated by GDM. Prevalence of GMD depends on environmental factor, dietary habits, social conditions, ethnicity and geography of patients and diagnostic criteria used for glucose intolerance.[2]

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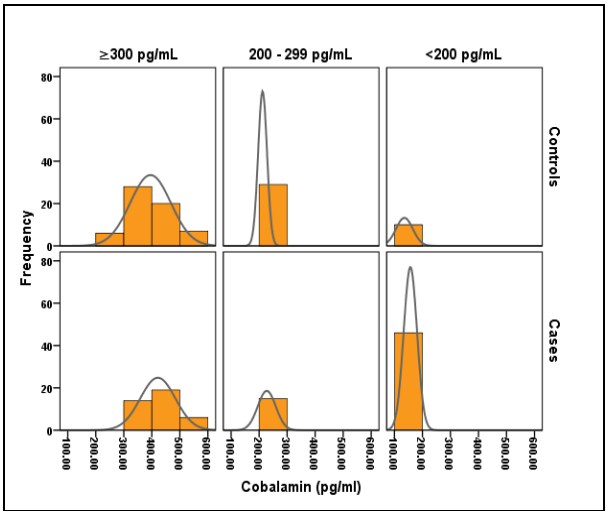
GDM prevalence of 4.2% to 26% has been reported for the South East Asia.[3,4] Risk of developing frank type 2 diabetes mellitus (T2DM) is increased seven times for the future pregnancies in those suffering from GDM.[5] Vitamin B12 is a vital micro nutrient essential for few peculiar biochemical reactions of nuclear maturation and metabolic reactions. Vitamin B12 is essential for the synthesis of methyl- cobalamin, S-adenosyl-methionine (SAM) and regeneration of methionine. Methyl – cobalamin is a major one carbon donor methyl donor for DNA methylation, nuclear maturation and cell division.[6,7] Nowadays, the vitamin B12 deficiency (hypovitaminosis B_{12}) has become increasing popular health problem.[8,9] Hypovitaminosis B_{12} is linked with glucose intolerance, insulin resistance (IR), gestational diabetes and T2DM in future.[10] Hypovitaminosis B_{12} in pregnant GDM women often results in intrauterine growth retardation, brain retardation, myelination defects, neural tube defects (NTDs), congenital malformations, macrosomia, neural tube defects, etc.[11] Hypovitaminosis B_{12} during pregnancy also puts mothers at grave complications of pre – eclampsia, eclampsia, abortions, etc. Pregnant women are at increased risk of hypovitaminosis B_{12} due

to the poor nutritional status, increased vitamin demand and poor dietary supply. Rapidly growing fetal tissue demands more vitamins, resulting in deficiency. Hypovitaminosis B_{12} is very common in the general population, pregnant mothers, GDM pregnant women, etc. Health issues of vitamin and mineral deficiencies are hotly hinted in the medical literature and hypovitaminosis B_{12} being very common and is a serious threat to the mother and fetus equally.[12,13] There is dire need to make the data available at national level for the hypovitaminosis B_{12} in pregnant GDM women for favorable maternal and fetal outcome. In this context, the present study was planned to determine the frequency of hypovitaminosis B12 levels in Pregnant Women suffering from Gestational Diabetes mellitus reporting at our tertiary care hospital.

MATERIALS AND METHODS

The present observational (case control) study was conducted at the Department of Pathology and Gynecology/Obstetrics, Indus Medical College, Tando Muhammad Khan, Pakistan. The study covered duration from January 2018 to September 2019. Study was conducted after the approval of ERC (ethical review committee). Technique of "Sampling for proportions" was used for sample size calculation as; 95 by using 5% α-level of significance, and "Power of test" of 90% for an assumed % of vitamin B12 deficiency in 51% GDM women and 21.9% in non – GDM.[5] Considering increase in power of study, 200 Pregnant women were equally divided into; Control (n=100) Non – GDM Pregnant women and Cases (n=100) GDM diagnosed Pregnant women. Pregnant women were selected by convenient sampling. Prior – set inclusion and exclusion criteria were followed for the control and case selection. Case was defined as a pregnant woman (2^{nd} & 3^{rd} trimester) showing fasting blood glucose (FBG) ≥100 mg/dL.[14] Gravida was defined as number of total conceptions irrespective of outcome. Gestational age was calculated from the first day of last menstrual period (LMP). Pregnant women of $2^{nd}/3^{rd}$ trimester with normal FBG qualified as control. Volunteer pregnant women of $2^{nd}/3^{rd}$ trimester with FBG ≥100 mg/dL, age 21 – 40 years qualified for GDM control. Volunteer GDM patients taking metformin and B_{12} vitamin supplements were excluded. Pregnant women with and without GDM suffering from major systemic disease, proton pump inhibitor intake, strict vegetarians, chronic liver diseases were also excluded. Participants were informed of the purpose of study, benefits and harms. Volunteers were informed that the data will be published in a medical journal for patient benefit. Participants were informed that biodata and blood findings will never be publicized individually. Expenses of laboratory investigations were borne by the researchers. A proforma was formulated prior to study for data entry and maintained confidentiality of patient data. Clinical history, physical examination and laboratory findings were noted. Volunteers were requested to abide by the study protocol strictly, full co-operation and follow ups. Volunteers were asked to sign the consent form and willingness for blood samples. Volunteer women were asked for blood sampling. Blood samples were collected by senior staff nurse as per standard criteria after securing sterilization protocol. Blood was collected by venesection from ante – cubital vein in a Disposable syringe (BD, USA). 5 ml blood was taken from peripheral vein; 3 ml was taken in EDTA tube and 2 ml in plain tubes. Sera were taken by centrifuging blood sample (x3000 rpm, 15 min.). Analysis of hemoglobin, Hct, RBC counts and Platelet counts were performed by researcher / consultant Pathologist. Sera were used for the estimation of blood glucose (hexokinase) and HbA1c (Colorimetric method) and vitamin B12 by ELISA assay. Hypovitaminosis B12 was defined as <300 pg/ml. Vitamin B12 levels were categorized as ≥300 pg/mL – as normal, 200 - 299 pg/mL – as marginal deficiency and <200 pg/mL – deficiency.[15] Research variables were typed in a Microsoft Excel sheet and Statistical analysis on SPSS software 21.0 (IBM, Inc USA). Continuous variable output was tabulated as mean +/- SD and analyzed by Student`s t-test. Categorical variable output was cross tabulated as frequency and % and analyzed by Chi-square test. Numerical data association was analyzed by Pearson`s correlation and output were tabulated as correlation co – efficient (r) value with statistical significance. Statistical significance was taken at 95% CI (P≤ 0.05).

RESULTS



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

Age, gestational age, gravida, hemoglobin, hematocrit, RBC and platelet counts are shown in table 1 in control and cases. Random blood glucose (RBG), fasting blood glucose (FBG) and Glycated HbA_1 (HbA1c) shows

statistically significant difference between control and cases (P <0.05).

Table No.1: Demographic and Laboratory findings of control and cases

Parameter	Control (n=100)	Cases (n=100)	P
	Mean± S.D (SEM)	Mean± S.D (SEM)	
Age (years)	30.3±1.59 (0.15)	30.1±1.27 (0.12)	0.93
Gestation age (weeks)	33.7±2.52 (0.25)	34.6±2.44 (0.24)	0.47
Gravida	2.3±0.48 (0.04)	2.45±0.49 (0.04)	0.10
Hct (%)	37.8±3.36 (0.33)	38.19±3.35 (0.33)	0.93
Hb (g/dl)	10.8±0.58 (0.05)	10.74±0.70 (0.07)	0.08
RBC counts ($x10^6/\mu L$)	3.21±0.45 (0.04)	3.19±0.48 (0.04)	0.12
Platelet ($x10^9/\mu L$)	3.74±0.39 (0.03)	3.61±0.40 (0.04)	0.65
FBG (mg/dl)	88.6±7.8 (0.78)	154.9±47.35 (4.73)	0.0001
RBG (mg/dl)	136.5±9.07 (0.90)	227.8±87.31 (8.73)	0.0001
HbA1c %	5.3±0.62 (0.11)	7.51±1.37 (0.13)	0.0001
Vitamin B_{12} (pg/ml)	316.2±116.9 (11.6)	270.5±131.1(13.10)	0.023

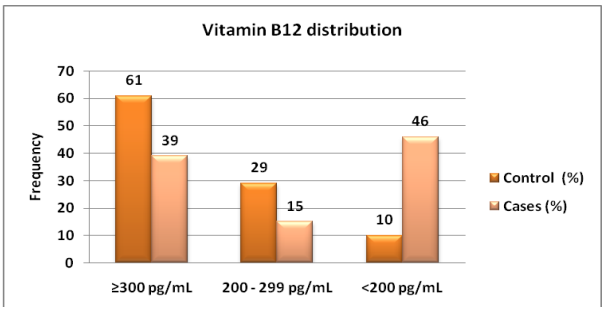
Table No.2: Frequency distribution of vitamin B_{12} in control and cases

Vitamin B_{12}	Control %	Cases %	X^2-value	P
≥300 pg/mL	61	39	201.0	0.0001
200 - 299 pg/mL	29	15		
<200 pg/mL	10	46		
Total	100	100		

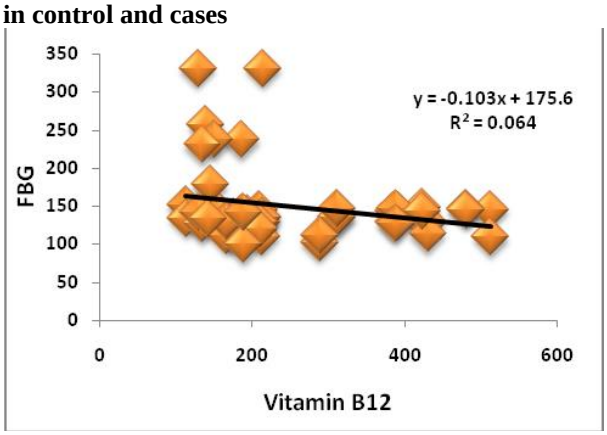
Table No.3: Pearson`s Correlation of serum cobalamin in cases

	r-value	P-value
Age	- 0.262	0.0001
Gestational Age	- 0.238	0.001
Gravida	- 0.277	0.0001
Fasting Blood Glucose	- 0.284	0.0001
Random Blood Glucose	- 0.229	0.0001
Glycated Hb A1(HbA1c)	- 0.014	0.083

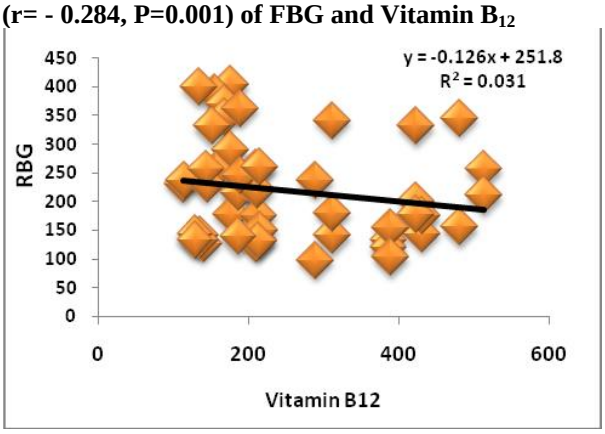
Vitamin B12 shows significant negative correlation with age (r= - 0.262, P=0.0001), gestational age (r= - 0.238, P=0.001), gravida (r= - 0.277, P=0.0001), FBG (r= - 0.284, P=0.0001) and RBG (r= - 0.229, P=0.0001). HbA1c (r= - 0.014, P=0.083) reveals non – significant correlation (Table – 3). Scatter plots 3 – 5 show the inverse correlation of vitamin B12 with FBG, RBG and HbA1c.



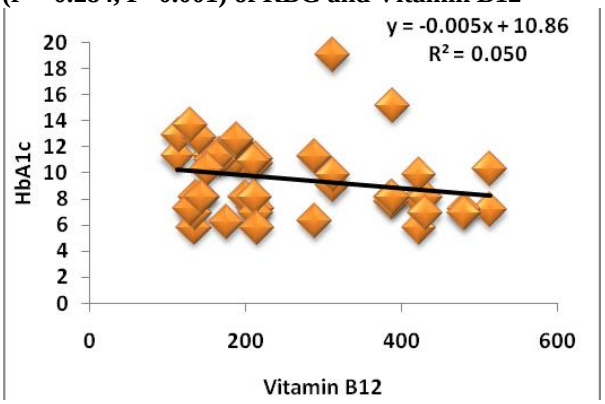
Graph No.2: Frequency distribution of vitamin B_{12} in control and cases



Graph No.3: Scatter plot shows inverse correlation (r= - 0.284, P=0.001) of FBG and Vitamin B_{12}



Graph No.4. Scatter plot shows inverse correlation (r= -0.284, P=0.001) of RBG and Vitamin B12



Graph No.5. Scatter plot shows non – significant inverse correlation (r=-0.014, P=0.083) of HbA1c and vitamin B12

DISCUSSION

The present hospital-based study reports hypovitaminosis B_{12} in 61% of GDM cases and 39% of controls (P=0.0001). Findings of hypovitaminosis in GDM cases is in agreement with previous studies.[5,16-19] A previous study (Ambreen *et al* 2017)[19] reported hypovitaminosis in 67% of GDM cases and 39% in control the findings are in full agreement with the present study. A previous retrospective study[5] from UK reported severe hypovitaminosis B12 in GDM cases compared to non- GDM pregnant women. They further added the risk of GDM is 2.59 times high in hypovitaminosis B12 pregnant women compared to those with normal vitamin B12.[5] The findings are in keeping with the present study. Another previous study[20] reported hypovitaminosis B12 is very common even in non – GDM pregnant women A previous study[21] from South East Asia reported similar observation of hypovitamosis B12 in gestational diabetes mellitus cases. This previous study reported hypovitaminosis B12 was positively associated with adiposity, insulin resistance (IR) and GDM. A previous study[20] reported hypovitamosis is prevalent in the South East Asia in GDM cases. They proved the cause effect relationship of hypovitaminosis B12 and GDM and the risk was doubled in cobalamin deficient pregnant women. The observations of this previous study are in consistent with the present study. However, the cause effect relationship was not ascertained in the present study due to the study design. Knight et al[22] studied a cohort of pregnant women of British ethnicity and analyzed the vitamin B12 levels. They reported severe hypovitaminosis B12 in the indigenous population despite nutritious dietary habits.[22] In present study, hypovitaminosis B12 in GDM cases was severe enough with vitamin levels of 270.5±131.1 pg/ml compared to 316.2±116.9 pg/ml in control (P=0.023). Other noteworthy finding of present study is vitamin B12 shows significant negative correlation with age, gestational age, gravida, FBG and RBG. However; HbA1c shows non – significant correlation with vitamin B12 (r= - 0.014, P=0.083). The findings of inverse correlation are in agreement with previous studies.[4,22-25] A previous study[22] reported vitamin B12 shows negative correlation with fasting blood glucose (r= -0.09; p=0.006) similar to noted in the present study. The present study proves negative correlation of vitamin B12 with age, gestational age, gravida, FBG and RBG. In contrast, to above study, the HbA1c shows non – significant correlation with vitamin B12 (r= -0.014, P=0.083). A previous study[4] reported inverse association of vitamin B12 with fasting blood glucose (β= -0.29, p=0.004), gravidity (β = -0.28, p=0.01) and gestational age (β = -0.57, p=0.21). The findings are highly consistent to the present study as we found negative correlation of vitamin B12 with age, gestational age, gravida, FBG and RBG. Previous studies[26,27] reported vitamin B12 deficiency is prevalent in the normal population of the country, hence the fact of increased demand in GDM may create more severe hypovitamosis B12 in pregnant women. Few of previous studies[28-30] suggested the hypovitaminosis B12 is common in diabetics due to the metformin therapy, but this was exclusion criteria in the present study, hence the findings point towards more prevalent vitamin deficiency. The finding of 61% hypovitamosis B12 in GDM women suggests the gravity of the problem that may result in severe fetal malformations and adverse maternal outcome, and these may be prevented simply by vitamin B12 supplements particularly in the developing countries where malnutrition is prevailing. Findings of present study in context of national and international literature, is of opinion of timely screening of vitamin B12 and vitamin supplementation. Only limitation of present study is a small sample size, hence findings cannot be generalized for other parts of country. Study strength lays in its prospective study design, inclusion and exclusion criteria. It is suggested the vitamin B12 screening may be mandatory for women suffering from gestational diabetes mellitus and timely vitamin B12 supplements be given for fetal and maternal benefit.

CONCLUSION

The present study reports hypovitaminosis B_{12} in 61% of GDM cases. Vitamin B12 shows significant negative correlation with age, gestational age, gravida, fasting and random blood glucose. Severe hypovitamosis B12 in GDM women suggests the gravity of the problem that may result in severe fetal malformations and adverse maternal outcome, and this may simply be prevented by vitamin B12 supplements particularly in the developing countries where malnutrition is prevailing.

Author's Contribution:

Concept & Design of Study:	Inayatullah Memon
Drafting:	Khadim Hussain, Momna Khan
Data Analysis:	Sadia Nizamani, Ghulam Abbas Soomro, Syed Ali Akbar Shah
Revisiting Critically:	Inayatullah Memon, Khadim Hussain
Final Approval of version:	Inayatullah Memon

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

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